ECOGEOGRAPHIC, GENETIC AND TAXONOMIC STUDIES OF THE GENUS *LATHYRUS* L.

BY

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ABSTRACT

Lathyrus species are well placed to meet the increasing global demand for food and animal feed, at the time of climate change. Conservation and sustainable use of the genetic resources of *Lathyrus* is of significant importance to allow the regain of interest in *Lathyrus* species in world.

A comprehensive global database of *Lathyrus* species originating from the Mediterranean Basin, Caucasus, Central and West Asia Regions is developed using accessions in major genebanks and information from eight herbaria in Europe. This Global *Lathyrus* database was used to conduct gap analysis to guide future collecting missions and *in situ* conservation efforts for 37 priority species. The results showed the highest concentration of *Lathyrus* priority species in the countries of the Fertile Crescent, France, Italy and Greece. The region extending from South-Central Turkey, through the western Mediterranean mountains of Syria to the northern Bekaa valley in Lebanon, and precisely the area around the Lebanese / Syrian border near Tel Kalakh region in Homs, was identified as the hotspot and the overall priority location for establishing genetic reserves. The gap analysis for *ex situ* conservation shows that only 6 species of the 37 priority species are adequately sampled. Showing a need for more collecting missions in the areas underrepresented, and for collecting closely related wild species of *Lathyrus* L. Six priority *Lathyrus* species have no *ex situ* collections requiring also further targeted *ex situ* collecting.

Core subsets of *Lathyrus* species were identified by using several methods to develop manageable subsets which capture most of the variation from the original dataset and with high probability of finding sought traits. MaxEnt, PowerCore programs and R language

platform facilitated subsets were derived from 2674 accessions belonging to 31 *Lathyrus* species originating from the Mediterranean Basin and the Caucasus, Central and West Asia regions. Focused Identification of Germplasm Strategy (FIGS) was also used to derive a heat and drought tolerance subset based on maximum temperature and aridity index. PowerCore had the highest Shannon diversity index based on species, but does not capture enough accessions within species, which could be due to low number and nature of variables considered. MaxEnt subset and random subsets selected on the basis of taxon and geographic representativity appear to capture most the variability in the original population. The diversity index could be improved by adding accessions of species not included in the selected random samples using any of the methods. FIGS has allowed for the selection of more accessions of species well known for their adaptation to drought and heat. These subsets, with manageable size and higher probability of finding the sought traits, will allow to link conservation with utilization of genetic resources and will reduce the pressure of regeneration of species with cross-pollination, as is the case of some species of *Lathyrus*.

Molecular characterization by using Amplified Fragment Length Polymorphism (AFLPs), along with the morphologic observation were used to clarify the taxonomic and phylogenetic relationships within and between the sections and the species of the genus *Lathyrus*. The results showed that the Sect. *Aphaca, Clymenum, Lathyrostylis* and large part of *Lathyrus* section could be differentiated either by using morphological characters or AFLP markers. In addition, the sections *Orobus, Pratensis* and *Orobastrum* could also be separated when using model-based clustering analysis. The sections *Linearicarpus* and *Nissolia* stayed grouped when applying different clustering methods to morphological characters and AFLP markers. Both morphological characters and polymorphic markers were able to assign efficiently the species and sub-species to their respective sections, however, morphological

characters allowed the discrimination of all species compared to AFLP markers. The used of STRUCTURE program improved further the classification of sections, but most importantly could highlight the genetic relationships among species.

A Field Guide for the "Grass pea and Chicklings (*Lathyrus* L.)" of the Mediterranean Basin and the Caucasus, Central and West Asia regions is produced including 76 taxa with line drawings for 54 taxa, to assist local plant genetic resources workers in species identification. This aid is using different illustrations (line drawings, photographs, paintings, etc.) of the key features of the species, hence avoiding recourse to complex botanic terminology. In addition, it includes well detailed texts containing scientific and vernacular names, diagnostic descriptions, iconography, and alliances to other species, distribution maps, phenology, ecological preferences, geographic distribution and conservation status.

DEDICATION

To my beloved late father (1924-2002)

To my mother who always prayed for my success

To my wife Rasha and children Abdullah, Amr, Bana and Leen

To my brothers and sister

for their understanding and support during my studies and preparation of the thesis

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LIST OF ABBREVIATION

AP-PCR - Arbitrary primed PCR

β-ODAP (beta-N-oxalyl-diamino-propionic acid

IPGRI – International Plant Genetic Resources Institute (Bioversity International).

TGGE - Thermal gradient gel electrophoresis

AFLP - Amplified fragment length polymorphism

AFLP - Amplified Fragment Length Polymorphism

ATP - Adenosine Triphosphate

BBS - Bangladesh Bureau of Statistics

BOAA - beta-(N)-oxalylamino-L-alanine acid

CAPS - Cleaved Amplified Polymorphic Sequence

CBD - Convention on Biological Diversity

cDNA – complementary DNA

CGIAR -Consultative Group on International Agricultural Research

CLIMA – Centre for Legumes in Mediterranean Agriculture, Australia

cpDNA – chloroplast DNA

CSA - Central Statistical Agency

CWANA - Central and West Asia and North Africa region

CWR – Crop Wild Relatives

DAF - DNA Amplification Fingerprinting

DAMD - Direct Amplification of Minisatellite-region DNA

DGGE - Denaturing Gradient Gel Electrophoresis

ECPGR - European Cooperative Programme for Plant Genetic Resources (ECPGR) (formerly "European Cooperative Programme for Crop Genetic Resources Networks -

EDTA - Ethylenediaminetetraacetic acid

EURISCO - European PGR collection catalogue

GBIF - Global Biodiversity Information Facilities

GCDT - Global Crop Diversity Trust.

GEF-The Global Environment Facility

GIS - Geographical Information System

GP – Gene Pool

GRIN - Genetic Resources Information Network

GRS - Genetic Resources Section, ICARDA

HD – Heteroduplex

ICAR - Indian Council of Agricultural Research

ICARDA - International Center for Agricultural Research in the Dry Areas

ILDIS - International Legume Database and Information Service

IRLC - Inverted Repeat Lacking Clade

ISSR - Inter-simple Sequence Repeat Amplification

ITPGRFA - International Treaty on Plant Genetic Resources for food and Agriculture

IUCN - International Union for Conservation of Nature

MAAPS - Multiple Arbitrary Amplicom Profiling

MOAC - Ministry of Agriculture and Cooperatives

mtDNA – mitochondrial DNA

OPA - Orthogonal Projection Approach

PCA - Principal Coordinate Analysis

PCR – Plant Genetic Resources

RAPD - Random Amplified Polymorphic DNA

RFLP - Restriction Fragment Length Polymorphism

SCAR - Sequence Characterized Amplified Regions

SINGER – System-wide Information Network of Genetic Resources

SNP – single nucleotide polmorphism

SPAR - Single Primers Amplification Reaction

SRAP/EST - Sequence-Related Amplified Polymorphism/Expressed Sequence Tags

SSCP - Single Strand Conformation Polymorphism

SSR - Simple Sequence Repeats

STMS - Sequence-tagged Microsatellite

STS - Sequence Tagged Site

TAQ – Thermus Aquaticus

TDWG - Biological Sciences Commissions on Taxonomic Database

TG – Taxon Groups

UNCCD - United Nations Convention to Combat Desertification

UNCED - United Nations Conference on Environment and Development

UPGMA - Unweighted Pair Group Method with Arithmetic Average

USDA - United Stated Department of Agriculture

VNTR - Variable number of Tandem Repeats

WDPA - World Database on Protected Areas

CHAPTER ONE

INTRODUCTION

1.1 Food security and agrobiodiversity: importance and threats

The international community has recognized that land degradation, climate change and loss of biological diversity are major global challenges for sustainable development. Three important international treaties: the Convention on Biological Diversity (CBD, 1992), United Nations Convention to Combat Desertification (UNCCD, 1994), and the United Nations Framework Convention on Climate Change (UNFCCC or FCCC, 1992) were launched during the United Nations Conference on Environment and Development (UNCED), informally known as the Earth Summit, held in Rio de Janeiro from 3 to 14 June 1992. The three conventions are interdependent and share many of the goals.

Conservation and sustainable use of biodiversity along with the fair and equitable sharing of benefits arising from the use of genetic resources are the major objectives of the CBD signed by more than 195 countries/parties.

Biodiversity, in general is vital to maintaining life on the planet and without a rich range of plants in the world, humans would not be able to survive as plants play a key role in the balance of the ecosystem regulating carbon dioxide in the air and providing food/feed to the majority of living creatures on Earth. Genetic variability within populations is essential for the survival and future security of any species and without a rich pool of genes most species would become endangered due to the lack of adaptability to changing environments. This is especially important with the recent effects of climate change, where ecosystems are

under extreme and sudden pressures of recurrent droughts and extreme temperatures, and the genetic variability is vital to survive and adapt to the associated biotic and abiotic stresses. Besides its intrinsic ecological, genetic, social, economic, scientific, educational, cultural, recreational and aesthetic values, biological diversity is considered as an essential part to contribute to eradicate poverty and achieve food security and sustainable development.

Agricultural biodiversity, is an important part of the biodiversity directly related to human well-being as it includes all components relevant to food and agriculture, and all components that constitute the agricultural ecosystems (agro-ecosystems) including the variety and variability of animals, plants and micro-organisms, at the genetic, species and ecosystem levels, which are necessary to sustain key functions of the agro-ecosystem, its structure and processes (CBD, 1992). Agricultural biodiversity provides in addition to food and income, raw materials for clothing, shelter, medicines, breeding new varieties, and performs several services including maintenance of soil fertility and biota, and soil and water conservation, all of which are essential to human survival. Agricultural biodiversity includes: plant genetic resources, including crops, wild plants harvested and managed for food, trees on farms, pasture and rangeland species; animal genetic resources, including domesticated animals, wild animals hunted for food, wild and farmed fish and other aquatic organisms; and microbial and fungal genetic resources.

An important dimension of agricultural biodiversity is its management by communities which adds farmers' selection to natural evolution. The maintenance of this biodiversity is essential for the sustainable production of food and other agricultural products and the benefits these provide to humanity, including food security, nutrition and livelihoods. Nearly one third of the world's land area is used for food production

If for no other reason, humans should conserve biodiversity for selfish means. Plant crops are a staple part of the diet for every human being and to not protect the diversity of these important crops for future utilisation would be extremely foolish. In the drylands and mountainous areas, local agricultural biodiversity still play a crucial role in sustaining the livelihoods of rural poor.

Article 1 on the objectives of the Convention on Biological Diversity (CBD, 1992) clearly states the importance of preserving biodiversity for human utilisation. Of all the threats to biodiversity, anthropogenic disturbance is the most damaging. Humans put a huge pressure on the ecosystem for a number of underlying reasons including increased population, poverty of rural communities, non appropriate and non enforcement of policies for land use and limited public awareness. As a result, irreversible damage to unique habitats has been taking place at a rapid speed over many decades to provide more land for agriculture in a struggle to provide enough food for an ever increasing global population.

Climate change is another important threat to global biodiversity. Climate change is a natural process; however, anthropogenic activities over recent decades have caused the rate of climate change to be accelerated. By 2100, 10-30% of species globally could be at high risk of extinction (Fischlin *et al.,* 2007). This also means there could be huge rates of genetic erosion in species, severely reducing the adaptation capabilities of species to new and unstable ecosystems.

Other threats to biodiversity include limited distributional range and invasive aggressive alien species, but these too can stem from human damage and interference in delicate ecosystems. Fires, urbanization and quarries are also affecting natural habitats. For the farming systems, the introduction of improved varieties and mainly the introduction of

new crop species (fruit tree mainly) are replacing the landraces maintained within the traditional farming systems. In a study conducted in four countries of the Fertile Crescent, landraces for crops and local breeds for livestock are still predominately used but their importance is decreasing with the increasing effects of recurrent droughts (Mazid *et al.*, 2005).

The loss of agrobiodiversity will directly affect the livelihoods of local communities in the remaining biodiversity rich areas and will prevent breeding programs from valuable genes needed to cope with the challenging biotic and abiotic stresses, and developmental actions from adapted genetic material needed to rehabilitate degraded ecosystems and farming systems.

Plant diversity plays a pivotal role in the functioning of all natural ecosystems, as well as providing direct benefits in terms of food and medicine for humans and foodstuffs for wild and domesticated animals. The fundamental importance of biodiversity conservation tied to sustainable exploitation by humankind is central to the Convention on Biological Diversity (CBD, 1992). More specific reference to socio-economically important plant biodiversity is made in the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, 2004). These two international binding conventions/agreements provide a fairly broad framework for plant conservation linked to sustainable and equitable use of resources but lack any explicit strategy for achieving the long-term objectives (http://www.biodiv.org/programmes/cross-cutting/plant/) to halt loss of plant diversity with specific conservation targets that are to be achieved by 2010. Effective conservation and sustainable use of biodiversity require:

- Use of complementary *ex situ* and *in situ* conservation methodologies;

- National, regional and international conservation coordinated efforts;
- Linking conservation to utilization;
- Empowering the custodians of biodiversity;
- Better understanding of biology, distribution, threats, adaptation of various species in genepools;
- Promoting education and awareness about the importance of conserving plant diversity,
- Building capacities of national systems for the conservation of genetic resources.

The genetic diversity of the genus *Lathyrus* is of significant importance, particularly for its potential use within the rainfed cropping systems of many countries and as a genetic resource for the improvement of *Lathyrus sativus* L. used for both feed in many parts of the world and food in poor regions.

1.2 Conservation techniques

Two major types of conservation were defined: ecological and genetic. Ecological conservation attempts to preserve an ecological niche, rather than just concentrating on protecting a single or groups of species. Genetic conservation, however, focuses on preventing the genetic erosion of a single species and the component of its genepools (Maxted *et al.*, 1997b).

Two major techniques are used to conserve biodiversity: *ex situ* and *in situ* conservation which are defined by CBD (1992) as: "*In-situ conservation* means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or

cultivated species (on-farm conservation), in the surroundings where they have developed their distinctive properties; "*Ex-situ conservation* means the conservation of components of biological diversity outside their natural habitats" (CBD definition, UNCED, 1992). Both strategies are equally important and should be regarded as complementary (Thormann *et al*. 2006; Engelmann and Engels, 2002; Dulloo *et al*., 1998; Maxted *et al*., 1997a).

In situ conservation of crop wild relatives has gained increasing attention in many countries, as demonstrated by their inclusion in the many national reports drafted for the Second report on the *State of the World's Plant Genetic Resources for Food and Agriculture* (FAO, 2010, Dulloo *et al.,* 2010).

There has been a growing interest to promote *in situ* conservation of plant genetic resources, because of the urgent need to protect threatened natural and agro-ecosystems. The contemporary conservationist when formulating an overall conservation strategy should think in terms of applying a combination of different techniques, including both *in situ* as well as *ex situ*, where the different methodologies complement each other (Maxted *et al.*, 2003). With the majority of research in the past having been focused on developing techniques for the *ex situ* conservation of plant genetic resources, there is now a need to redress the balance and to depend on the experience of other biological disciplines, to provide a firm scientific base for *in situ* genetic reserve conservation (Maxted *et al.*, 2003).

A model for *in situ* genetic conservation should provide a generalized methodological framework that can be applied by researchers to establish and implement genetic reserve and on-farm conservation projects and these will form part of an overall conservation strategy for preservation of a crop gene pools. However, it is stressed that the methodologies mean to be prescriptive or to imply that any single methodology would be appropriate for all situations; they should be presented as a reference point from which to explore the application of *in situ*

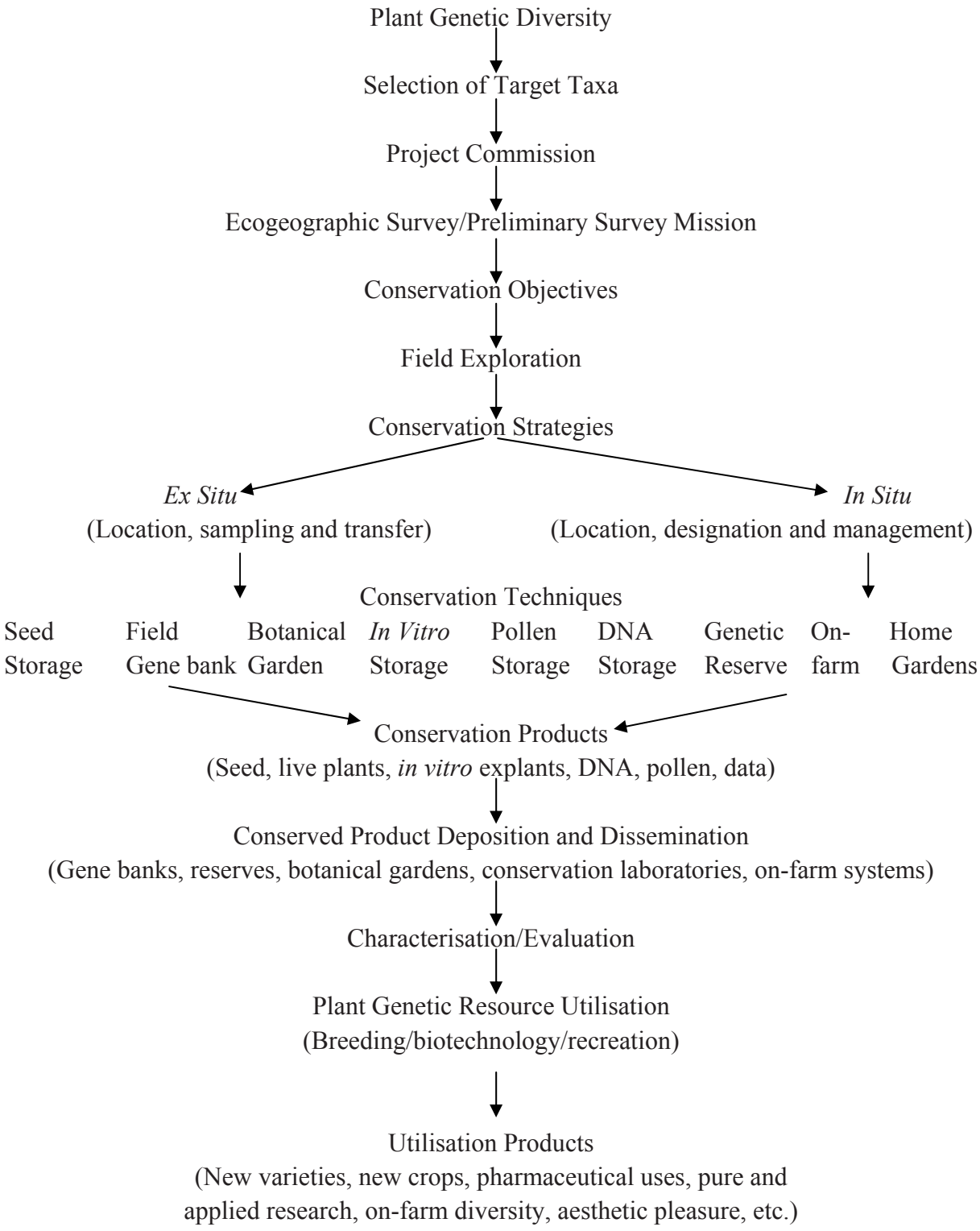
conservation techniques. Another key point that requires reiteration is that whenever possible an *in situ* conservation project will require a true partnership with local communities and full involvement of key stakeholders (Maxted *et al.*, 1997a).

It is important to stress before discussing this methodology, however, that no model, methodology or scheme should be followed slavishly. In this case the methodology proposed is meant to act as a guide to some of the important issues that require discussion. This general methodology will almost invariably require adaptation for each particular taxon and each situation where it is to be applied (Maxted *et al.* 2003)

Conservation of crop relatives or other wild species in a genetic reserve involves the location, designation, management and monitoring of genetic diversity in a particular, natural location (Maxted *et al.,* 1997). This technique is the most appropriate for the bulk of wild species, whether closely or distantly related to crop plants, because it can be relatively inexpensive, when the management regime is minimal, it is applicable for orthodox and non-orthodox seeded species, permits multiple taxon conservation in a single reserve and allows for continued evolution. However, the disadvantages of conservation in a genetic reserve are that the conserved material is not immediately available for plant breeding or other form of utilization and, if the management regime is minimal, little germplasm characterization or evaluation data may be available (Maxted *et al.,* 1997). In the latter case, often the reserve manager may even be unaware of the complete specific composition of the reserve (Maxted *et al.*, 2003)

Several authors working independently have specifically proposed or illustrated methodologies for genetic reserve conservation (Jain, 1975 and Maxted *et al.*1997a). The most detailed methodology for plant genetic conservation is shown in the following scheme.

A model of plant genetic conservation (adapted from Maxted *et al*., 1997a).



The principal aim of *ex situ* conservation is to maintain seeds and other germplasm materials alive as long as possible and to reduce the frequency of regeneration that may cause the loss genetic diversity (Dulloo *et al.,* 2010). Research focus of *ex situ* conservation should be to enhance our understanding of the responses of a wide diversity of species (in particular crop wild relatives (CWR) as well as neglected and underutilized species (NUS), including those bearing recalcitrant seeds) to both single and different storage conditions and methods, with the aim of providing conservationists with information on suitable options for conserving given species (Dulloo *et al.,* 2010).

It is very important to back up any *in situ* interventions with complementary *ex situ* conservation in genebanks as seed, pollen, living plants (in field genebanks or in botanic gardens), tissue culture, or cryopreservation, depending upon the biology of species to be conserved (Dulloo *et al.,* 2010). The most effective conservation strategies incorporate both *in situ* and *ex situ* techniques to complement each other where possible.

The project on "conservation and sustainable use of dryland agrobiodiversity in Jordan, Lebanon, Palestine and Syria" developed the key elements for a holistic conservation strategy including management plans to promote community-driven *in situ*/on-farm conservation of landraces and wild relatives of crops of global importance (Amri *et al.*, 2005a). These key elements include: the assessment and monitoring of the status and threats of plant populations, identification of biodiversity rich areas, and development of their management plans in collaboration with key stakeholders. These management plans include technological options, socio-economic options including add-value and alternative sources of income, institutional and policy options, along with actions to increase public awareness on

the importance of conserving biodiversity and environment (Amri *et al*., 2005). These actions need to be supported by local, community, national, regional and international efforts.

1.3 Importance and justification for the genus *Lathyrus* L.

Grass pea (*Lathyrus sativus* L.), known as chickling vetch, Indian vetch, is an annual legume crop of economic and ecological significance in several countries in South Asia and Sub-Saharan Africa, and to a limited extent in some countries of Central and West Asia, North Africa, southern Europe and South America (Smartt, 1990; Campbell *et al.,* 1994; Siddique *et al.,* 1996; Haque *et al.,* 1996; Kislev, 1989; Getahun *et al.,* 1999; Hanbury *et al.,* 1999; Mera *et al.,* 2000; Milczak *et al.,* 2001; Vaz-Patto *et al.,* 2006; Yan *et al.,* 2006). It is grown mainly for food in India, Bangladesh, Nepal, Pakistan and Ethiopia, and for feed and fodder in other countries (Siddique *et al*., 1996; Campbell *et al.,* 1997; Getahun *et al.,* 2005; Vaz-Patto and Rubiales, 2009). In West Asia and Australia dry areas, the use of grass pea in rotation with cereals is encouraged (Abd-El-Moneim and Cocks, 1993; Hanbury *et al.,* 2000a). Its cultivation is also encouraged in countries of North America, Latin America, Australia and Southern Europe and in China to adapt to the adverse effects of climate change, exploitation of marginal lands and to break the continuous wheat cultivation practice (Campbell *et al.,* 1994; Hanbury *et al..,* 1995 and 1999; Siddique *et al.,* 1996; Mera *et al.,* 2000; Falco and Pardo, 2000; Milczak *et al.,* 2001; Crino *et al.,* 2004; Yang and Zhang, 2005; Vaz-Patto *et al.,* 2006; Polignano *et al.,* 2009).

The seeds of grass pea are rich in crude protein (24-31%) and complement cereals in amino acid composition for a balanced diet of poor people in its major production zones (Aletor *et al.,* 1994; Akalu *et al*., 1998; Hanbury *et al.,* 2000a). It also contains high amount of L-homoarginine, which acts as precursor for lysine in higher animals (Quereshi *et al.,*

1977). It constitutes the only food crop producing green and forage where other crops are decimated by droughts or floods in other areas. However, in drier years, excessive human consumption of the grains could cause a neurological disorder, lathyrism, caused by the presence of a neurotoxin in the seed known as either beta-N-oxalyl-diamino-propionic acid (beta-ODAP) or beta-(N)-oxalylamino-L-alanine acid (BOAA). The toxicity results in irreversible paralysis, characterized by lack of strength in, or inability to move the lower limbs. It is particularly prevalent in some areas of Bangladesh, Ethiopia, India and Nepal, and affects more men than women.

The total acreage of grass pea is estimated at 1.50 million ha with annual production of 1.20 million ton, with 0.92 million ha in South Asia and 0.63 million ha in Sub-Saharan Africa (ICAR, 2009; MOAC, 2009). Its area has significantly decreased in India and Nepal following the ban of its cultivation by governments (ICAR, 2009; MOAC, 2009). But grass pea cultivation is still important in Bangladesh where it occupies the first position among the pulse crops (BBS, 2009), and in Ethiopia (CSA, 2010), because of its ability to produce under harsh conditions (Lu *et al.,* 1990; Tadesse *et al.,* 1997).

Grass pea fixes 108-125 kg ha^{-1} which satisfies its needs in nitrogen and provides the excess to following crops (Ahlawat *et al.,* 1981; Peoples *et al*., 1995 and 2008; Muehlbauer and Tullu, 1997). It can tolerate extreme temperatures and droughts and water-logging (Lal *et al.,* 1986; Campbell *et al.,* 1994; Tadesse and Bekele, 2003). Several other *Lathyrus* species are cultivated for human consumption, animal feed, and fodder, as well as for ornamental purposes but there is potential for further exploitation of the *Lathyrus* gene pool for the same purposes. *Lathyrus* species are also important as soil nitrifiers and as dune stabilizers.

Despite these advantages, relatively little research efforts have been directed to

improvement of grass pea, a highly under-utilized crop. Limited efforts on its improvement through genetic and agronomic manipulations were initiated in India, Canada, Bangladesh and Ethiopia but interest is renewed in grass pea with the growing concerns with climate change (Siddique *et al.,* 1996; Hanbury *et al.,* 1999; Mera *et al.,* 2000; Milczak *et al.,* 2001; Falco and Pardo, 2000; Crino *et al.,* 2004; Yang and Zhang, 2005; *Vaz-Patto et al.,* 2006; Polignano *et al.,* 2009; Grela *et al*., 2010).

1.4 Problem elucidation

In reference to the financial constraints to support any conservation technique, there are priorities to decide which species should be conserved. The main factor to decide whether a species is of high priority is the socio-economic value associated with that species. If the species can be utilised by humans and be a source of income for growers then the conservation priority is high and this categorizes it as prioritizing taxa (Maxted *et al.*, 1997a). It is vital and logical for humans to preserve and protect species in which they have a direct interest. These interests are numerous, some are: nutrition and sustenance, economic value, cultural significance, building materials and medicinal qualities. It is essential to conserve crops and plants that humans rely on. If genetic erosion of species isn't slowed now, then the future will be very unwelcoming for the species concerned, and humans that rely on these species. This would mean that crops would have a narrower range of genes for adaptation to environments which could become very different in the future, and if they can't adapt then they may become extinct or endangered. This would put severe pressure on the human race as food sources would become more fragile and scarce and many people would likely perish. Therefore, is it vital that crops, crop wild relatives and other socio-economic plants are conserved for utilization by humans to prepare for an uncertain future?

In spite of secondary priority level of *Lathyrus* L. within the regional conservation strategies developed by the Global Crop Diversity Trust (GCDT, 2009) and because of its importance as a survival food for some of the poorest people in the world, its inherent adaptation to harsh conditions, yet recognizing the dangers that excessive consumption can cause, *Lathyrus* species deserve due importance for the conservation and sustainable use of its genetic resources for breeding and rehabilitation purposes. Lathyrism needs to be addressed as a matter of some urgency – with the breeding of zero or very-low neurotoxin varieties being the most promising solution, requiring access to suitable genetic resources including wild relative species. The crop has been recognized as an important crop for which there is a high degree of international inter-dependence with respect to its genetic resources and as such included in the Annex 1 of the ITPGFA.

However, local landraces and cultivars and wild species are being lost by various degradation factors including the switch to alternative crops and destruction of natural habitats, potentially limiting the progress that can be made through genetic enhancement and the availability of genetic resources for the rehabilitation of degraded ecosystems. Therefore, the collection, conservation, characterization, study of genetic diversity and utilization of the genus *Lathyrus* deserve ample attention within the research efforts. There is an urgent need to conserve the genetic diversity of the genus using both *ex situ* (gene banks) and *in situ* (natural habitats) conservation methods. Fortunately, some significant collections have already been assembled and are maintained in a number of different institutes throughout the world including International Centre for Agricultural Research in the Dry Areas (ICARDA), France, Bangladesh, etc.

1.5 Research aims and objectives

This study will add to the existing knowledge in the literature and will use different approaches for the critical assessment of the genetic diversity towards its efficient conservation and sustainable use. Molecular markers and morphological characterization will be performed to study the diversity of *Lathyrus*. The genetic relationships between of different taxa in *Lathyrus* will be determined and ecogeographic factors related to the diversity will be studied. By studying patterns of diversity, gaps in *ex situ* collections will be determined and appropriate sites for *in situ* conservation will be identified. Multivariate analysis of collected data will help in identifying a core collection and Focused Identification of Germplasm Strategy will be introduced to construct best sets for targeted traits which will facilitate further evaluation, breeding and use. Within the major taxa, distinct groups (genepools) will be formed by using geographically constrained clustering methods. The genetic relationships between these groups will be studied.

The study will result in a better understanding of *Lathyrus* taxonomy and the genetic relationships between taxa and the genetic diversity within taxa. Strategies for improved conservation of the species will be developed. Distinct groups will be identified within the taxon (genepools) to assist agronomists and breeders in utilizing the conserved germplasm.

An integrated eco-geographical study is carried out for *Lathyrus* species found in the Mediterranean Basin and the Caucasus, Central and West Asia Regions, using specimens from different herbaria to understand the ecological and geographical distribution of the genus *Lathyrus* in these regions.

A Field Guide for the “Grass pea and Chicklings (*Lathyrus* L.)” of the Mediterranean Basin and the Caucasus, Central and West Asia Regions is produced including 76 taxa with

line drawings for 54 taxa, to assist local plant genetic resources (PGR) workers in species identification. This will be an easy to use identification aid, which will avoid the jargonistic pit-falls of conventional taxonomic keys. This aid is using different illustrations (line drawings, photographs, paintings, etc.) of the key features of the species, hence avoiding recourse to complex botanic terminology. This botanical field guide includes in addition to line drawings, paintings or photographs, well detailed texts containing scientific and vernacular names, diagnostic descriptions, iconography, and alliances to other species, distribution maps, phenology, ecological preferences, geographic distribution and conservation status. DELTA software is used to produce Lucid outputs in accordance to the characterization and observation of the morphological characters for *Lathyrus* species studied. These outputs aimed to be user-friendly and therefore more widely accessible. Both DELTA and Lucid have flexibility; characters can be incorporated within the keys, and therefore subjectivity can also be flexible. The aim and objectives of the thesis can be summarized as:

Aim: Contribution to efficient conservation and use of genetic resources of *Lathyrus* species of the Mediterranean Basin and the Caucasus, Central and West Asia Regions

Objectives:

- Better understanding of taxonomy and phylogeny of *Lathyrus* species of the Mediterranean Basin, the Caucasus, Central and West Asia regions using characters and molecular techniques;
- Produce a Field Guide for the "Grass pea and Chicklings (*Lathyrus* L.)" of the Mediterranean Basin and the Caucasus, Central and West Asia Regions for easy species identification.
- Recommend areas for further collecting missions and for *in situ* conservation of

priority *Lathyrus* species;

- Introduce methodology for selecting core collection sets and best bet set for adaptive traits.

1.6 Research plan:

a) Undertake a literature review of the taxonomic history and genetic diversity of the genus *Lathyrus*.
b) Collate and analyze ecogeographic data in the Mediterranean Basin and the Caucasus, Central and West Asia regions of the genus *Lathyrus.*
c) Collate existing and record novel characterisation information for accessions of all *Lathyrus* species held at ICARDA and originated from the Mediterranean Basin and the Caucasus, Central and West Asia regions.
d) Develop and produce identification aids and a field guide for *Lathyrus* species distributed in the Mediterranean Basin and the Caucasus, Central and West Asia regions.
e) Review current conservation strategies for the genus *Lathyrus*.
f) Carry out molecular investigations of representatives of *Lathyrus* section *Lathyrus* to clarify taxonomic relationships.
g) Determine areas of high taxonomic and genetic diversity using a combination of molecular and GIS techniques for the genus *Lathyrus.*
h) Use GIS tools to predict the distribution of the species in relation to edaphic and meteorological data and undertake gap analysis for the genus *Lathyrus* in the Mediterranean Mediterranean Basin and the Caucasus, Central and West Asia regions.
i) Formulate conservation and use strategies for *Lathyrus* species (target *ex situ* collecting, core collection, potential locations for genetic reserve and on farm programmes), together with a review of actual and potential usage.

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CHAPTER TWO

LITERATURE REVIEW

The genetic diversity of the genus *Lathyrus* (Grass pea and chicklings) is of great importance, particularly for potential use in rain-fed cropping systems of many countries (Campbell *et al.*, 1994) and as a source of genes for the crop improvement of *L. sativus* L. Several species are cultivated for human consumption, animal feed, and fodder, as well as for ornamental purposes (Sarker *et al.*, 1997), but there is potential for further exploitation of the *Lathyrus* gene pool. Therefore, the collection, conservation, characterization, study of genetic diversity and utilization of the genus *Lathyrus* deserves ample attention as a priority research area. There is an urgent need to conserve the genetic diversity of the genus using both *ex situ* (e.g. gene banks) and *in situ* (e.g. within natural habitats) conservation techniques. This will permit a critical assessment and monitoring of the genetic diversity, evolution and genetic erosion of the genus, as well as enhancing its exploitation (Sabanci, 1996).

Lathyrus is a large genus containing around 160 species (Lewis *et al*., 2005), mainly located in Europe, Asia and North America, and extending to temperate South America and tropical East Africa, but the genus has its centre of diversity primarily in the Mediterranean and Irano-Turanian regions (Kupicha, 1981). It is adapted to temperate regions but can also be found at high altitudes in tropical Africa. Endemic species are present in all continents, except Australia and Antarctica (Kupicha, 1981).

L. sativus L., *L. cicera* L. and *L. ochrus* (L.) DC. provide important human food, animal feed and fodder sources. *L. sativus* is widely cultivated for human consumption, as

well as for fodder and green manure. Its primary ceners of cultivation are in Southern Asia, particularly in Bangladesh, China, India, Nepal, and Pakistan, and Sub-Saharan Africa particularly in Ethiopia (Asthana, 1996), with more limited production in southern Europe and West Asia. *L. cicera* is cultivated in Greece, Cyprus, Iran, Iraq, Jordan, Spain and Syria and *L. ochrus* is cultivated in Cyprus, Greece, Syria and Turkey (Saxena *et al*., 1993). Some other species are used as minor forage or fodder crops: *L. hirsutus* L. is cultivated in southern United States as a fodder species and *L. clymenum* L. is cultivated in Kos, Greece (Sarker *et al.*, 2001). It is an important low risk aversion crop because it has relatively good tolerance to water-logging (in the case of flooding), good ability to grow on residual moisture after the end of the rains or in case of drought, and because it requires low production costs (Tadesse *et al.,* 1997,).

Grass pea is nutritionally equivalent with other grain legume species, containing up to 30% crude protein (which is high in lysine), about 60% carbohydrates and 0.6% fat (Hartman *et al*., 1974). The grass pea is favoured for its ability to mature and produce a yield in times of drought when other crops have failed. The seed, however, may contain 0.1-2.5% of the water soluble non-protein amino acids ODAP (β-N-oxalyl-α,β diaminopropionic acid) or BOAA (l-3-oxalylamino-2-amino propionic acid), which have been found to be neurotoxins, the causative agent of crippling, irreversible neurological disorder, lathyrism (Barrow *et al.*, 1974; Rutter and Percy, 1984; Kaul and Combes, 1986), which leads to paralysis of the lower limbs. These neurotoxins need to be genetically removed or reduced to below critical values if *Lathyrus* is to gain importance as a food crop (Abd El Moneim and Cocks, 1993). At present, several grass pea - producing countries are involved in the development of very low or toxin-free *L. sativus* varieties (Malek *et al.*, 1996; Tadesse *et al.,* 1997). Additionally, the primary, secondary and tertiary gene pools may play an important role for the genetic

improvement of cultivated *Lathyrus* species including for lowering ODAP content. For example, a toxin-free gene has been identified in *L. tingitanus* L., which is being used to develop toxin-free grass pea varieties in China (Zhou and Arora, 1996) (Sarker *et al.,* 2001).

Several species within the genus are cultivated as ornamentals such as sweet pea (*L. odoratus* L.), everlasting pea (*L. latifolius* L.) and *L. sylvestris* L. A number of other species, particularly in section *Lathyrus*, have potential for the development as new horticultural species (Davis, 1970). The popular ornamental garden sweet pea *L. latifolius* L. (the Broad-leaved Everlasting Pea), *L. sylvestris* L. (the Narrow-leaved Everlasting Pea,) and *L. grandiflorus* Sibth. & Smith (the Two Flowered Pea) are also commonly grown as ornamental species, and many cases occur in the wild because of garden escapes (Baggott, 1997). Due to the potential the genus has as a food, feed and fodder crop, as well as its extensive cultivation as an ornamental, it is necessary to collect and conserve as much as possible of the available cultivars and landraces, as well as the wild species. Table 2.1 provides a list of those species known to be historically or currently cultivated for agriculture or horticulture (Kearney, 1993; Sarker *et al.,* 2001).

Table 2.1. Historic or current cultivated species of *Lathyrus* (Kearney, 1993).

Species	Use	Use Status	Location
L. annuus	Pulse, Fodder	Rare	Europe, N. Africa
L. aphaca	Fodder	Rare	India
L. blepharicarpus	Pulse	Historic	Near East
L. cicera	Pulse, Fodder,	Rare	S. Europe, N. Africa
L. clymenum	Pulse	Rare	Greece
L. gorgoni	Fodder	Historic	Middle East
L. hirsutus	Forage	Common	U.S.A.
L. latifolius	Horticulture	Common	Europe
L. ochrus	Pulse, Fodder	Rare	Greece, Middle East
L. odoratus	Horticulture	Common	Widespread
L. pratensis	Forage	Rare	S. Europe, N. Africa

L. rotundifolius	Horticulture	Common	Widespread
L. sativus	Pulse, Forage	Common	Widespread
L. sylvestris	Forage	Rare	S. Europe, N. Africa
L. tingitanus	Fodder	Rare	N. Africa
L . tuberosus	Tubers	Rare	W. Asia

Many farmers consider members of the genus as noxious weeds (Gams, 1924; Aarssen *et al.*, 1986). *L. annuus* L. and *L. hierosolymitanus* Boiss. are persistent and particularly difficult to control. *L. aphaca* L. can cause problems in cereals: their twining habit can make mechanical harvesting very difficult and may cause lodging of the crop and could increase the incidence of fungal diseases (Aarssen *et al.*, 1986).

The genus is well placed to help meet the increasing global demand for animal feed and to provide crops for a diversity of farming systems, particularly when low neurotoxin lines will be available. To prevent genetic erosion and extinction, *Lathyrus* conservation has been given priority by Bioversity International (former IBPGR and IPGRI) since 1985. Many national programs and international bodies have launched germplasm collection and conservation activities of this under-utilized genus (Sarker *et al.*, 2001). However, to date, an extensive and systematic approach to collect, conserve and evaluate *Lathyrus* has not been adopted. Furthermore, it is necessary to study the genetic diversity of the available collections in order to understand their full utilization potential (Maxted *et al.*, 2003).

2.1 Taxonomy history of genus *Lathyrus* L.

2.1.1 Leguminoseae family and sub-family taxonomy

Lathyrus is one of 727 genera of the Leguminosae Juss family which is currently divided into three subfamilies and 36 tribes and of about 19,325 species (Lewis *et al*., 2005). Leguminosae is the third largest family of flowering plants after the Asteracea or Compositae and Orchidacae. Compared with the families and many others, the Leguminosae are notably 'generalists' ranging from forest giants to tiny ephemerals, with great diversity in, their methods of acquiring the essentials for growth, reproduction and defense (Polhill *et al.* 1981). The family is to be found in all terrestrial habitats from the equator to the polar fringes, it has much of its diversity centered in areas of varied topography with seasonal climates (i.e. in the case of *Lathyrus* the Mediterranean basin). The adaptability of legumes enhances their great economic importance, which is likely to increase with increased human pressure on marginal lands (Polhill *et al.* 1981). Legumes are to be found as major components of most of the world's vegetation types and many have the ability to colonize marginal and barred lands because of their capacity to fix atmospheric nitrogen through root nodules (Sprent, 2001).

The Leguminosae family consists of three sub-families: Sub-family Caesalpinioideae consisting of 4 tribes and about 2250 species; sub-family Mimosoideae with 4 tribes and about 3270 species; and the sub-family of interest to us, the Papilionoideae comprising 28 tribes and about 13,800 species. The Papilionoideae widely distributed from rainforest to the edge of dry and cold deserts (Polhill, 1981). This sub-family is probably second only in economic importance to the Graminae, yielding pulses, timber, vegetables extracts and ornamental plants (Townsend & Guest, 1974).

2.1.2 The tribe *Fabeae*

The tribe Fabeae was described by Reichenbach in 1832. The recent literature uses the invalid old synonym Viceae (Bronn) DC Bronn.) DC. (1825) (*sensu* Polhill and Raven, 1981) (Lock and Maxted 2005). Viceae traditionally has included numerous genera with relatively small numbers of species in each; *Abacosa* Alef., *Aphaca* Miller, *Arachus* Medic., *Arbus* L., *Atossa* Alef., *Bona* Medic., *Cicer* L., *Cicerula* Medik, *Clymenum* Miller, *Cracca* (Riv) Medik, *Cujunia* Alef., *Endusia* Benth. & Hook., *Ervilia* Link., *Ervum* L., *Faba* L., *Graphiosa* Alef., *Hypechusa* Alef., *Lastila* Alef., *Lathyrus* L., *Lens* Mill., *Navidura* Alef., *Nissolia* L. non Jacq., *Orobus* L., *Parallosa* Alef., *Pisum* L., *Sallunia* Alef., *Swantia* Alef., *Tuamina* Alef., *Vicia* L. *Vicilla* Schur and *Wiggersia* Alef (Bronn) DC Bronn.) DC. (1825). In recent years the number of genera has decreased therefore increasing the number of species per genus. The Fabeae generic classification has stabilized into a generally accepted grouping of five genera, Kupicha (1981) includes 5 genera: *Vicia* L., *Lathyrus* L., *Lens* Mill., *Pisum* L. and *Vavilovia* A. Fedorov and 350 species. She commented that the Vicieae, narrowly defined, excluding *Arbus* Adans. and *Cicer* L., "form a small, distinct group with several specialized features: tendrilous leaves; unusual stem vasculature; precise and elaborate floral details." Kupicha (1981) provides the following key to the genera included:

1. Style dorsally compressed, folded longitudinally, with margins meeting adaxially, and pubescent on adaxial (inner) face…….....2
2. Annuals; leaves tendrilous, usually with more than one pair of leaflets; stipules large, foliaceous; leaflets conduplicate in bud ..……..*Pisum*
3. Perennials; leaves mucronate to shortly tendrilous, unijugate; stipules small; leaflets supervolute in bud ……...……..…. *Vavilovia*

1. Style not as above (i.e. not folded longitudinally) ……..…… 3

3. Style dorsally compressed, pubescent only on adaxial (inner) face ……..…. 4

4. Leaflet ptyxis supervolute …… *Lathyrus*

4. Leaflet ptyxis conduplicate..……..…… 5

5. Seeds lenticular ……..……..….. *Lens*

5. Seeds +/- spherical ……..….. *Vicia* p.p. (*V. koeieana* + *V. ervilia*)

3. Style not as above; if dorsally compressed then pubescent all round or only on abaxial face ……..…….*Vicia*

The group is generally recognized by its twining habit and multi-foliolate, tendrilous leaves, although not all taxa have all three characters. Kupicha (1981) provides the following description of the Vicieae:

> "Vicieae (Adans.) DC. (1825)**,** nom conserve. Prop. Perennial and annual herbs with erect or more usually climbing or sprawling habit; indumentum of simple smooth-walled hairs and short-stalked glandular hairs; stems with cortical vascular bundles in the internodes, often winged; primary shoot almost always of limited growth, plants proliferating from basal nodes; leaves epulvinate, ex-stipellate, alternate, distichous, paripinnate with the rachis ending in a tendril or mucro or very rarely imparipinnate; leaflets entire (rarely dentate), many-paired to unijugate; rarely (in *Lathyrus)* leaves phyfiotlic or reduced to a tendril and stipules; stomata anomocytic; stipules semisagittate or hastate or variously divided; leaflet ptyxis supervolute or conduplicate; flowers in auxiliary racemes or sometimes solitary, very rarely in panicles; bracteoles rarely present; wing-petals superficially adnate to keel

by thumb-and-pocket configuration; keel-petals united along lower edge; staminal tube diadelphous but vexillary stamen with flattened filament lightly adhering to its neighbours; mouth of tube oblique or truncate; anthers introrse, versatile, of equal size, filaments slender or *(Pisum and Vavilovia)* dilated at apex; pollen grains rectangular-elliptic in equatorial view, endoapertures 117-118 of height of polar axis, with heavily thickened margin; style borne at right-angles to ovary, usually compressed dorsally or laterally, pubescent (distribution of hairs various), sometimes spathulate and/or contorted; stigma terminal, rarely (*Lathyrus*) bipartite; legume ± linear, laterally compressed, (1-)2-many-seeded, usually dehiscent, occasionally winged, sometimes with 'woolly' or (rarely) membranous partitions between the seeds *(Vicia);* geocarpy occasional in *Vicia, Lathyrus and Pisum;* seed compressed-spherical, with long to short hilum; testa smooth or variously rough-textured; lens (boss) near hilum or opposite; vascular bundle continuing past chalaza, unbranched; endosperm absent; radicle long and curved. Seedling hypogeous; radicle and hypocotyl triarch, rarely tetrarch; transition region between root and stem in epicotyl; first scale leaf (cataphyll) borne on side of plumule away from cotyledons. x = 7(6, 5), polyploidy rare. Canavanine sometimes present (*Vicia* p.p.). 5 genera with temperate distribution."

Vicieae is economically the most important in the temperate world and the adaptability of the Leguminosae enhances their great economic importance, which is likely to increase with increased human pressure on marginal lands (Polhill *et al.*, 1981).

Lock and Maxted, 2005 adopted Polhill statements in 1981 that "Fabeae a well-defined tribe, forming part of the temperate epulvinte series". It contains five genera, of

which (*Lathyrus* and *Vicia*) are large. The tribe as a whole is centered in the Irano-Turanian region of the Mediterranean. *Lathyrus* and *Vicia*, each with about 160 species, have very similar distribution centered on the Mediterranean but extending throughout Europe, N. Asia and N and tropical Africa, with secondary centers in N. America and S. America. One large group of species, some in *Vicia* and some in *Lathyrus*, are superficially extremely similar and can only be distinguished by technical characters of the style. This group was in the past recognized as the genus *Orobus* L. (Kupicha, 1981a). *Lens* has 4-6 species and *Pisum* 2-3. Both include important crop plants and, perhaps because of this, their taxonomy is controversial. Both are E. Mediterranean genera with outlying species. The monospecific genus *Vavilovia*, sometimes included in *Pisum*, is confined to mountainous habitats in W Asia. Kupicha (1981a) was unable to suggest a closest relative to the tribe and excluded *Abrus* (Abreae) and *Cicer* (Cicereae) from it (Lock and Maxted, 2005).

In 1995, Chappill placed Fabeae (as Viceae) in one group with Astragalineae, Galeginae, Loteae, Coronilleae, Cicereae and Trifolieae based on morphological analysis. While Doyle (1995) included these sub-tribes and tribes (except Loteae and Coronilleae) in a clade characterized by the loss of the inverted repeat lacking clade (IRLC), with Carmichaelieae which is included in Galegeae *sens*. *lat.* Cicereae, Galegeae, Hedysareae, some Millettieae and Trifolieae.

While Fabeae (as Viceae) forms a clearly monophyletic group in which *Pisum* is sister to *Lathyrus*, and these two emerge as a well supported clade within a paraphyletic *Vicia* (Steele and Wojciechowski, 2003) and (Wojciechowski *et al.* 2004). A sub-clade of *Vicia* species is sister to *Lens*. Within *Lathyrus*, the cpDNA restriction site phylogeny of Asmussen & Liston (1998) agrees in general with dividing the genus into sections previously recognized

using classical taxonomic methodology (Kupicha, 1983; Steele and Wojciechowski, 2003). A basic information for the whole tribe of Fabeae provided by the Vicieae Database Project (Alkine *et al.* 1983a & b). The Fabeae is considered to comprise 5 genera (*Vicia*, *Lens*, *Lathyrus*, *Pisum* and *Vavilovia*) with about 329 species (Lock and Maxted, 2005).

2.1.3 The genus *Lathyrus* L.

The genus *Lathyrus* slightly larger than the sister genus *Vicia,* is easier to be identified, it has more clear vegetative characters than *Vicia* (Kupicha, 1983). It has an interesting floral variation similar to that of *Vicia*. The morphology and taxonomy of *Lathyrus* have been studied by several scientists (Bassler,1966, 1973 & 1981; Kupicha, 1983). The extensive use of characters has led to a great improvement in the infra-generic classification of *Lathyrus*. But, there is still a need to study this genus in more details to be well known.

The genus *Lathyrus* L. is a member of the legume tribe Fabeae (which is known as Vicieae) of the *Papilionoideae* along with *Vicia* L.; *Lens* Mill.; *Pisum* L. and *Vavilovia* A. Fedorov.

The precise generic boundaries between *Lathyrus* and *Vicia* have been much debated which has led to an abundant and complex synonymy, the problem being the *Oroboid* species that appear to form a bridge between the two genera (Kupicha, 1981). Possibly due to its socio-economic potential, the genus *Lathyrus* has proved a popular group to study and more than 20 major classifications of the genus have been produced post-Linnaeus.

2.1.3.1 Major and accepted classifications of *Lathyrus* L.

Lathyrus contains some 160 species (Lewis *et al*., 2005) distributed throughout temperate regions of the northern hemisphere and extends into tropical E. Africa and into S. America. The six important studies that resulted in generic classifications are reported below:

a) Before the classification of Gordon (1848), botanists had accepted the two Linnaean genera *Lathyrus* and *Orobus*, which were separated based on different criteria by different authors. Gordon united the two into *Lathyrus*, and stated that this genus is characterized by a dorsally compressed style pubescent on the adaxial face, thereby excluding the 'oroboid' members of *Vicia* (Kupicha, 1981). Gordon recognized six sections within *Lathyrus*: *Eulathyrus*, *Cicerula, Clymenum*, *Nissolia*, *Aphaca*, and *Orobus* (Kupicha, 1983).

b) Boissier (1872) reinstated *Orobus* as a distinct genus to include those members of sect. *Orobus* that lacked tendrils. The remaining species that possessed tendrils were placed in *Lathyrus* sect. *Orobastrum* (Kupicha, 1983).

c) Bassler (1966) published a taxonomic study of ' *Lathyrus* subgen. *Orobus* (L.) Baker. All the perennials members of sect. *Orobus sensu* Gordon were put in a separate unit. Bassler recognized the following sections within subgen. *Orobus*: *Orobus*, *Lathyrostylis* (as *Platystylis*), *Orobon*, *Pratensis*, *Eurytrichon* and *Neurolobus* (Kupicha, 1983).The annual species that were excluded from subgen. *Orobus* formed, by implication, the small and fairly well defined sect. *Orobastrum*, as in Davis (1970) (Kupicha, 1983).

d) Czefranova (1971) studied the Eurasian species of *Lathyrus* and she divided the genus

into six subgenera: *Orobus*, *Lathyrus*, *Clymenum*, *Nissolia*, *Cicerula* and *Aphaca.* Subgenus *Orobus* contained five sections: Sect. *Orobus*; including members of sect. *Lathyrostylis* as well as *L. linifolius*, *L. montanus*, *L. vernus* and *L. venetus*. Sect. *Lathyrobus*, including the 'oroboid' species such as the perennials with multijugate leaves, broad pinnate-veined leaflets, no tendrils and many-flowered inflorescence, and sections *Eurytrichon*, *Pratensis* and *Neurolobus* (Kupicha, 1983). Subgen. *Lathyrus* contained the three sections *Lathyrus*, *Orobon* and *Orobastrum sensu* Davis (Kupicha, 1983).

e) Kupicha (1983) published her study on "The infrageneric structure of *Lathyrus*" and defined 13 sections of genus *Lathyrus,* which is considered the most comprehensive classification. She stated that the Eurasian species have been classified in a broadly similar manner by all authors. The five groups *Clymenum*, *Aphaca*, *Nissolia*, *Cicerula* and *Lathyrus* (which, except the last, are composed entirely of annuals) are generally accepted, while the remaining species, mostly perennials, have been assigned to progressively smaller, more numerous and better-defined sections. Table 2.2 summarized the classification and geographic distribution of *Lathyrus* (Kupicha, 1983).

f) Asmussen and Liston (1998) adopted Kupicha's study and supported Kupicha's sections *Orobus, Lathyrostylis,* and *Clymenum* but disagreed on the circumscription of the remaining sections.

g) Kenicer *et al.,* 2005 have adopted Kupicha's system as modified by Asmussen and Liston (1998). Morphological homoplasy has often been cited as the principal challenge in the classification of *Lathyrus* (Barneby and Reveal, 1971; Kupicha, 1983)

and the Fabeae (Gunn and Kluve, 1976; Kupicha, 1981; Steele and Wojciechowski, 2003). They stressed that the misinterpretation of homoplasious characters underlies an apparently flawed reclassification of the tribe (Roskov *et al.,* 1998). Roskov and colleagues based their revision on the same vegetative characters used by Linnaeus and his contemporaries, such as stipule form, leaflet number, and presence and strength of tendrils—features now recognized as homoplasies (Simola, 1968; Kupicha, 1981, 1983). Sectional classifications of *Lathyrus* by Bassler (1966, 1971, 1973, 1981), Czefranova (1971), and Kupicha (1983) attempted to account for convergence in characters and possible reversal of character states. The groups defined by these authors are based on combinations of character states in which one or more states may be absent for some taxa within a group. Such reliance on preponderance of shared characters rather than on diagnostic synapomorphies hinders the demonstration of sectional monophyly based on morphology (Kenicer *et al.,* 2005). The following figure summarises the most important taxonomic studies of genus *Lathyrus* (Figure 2.1)

Gordon (1848)	Boiossier (1872)	Bassler (1966)	Davis (1970)	Czefranova (1971)	Kupicha (1983)	Dogan, Kence and Tigin (1992)	Asmussen & Liston (1998)	Kenicer et. al,(2002)
					Notolathyrus		*Orobus*	*Notolathyrus*
Orobus	*Orobus*	*Orobus*	*Orobus*	*Lathyrobus*	*Orobus*	*Orobus*		*Orobus*
	Orobastrum	*Lathyrostylis*	*Lathyrostylis*	*Orobus*	*Lathyrostylis*	*Lathyrostylis*	*Lathyrostylis*	*Lathyrostylis*
		Pratensis	*Pratensis*	*Pratensis*	*Pratensis*		*Pratensis*	*Pratensis*
		Eurytrichon		*Eurytrichon*				
Aphaca	*Aphaca*		*Aphaca*	*Aphaca*	*Aphaca*	*Aphaca*	*Aphaca*	*Aphaca*
Orobus	*Orobastrum*	*Neurolobus*	*Neurolobus*	*Neurolobus*	*Neurolobus*		*Neurolobus*	*Neurolobus*
		Orobon	*Orobon*	*Orobon*	*Orobon*	*Orobon*	*Lathyrus*	
Eulathyrus	*Eulathyrus*		*Lathyrus*	*Lathyrus*	*Lathyrus*			*Lathyrus*
						Lathyrus		
Cicerula	*Cicerula*		*Cicerula*	*Cicerula*		*Gorgonia*		
						Cicerula	*Cicerula*	
Orobus	*Orobastrum*		*Orobastrum*	*Orobastrum*	*Orobastrum*	*Clymenum*	*Orobastrum*	
					Linearicarpus		*L. sphaericus*	*L. sphaericus*
							L. angulatus	*L. angulatus*
					Viciopsis			
Nissolia	*Nissolia*		*Nissolia*	*Nissolia*	*Nissolia*	*Nissolia*	*Nissolia*	*Nissolia*
Clymenum	*Clymenum*		*Clymenum*	*Clymenum*	*Clymenum*	*Clymenum*	*Clymenum*	*Clymenum*
							L. gleospermus	

Figure 2.1: The most important taxonomic studies of genus *Lathyrus* L. This figure is compiled from Kupicka, 1993 and Kenicer *et al.*, 2005.

Lathyrus is well represented in the New World by two separate endemic groups in North and South America (Kupicha, 1983). They have been included in an infrageneric classification, but both groups have been revised on a regional basis (Hitchcock, 1952; Burkat, 1935, 1942) and their vegetative and floral characters are described in recent surveys (Simola, 1968; Gunn & Kluve, 1976).

Table 2.2. Summary of the classification and geographic distribution of *Lathyrus* (Kupicha, 1983).

Section	**2.1.4 Species**	**Geographical Distribution**
Orobus (L.) Godr.	54 species	Europe, W. and E. Asia, N.W. Africa and N and C.

		America
Lathyrostylis (Griseb.) Bassler	20 species	C. and S. Europe, W. Asia and N.W. Africa
Orobon Tamamsch.	1 species	Anatolia, Caucasia, Crimea and Iran
Lathyrus L.	33 species (incl. *L. annuus*, *L. blepharicarpus*, *L. cicera*, *L. gorgoni*, *L. hirsutus*, *L. latifolius*, *L. odoratus*, *L. rotundifolius*, *L. sativus*, *L. sylvestris*, *L. tingitanus*, L . *tuberosus*)	Europe, Canaries, W. and C. Asia and N. Africa
Pratensis Bassler	6 species (incl. *L. pratensis*)	Europe, W. and C. Asia and N.W. and N. E. Africa
Aphaca (J.Mill.) Dumort.	2 species (incl. *L. aphaca*)	Europe, W. and C. Asia and N. Africa
Clymenum (J.Mill.) DC. ex Ser.	3 species (incl. *L. clymenum*, *L. ochrus*)	Mediterranean
Orobastrum Boiss.	1 species	Mediterranean, Crimea and Caucasia
Viciopsis Kupicha	1 species	S. Europe, E. Anatolia and N. Africa
Linearicarpus Kupicha	7 species	Europe, W. Asia and N. and E. Africa
Nissolia (J.Mill.) Dumort.	1 species	Europe, W. Asia and N.W. Africa
Neurolobus Bassler	1 species	W. Crete
Notolathyrus Kupicha	23 species	Temperate S. America and S.E. USA

The key to sections (Kupicha, 1983):

1. At least some of the leaves phyllodic; annuals..... .2

+ None of the leaves phyllodic; annuals and perennials…..... .3

2. All leaves phyllodic and without tendrils; phyllodes with parallel venation; stigma single; fruit not winged……..... .sect. 11. *Nissolia*

+ Lower leaves phyllodic; upper one with leaflets and tendrils; phyllodes with pinnate venation; sigma double; fruits winged……..……...sect. 7. *Clymenum*

3. Stipules hastate (in adult leave ……..…….4

+ Stipules semi-sagittate.……..…….7

4. Leaves without leaflets, except in seedling ……...sect. 6. *Aphaca*

+ All leaves with leaflets.....5

5. Leaves with two or more pairs of pinnate-veined leaflets…… .sect. 1. *Orobon* p.p.

+ Leaves unijugate, leaflets parallel-veined ……...6

1. Plants of Old World; leaves hypo-amphistomatic; wing petals with 'waisted' limb …...……..…..sect. 5. *Pratensis*

+ Plants of New World; leaves epi-amphistomatic; wing petals not 'waisted' ……..sect.3 *Notolathyrus*

7. Style Contorted; standard always stenonychioid 8

+ Style not contorted, or if so then limb of standard narrower than claw (*L. sulphureus*) 10

8. Tendrils absent; Perennials …..... 9

+ Tendrils present or if absent then plants annual ……...……..sect. 4. *Lathyrus* p.p

9. Leaves unijugate, hypostomatic; leaflets broadly ovate with pinnate venation. ……..…….. sect. 3. *Orobon*

+ Leaves 1-7-paired, epi-amphistomatic; leaflets lanceolate, with parallel venation …… sect. 2. *Lathyrostylis* p.p

10 Annuals……..11

+ Perennials……..15

11. Leaves unijugate……... 12

+ Leaves with two or more pairs of leaflets ...……..……...……. 13

12. Legumes strongly stipitate..…….……...… sect. 8. *Orobastrum*

+ Legumes not stipitate……..14

13. Leaf venation pinnate ……... sect. 9. *Viciopsis*

+ Leaf venation parallel …….…….…… sect. 10. *Linearicarpus*

14. Stems winged; leaves hypo-amphistomatic (*L. gorgoni*, *L. pseudo-cicera*) ……..……..……..…..…sect. 4. *Lathyrus* p.p.

+ Stems not winged; leaves epi-amphistomatic ……...…..….sect. 10. *Linearicarpus*

15. Leaves unijugate …….……..…..……..…… 16

+ Leaves with two or more pairs of leaflets …….……...……...…….……...…17

16. Stems strongly winged; flowers less than 1 mm long; plants of Crete …….. sect. 12 *Neurolobus*

+ Stems not or only weakly winged; flowers more than 1.5 cm long; plant of S. America …..... sect. 13. *Notolathyrus* p.p.

17. Legumes tomentose; plants of S America ……...…..…… sect. 13. *Notolathyrus* p.p.

+ Legumes ± glabrous; plants of N. America and Eurasia ……..... 18

18. Leaflets epi-amphistomatic, parallel-veined; leaf rachis etendrillous; stem not winged …….…...…sect. 2. *Lathyrostylis*

+ Leaflets usually hypostomatic and pinnate-veined; leaf rachis tendrillous or etendrillous; stem winged or unwinged; if leaves epi-amphistomatic then stem winged and tendrils present; if leaves parallel-veined then stem winged and/or leaves hypostomatic ……..…...… sect. 1. *Orobus* p.p.

The majority of the cultivated species are placed in *Lathyrus* section *Lathyrus*, possibly explaining why this section has received more taxonomic interest. This section was split by Davis (1970) and Czefranova (1971) into two sub-sections, *Cicerula* and *Lathyrus sensu stricto*, based on the type of style. However, Kupicha (1983) concluded that this was an artificial

separation based largely on the size of the flower and therefore re-merged *Cicerula* with *Lathyrus sensu stricto*. Kupicha (1983) taxonomy has been generally accepted but there remains an on-going debate trying to identify the wild progenitor of the cultivated *L. sativus* within sect. *Lathyrus*. To this end the taxonomic relationships within section *Lathyrus* were studied by Yunus (1990), Yunus and Jackson (1991) and Kearney (1993) and their ecogeographic distribution were studied by Baggott (1997). The progenitor of *L. sativus* remains unknown, but several Mediterranean candidate species have been identified and they resemble the cultigen's morphologically, namely *L. cicera*, *L. marmoratus* Boiss., *L. blepharicarpus* Boiss. and *L. pseudocicera* Pampan.

Kupicha (1983) described the genus *Lathyrus* as follows:

> ***"Lathyrus* L., Sp. Pl. 729 (1753).**
>
> Perennial and annual herbs, eglandular; with erect or more usually climbing or sprawling habit; rootstock occasionally tuberous. Stems winged or un-winged, always with complete replacement of cortical vascular bundles at the nodes. Leaves hypostomatic to epi-amphistomic, paripinnate (except in the phyllodic L. *Nissolia* and in the adult leaves of members of sect. *Aphaca*), ending in tendril or mucro; leaflets 1-8-paired (frequently 1-paired), entire, with supervolute venation and brochidodromous, veins pinnate or parallel (ranging from basal and parallel to pinnate and anastomosing). Leaves occasionally phyllodic or reduced to stipules and a tendril. Stipules entire and rarely toothed; semisagittate or hastate. Inflorescence racemose, 1-many-flowered. Calyx usually actinomorphic, sometimes with oblique mouth and teeth of unequal length. Standard oblong to stenonychioid usually bossed or pouched at the fold. Wings very rarely with 'pleat' in upper edge of limb. Staminal tube usually truncates at apex, rarely oblique. Style dorsally compressed, pubescent on adaxial face, sometimes spathulate and/or contorted; stigma sometimes double. Legume compressed, sessile, rarely stipitate, sometimes winged, occasionally bearing glandular or tuberculate hairs, rarely villous, rarely with

membranous or woolly partitions between the seeds, legume 2-many-seeded. Seeds with long to short hilum; testa smooth or rough; lens always near hilum; free amino acid canavanine absent, lathyrine often present. x=7, polyploidy rare.

Lectotype: *L. sylvestris L.* [*Lathyrus* has been lectotypified twice, by *L. sativus* L. (Britton & Brown, 1913:412) and by *L.* sylvestris (Green, 1929:175); no reason was given for the first choice and the second is preferred because *L. sativus* is the type of the segregate genus *Cicerula* Medik. (1787) whereas *L. sylvestris* has invariably been treated as part of sect. *Lathyrus* (*Eulathyrus*)]' (Kupicha, 1983)".

An amended list of *Lathyrus* species based on Kupicha (1983) classification is provided in Appendix 2.1 with a traditional dichotomous key in Appendix 2.2.

2.1.3.2 Crop and crop wild relatives (CWR) species (genepools and taxon groups)

Three main *Lathyrus* species are grown and used for human consumption: *L. sativus, L. cicera*, and *L. ochrus* and to a lesser extent *L. clymenum.* Another species that is occasionally grown for human consumption – but for its edible tubers rather than its seed - is *L. tuberosus*, known as the tuberous pea or earthnut pea.

Lathyrus sativus is known in English as grass pea, blue sweet pea, chickling vetch, Indian pea, Indian vetch, or white vetch. The ILDIS database lists 44 different vernacular names for the species and three synonyms: *L. asiaticus* (Zalkind) Kudr., *L. sativus* L. and *L. sativus* L. subsp. *asiaticus* Zalkind (Allkin *et al.*1986; Roskov, 2005).

L. cicera (synonym: *L. aegaeus* Davidov) lacks a common vernacular name in English, while *L. ochrus (synonym: Pisum ochrus L.)* is reportedly known as winged vetchling (Polunin, 1969). Additional information on grass pea, including its taxonomy, origin, properties and uses, genetic resources, breeding, ecology, agronomy and future

prospects can be found in Campbell (1997).

Several *Lathyrus* species have potential commercial importance, especially for their ornamental value or as forages and feed, including:

- *L. aureus (*Golden Pea*)*
- *L. annuus* (Red Fodder Pea)
- *L. japonicus* (Sea Pea)
- *L. latifolius* (Everlasting Pea)
- *L. linifolius* (Bitter Vetch)
- *L. nervosus* (Lord Anson's Blue Pea)
- *L. nissolia* (Grass Vetchling)
- *L. odoratus* (Sweet Pea)
- *L. pratensis* (Meadow Vetchling)
- *L. sphaericus*, (Spring Vetchling)
- *L. sylvestris* (Flat Pea-vine)
- *L. tingitanus* (Tangier Pea)

Genetic diversity studies of the genus have been carried by Yunus (1990) and Kearney (1993) who focused on grass pea and its close relatives in the section *Lathyrus*. These have been found to be predominantly self-pollinating, with anther dehiscence usually occurring before the flower has fully opened, although outcrossing up to 30% has been reported (Ben Brahim *et al*., 2001; Chowdhury and Slinkard, 1997; Rahman *et al*., 1995). High outcrossing (27.8%) has been reported in varieties with red flowers followed by pink (19.4%) and white (9.8%) in Bangladesh (Rahman *et al.,* 1995). Inter-specific hybridization has been successful between *L. sativus* and two other *Lathyrus* species, though the production of successful

hybrids remains low. The first successful inter-specific cross was with *L. cicera* (Saw Lwin, 1956; Davies, 1957; 1958). Yunus (1990) crossed 11 species in section *Lathyrus* with *L. sativus*, and found that *L. cicera* and *L. amphicarpos* gave viable seeds. Other species formed pods but these did not form fully developed viable seeds. *L. cicera* is thought morphologically to be the closest relative of *L. sativus* (Jackson &, Yunus, 1984). Plitmann *et al,* (1986) arrived at the same conclusion, based on studies of pollen morphology, karyotype and flavonoid aglycones. It is possible to apply Harlan and De Wet's gene pool concept to this crossability information for *L. sativus* to elucidate its gene pools. The cultivated and wild species of *L. sativus* are included in the primary gene pool. Townsend and Guest (1974) suggested that the primary gene pool is poorly differentiated in terms of morphological characters, as there are no clear-cut discontinuities between the cultivated and wild forms. Although Smartt (1984) concluded that the white flowered, white seeded varieties are the most highly selected but Jackson and Yunus (1984) suggested that the blue flowered; small speckled seeded forms are primitive. Therefore, it could tentatively place the white flowered, white seeded varieties in GP1A and the blue flowered, small speckled seeded forms in GP1B. The secondary gene pool includes the other biological species that will cross with some difficulty with the crop species. Therefore GP2 includes: *L. chrysanthus*, *L. gorgoni*, *L. marmoratus* and *L. pseudocicera*, with which *L. sativus* can cross and produce ovules, and possibly more remotely *L. amphicarpos*, *L. blepharicarpus*, *L. chloranthus*, *L. cicera*, *L. hierosolymitanus* and *L. hirsutus*, with which *L. sativus* can cross and with which pods are formed (Sarker *et al.,* 2001). The tertiary gene pool includes species that can cross with the crop species only with use of specialized techniques such as embryo rescue and culture or the use of bridging species. The remaining species of the genus can be considered members of the tertiary gene pool (GP3)' requiring the production of translocations (Sarker *et al.,* 2001).

Cytogenetic studies in section *Lathyrus* show that the vast majority of species are diploid having the chromosome complement 2n=2x=14. There is some variation in karyotype, but the majority of chromosomes are sub-metacentric. In *L. sativus,* all seven pairs are sub-metacentric while two cross-compatible species (*L. cicera* and *L. amphicarpos*) have one pair metacentric and six pairs sub-metacentric. This indicates that some chromosome structural differentiation has occurred between genomes of different species. From meiotic studies of interspecific hybrids, it would seem that *L. amphicarpos* is structurally more differentiated from *L. sativus* than is *L. cicera*. In F_1 hybrids of *L. cicera* x *L. sativus* the configurations observed were 6II + 2I and 7II. In the hybrid of *L. amphicarpos* x *L. sativus*, multivalent were frequently observed, suggesting that translocation changes had occurred (Yunus, 1990; Yunus and Jackson, 1991; Sarker *et al,* 2001). Schifino-Wittmann (2001) studied the chromosome number and structure and the meotic behavior of accessions of seven *Lathyrus* species and concluded that all species had a conservative karyotype size but differed in total complement size by as much as 20% suggesting a decrease in chromosome size during evolution. The species included in different genepools are summarized as follows:

Primary genepool	Secondary genepool	Tertiary genepool
L. sativus	*L. chrysanthus* *L. gorgoni* *L. marmoratus* *L. pseudocicera* *L. amphicarpos* *L. blepharicarpus*	Other *Lathyrus* species

	L. chloranthus *L. cicera* *L. hierosolymitanus* *L. hirsutus*	

2.1.3.3 Use of allozymes and molecular techniques to elucidate taxonomic relationships

- ***Introduction***

Many works based on morphological characters, cytology and enzyme electrophoresis have studied the diversity and phylogeny of species of the genus *Lathyrus* (Yunus *et al.*, 1991). A variety of molecular techniques have been developed for measuring genetic variability, the most common techniques use isozymes, RFLP and numerous genetic marker assays based on PCR such as RAPD, simple sequence repeats (SSR) and AFLP (Karp *et al.*, 1996) with a wide choice of genetic markers now available to the conservation biologists (Haig, 1998). Since the 1960s, allozymes have been successfully used to characterize genetic diversity of rare plant species (Hamrick and Godt, 1990), although sometimes they have not shown enough discriminatory power to distinguish between individuals (Brauner *et al.*, 1992; Buso *et al.*, 1998). In the past decade, DNA techniques have gained position, especially those based on the polymerase chain reaction (PCR) such as microsatellites (or SSR), RAPD, ISSR and AFLP, in part because these molecular markers provide a larger number of potentially polymorphic loci than allozymes (Heun *et al.*, 1994). Furthermore, they also require small amounts of tissue, an aspect that is especially interesting in plant conservation where the least destructive technique should be considered (Rossetto *et al.*, 1995). Each of these molecular

markers exhibits different properties; two major classes can be identified based upon the type of expression: codominant markers and dominant markers (Adrian *et al.*, 2003). The information that may be extracted from them, and therefore, the numerical tools employed in each case are necessarily different. Allozymes and SSRs are codominant markers. This means that the two alleles present in a particular locus of a diploid organism are usually identifiable, and that heterozygotes can be distinguished from homozygotes, which is a prerequisite for estimation of allele frequencies in population genetic studies (Adrian *et al.*, 2003). Thus, these markers provide interval data (i.e. quantitative data such as allele frequency or genetic distances) and nominal data (i.e. qualitative data such as genotypes or alleles) (Adrian *et al.*, 2003). Classification techniques based on the use of molecular markers provide a much more accurate and powerful means of analyzing genetic relationships (Soltis *et al.*, 1992). Because molecular markers measure genetic diversity at the DNA level, they can account for the effects of selection, are not influenced by the environment, and are available in an almost unlimited number (Chtourou-Ghorbel, 2001).

- ***Use of allozymes***

The first description of the use of gel electrophoresis was made by Smities in 1955, and followed by Hunter and Markert in 1957, when they described the histochemical visualization of enzymes on gel (Ferguson, 1997). Isozyme electrophoresis techniques are used widely in different fields such as population genetics, systematic genetic and others and it is based on the detection of enzyme sub-units.

Isozymes used to differentiate the molecular forms of the same enzyme that catalyze the same reaction, but with different electrophoretic mobility (Markert and Moller, 1959). If the variants are encoded by different alleles at the same locus, they are termed allozymes

(Prakash *et al.*, 1969). They are revealed when tissue extracts are subjected to electrophoresis in various types of gels and subsequently submersed in solutions containing enzyme-specific stains. The main advantage of this technique is that it provides co-dominant markers, implying that heterozygotes can be determined. In addition the technique is simple to run, does no require sophisticated equipment and it is relatively inexpensive. Its main drawback is the limited number of variants that can be visualized**.**

- ***A brief review of the basic DNA molecular techniques***

Three different sources of DNA in plant cells could be used for studying the genetic diversity and phylogeny, the chloroplast genome (cpDNA), the mitochondrial genome (mtDNA) and the nuclear genome (DNA). The chloroplast genome (cpDNA) is maternally inherited in most angiosperm species and paternally inherited in most gymnosperms. It is highly abundant in leaves and therefore amenable to isolation. The entire cpDNA sequence is known for a few species and appears to be highly conserved in term of size, structure, gene content and order. The chloroplast genome can be used to detect variation at all taxonomic level, mtDNA is particularly suited to intra-specific and population level studies (Demesure *et al.* 1995). Primers are available which work across broad taxa and can be used for diversity studies at all taxonomic levels (Demesure *et al.*, 1995). In contrast, the mitochondria genome (mtDNA) is less abundant in leaves, there is less background knowledge, fewer probes are available and these have been less well characterized. The high rates of structural rearrangements and the relatively low rates of point mutations mean it is of limited use at interfamily and interspecific levels but the high frequency of rearrangements, which can be easily detected as RFLPs, mean that mtDNA can be very useful for detecting variation at the interspecific and population levels (Karp *et al.*, 1997). Primers for conserved regions of

mtDNA sequence are available (Demesure *et al.*, 1995). The only specific part of the nuclear genome that has been used for diversity studies is rDNA (ribosomal RNA) gene family (Zhang *et al.*, 1990). Ribosomal RNA genes are located at specific chromosomal (NOR) loci where they are arranged in tandem repeats which can be reiterated up to thousands of times. Each repeats unit comprises a transcribed region separated from the next repeat by an intergenic spacer (IGS). The transcribed region comprises an external transcribed spacer (ETS), and internal transcribed spacer (ITS) for 3 different genes (Karp *et al.*, 1997). Primers pairs have been designed which will enable amplification of the different regions in a wide range of organisms. These regions evolve at different rates and can thus be used at all taxonomic levels, although in practice it can be difficult to detect sufficient variation at the below-species level (Karp. *et al.*, 1997). The chloroplast genome appears highly conserved across species, whereas the mtDNA, which is less well characterized, has high rates of structural rearrangements and relatively low rate of point mutation (Karp *et al.*, 1996).

Numerous arrays of molecular techniques have been described to measure genetic variation in all taxa levels. These techniques vary in the way they resolve genetic differences, in the type of data that they generate, in the taxonomic level at which they can be most appropriately applied, and their technical and financial requirements. These techniques were made possible with the most significant discoveries in molecular genetics of the restriction enzymes or restriction endonucleases, which are able to cut DNA in both strands (Nathan and Smith, 1975). Each restriction enzyme recognizes a unique, specific sequence of, usually 4-6 base pair (bp) in length, termed a restriction site, where the enzyme cuts (or restricts) the DNA. In general, restriction sites will occur throughout the genome and, consequently, application of the enzyme to total genomic DNA (restriction of the DNA) results in the conversion into millions of fragments. The frequency of restriction sites will vary depending

on both the restriction enzyme and on the genome. Restriction enzymes that cut at sites that are of common occurrence (frequent cutters) in a given genome will result in very large number of small fragments, whereas restriction with an enzyme that cuts sites which occur rarely (rare cutters) will result in fewer, larger fragments being formed (Karp *et al.* 1997).

The DNA fragments generated from restriction by a specific enzyme will all share in common the same sequence at the end (i.e. the restriction site, or part therefore, where the cut was made) but will be of different sequence composition in the middle. The different fragments can be separated according to their length (and hence molecular weight) by electrophoresis. Specialized techniques are therefore required to detect the variation in the DNA of two different individuals. Some of these are based on the initial digestion of the DNA with restriction enzymes, while others depend on the use of a different enzymatic reaction made available thought the discovery of *Taq* polymerase, known as the polymerase chain reaction (PCR) (Ferguson, 1997). The discovery of *Taq* polymerase by Mollis and Fallona in 1987, allowed the automation of the exponential amplification of DNA fragments from the total genomic DNA (Kleppe, 1971).

The basic concept of PCR was first tested with Klenow polymerase. The use of *Taq* thermo-stable DNA polymerase allowed the cycling process to be automated, as only a single addition of enzyme is required (Karp *et al.* 1997). During running PRC, DNA fragment is defined by primer annealing sites. Primers are short stretches of DNA sequence, which are complementary to the opposite ends of the target sequence DNA (Karp *et al.* 1997). They anneal to the complementary sequences in the target and thus ‘prime’ the polymerase amplification. Primers provide the initial point for *Taq* polymerase to synthesis a corresponding second DNA strand. By alternation of primer annealing temperature and

extension temperature, a single sequence can be amplified exponentially and subsequently visualized (Ferguson, 1997). Since both of strands of a DNA molecule run in antiparallel orientation, the primer sequences point to each other. The usual distance between the priming sites (and hence the size of the amplified fragment) is between 100 bp and a few kilobase (kb), although the recent development of so-called 'long distance PCR' now allows amplification up to at least 40 kb (Karp *et al.* 1997). Primers may be arbitrary, in which case amplification will occur wherever the primer is able to anneal to a complementary sequence within the genome, semi-arbitrary in which case they are targeted to a known sequence such as part of a gene family, or a microsatellite, or specific sequence primers composed of complementary nucleotides of two flanking regions either side of a target of the genome. For such direct-targeted PCR the sequence of these flanks must be known (Ferguson, 1997).

A large number of molecular techniques utilizing one or other or both restriction endonucleases and PCR are now available for measuring genetic and botanical diversity. These can be summarized in three basic categories classified in relation to, whether the assays are PCR-based, and whether arbitrary/semi-arbitrary primers or specifically designed primers for know sequences are used (Karp *et al.*, 1997).

- Category 1: non-PCR based methods, e.g. RFLP, VNTR (used as robes in genomic hybridization)
- Category 2: arbitrary or semi-arbitrary primed/or multi-locus profiling techniques, e.g. RAPD, DAMD, AP-PCR, ISSR, DAF, SPARs, AFLPs, SAMPL
- Category 3: site targeted PCR techniques, e.g. PCR-SEQUENCING, TGGE, DGGE, CAPS, SSCP, HETERODUPLEX, and STMS.

A range of new generation DNA molecular diversity detection systems are being developed including Single Nucleotide Polymorphism (SNPs), Sequence-Characterized Amplified Region (SCAR), Cleaved Amplified Polymorphism Sequences (CAPS) and Sequence-Related Amplified Polymorphism/Expressed Sequence Tags (SRAP/EST), which will add momentum to the study of phylogenetic relationships and assess the genetic diversity among and within taxa at different levels (Deulvot *et al*. 2010).

A. Non-PCR based methods

a. Restriction fragment length polymorphism (RFLP)

This method has been used to measure botanical diversity over a wide range of species (Beckmann and Soller, 1983). The DNA is digested with restriction enzymes and resultant fragments are separated by gel electrophoresis. The restricted DNA fragments are then transferred to a filter by a process termed Southern Blotting (Karp *et al.* 1997). Then "Probes" are hybridized to the filter to determine if any differences exist between individuals. Probe is a short DNA fragment (about 800bp) which can be cloned, could be of unknown sequence or part of a cloned gene. The probe should be made with radioactive nucleotides or nucleotides that are labeled with non-radioactive labels such as digoxigenin, to facilitate visualization, so that bands will appear where the probe has hybridized to different fragments (Ferguson, 1997) Variations in fragment lengths between individuals or species can arise either when mutations alter restriction sites or as a result of insertions/deletions between them (Burr *et al.*, 1983).

RFLPs are highly reproducible among different laboratories and, there are co-dominant markers. However, a good supply of probes that can readily detect variation is required, and these can sometimes be difficult to find at the cultivar or within population

levels. New probes can be isolated from cDNA or genomic libraries, but this requires substantial skill and investment of resources. In addition, RFLPs are time-consuming, but can be easily automated. They also require large quantities of good quality DNA (10ug per digestion) (Karp *et al.* 1996). In addition, lack of polymorphism in some species has been a problem (Ferguson, 1997).

b. Variable number of Tandem Repeats (VNTRs)

Hypervariable regions, comprised of tandemly repeated DNA sequences are distributed within the genomes of higher organisms. There are two classes: 'microsatellites', or simple sequence repeats (SSRs), with the basic repeat unit is around 2-8 bp in length, and 'mini-satellite' for longer repeat unit of around 16-100 bp. Hybridization to restricted DNA with micro- or mini-satellite probes gives multilocus patterns which can resolve variation at the levels of populations and individuals (Beyermann *et al.* 1992). The variation results from changes in the number of copies of the basic repeat and is often referred to as Variable Number of Tandem Repeat (VNTRs). VNTR loci are, in principle, co-dominant markers, but in RFLP analysis they often behave as dominant markers (Arens *et al.* 1995). More commonly variation in VNTR loci is visualized through semi-arbitrary primers and PCR amplification (Ferguson, 1997).

B. Arbitrary or semi-arbitrary primed techniques

a. Random Amplified Polymorphic DNA (RAPD)

With the advance of PCR, techniques became available which overcome many of the limitations of probe-hybridization-based methods RFLPs. Among these a subset of closely related techniques was developed simultaneously which involves the use of single arbitrary

primers in PCR reaction, which results in the amplification of many discrete DNA products from all three plant genomes (Karp *et al.* 1997). Each product will be derived from a region of the genome that contains two short segments which share sequence similarity to the primer and which are on opposite strands and sufficiently close together for the amplification to work (Karp *et al.* 1997). These kinds of techniques have been collectively termed multiple arbitrary amplicom profiling (MAAPS) (Caetano-Annoles, 1994). The most commonly used version is RAPD analysis (Random Amplified Polymorphic DNA) in which the amplification products are separated on agarose gels in the presence of ethidium bromide and visualized under ultraviolet light (Williams *et al.* 1990). AP-PCR (Arbitrary primed PCR) (Welsh and McClelland, 1990) and DAF (DNA Amplification Fingerprinting) (Caetano-Annoles *et al.*, 1991) which differs from RAPD principally in primer length, the stringency conditions and the method of separation and detection of the fragments. Polymorphisms are detected based on the presence or absence of bands resulting mainly from sequence difference in the primer binding sites. These techniques do not need DNA probes or sequence information for primer design and do not involve blotting or hybridizing steps. The technique is quick, simple and efficient and requires only the purchase of a thermocycling machine and agarose gel apparatus. It requires small amounts of DNA (10ng per reaction) (Karp *et al.* 1997). Variation is detected at a high frequency and discrimination between closely related individuals is usually possible (Ferguson, 1997). However, it is absolutely critical to maintain strictly consistent reaction conditions in order to achieve reproducible profiles among different laboratories., Data quality is limited because MAAPs gives dominant markers (heterozygosity is not discernible) and because fragments of the same electrophoretic mobility do not necessarily consist of the same sequence And single bands may sometimes consist of several co-migrating amplification products which makes band identities difficult

to assign (Karp *et al.* 1997). MAAPs have alleviated some of the technical problems associated with RFLPs, but have brought other related mainly to marker quality. Due to their simplicity and high throughput, however, they have been widely used to resolve problems in plant breeding, genetics and to estimates relationships based on distance (Tingey and del Tufo, 1993; Waugh and Powell, 1992).

b. Amplified Fragment Length Polymorphism (AFLP)

AFLP is a recently developed method that is equally applicable to all species and is highly reproducible (Vos *et al.*, 1995). It combines restriction, digestion and PCR. It starts with restriction digestion of the genomic DNA with two specific enzymes, one a rare cutter and the other a frequent cutter. Adaptors are then added to the ends of the fragments to provide known sequences for PCR amplification. These adaptors are necessary because the restriction site sequence at the end of the fragments is insufficient for primer design. Short stretches of known sequence are added to the fragment ends through the use of ligase (joining) enzyme. If PCR amplification of the restricted fragments was then carried out, all the fragments would be amplified which, under current technology, would be resolvable on a single gel. Primers are thus designed so that they incorporate the known adaptor sequence plus 1, 2 or 3 additional base pairs, (any one out of the four possible: A, G, C or T) (Karp *et al.* 1997). PCR will only occur where the primers are able to anneal to the fragments that have the adaptor sequence plus the complementary base pairs to the additional nucleotides. The additional base pairs are thus referred to as selective nucleotides. If one selective nucleotide is used, more fragments will be amplified than if two are used, and even fewer fragments will be amplified with three selective nucleotides. For technological reasons, addition of more than three selective nucleotides results in some non-specific PRC amplification (Karp *et al.*

1997). Normally two separate selective rounds of PCR are carried out. In the first round only one selective nucleotide is used, whereas in the second round the same selective nucleotide plus one or two additional ones are used. In practice this results in between 50-100 fragments being amplified, which can be separated on a polyacrylamide gel by electrophoresis.

The amplified products are normally visualize after exposure to X-ray film, where radio-labelled primers are used, but the technique has been adapted to fluorescent, non-radioactive and silver staining procedures, and has been automated. AFLP provides an effective means of detecting several polymorphisms in a single assay (providing on average 100 bands per gel compared with 20 for RAPD), all the evidence so far indicates that they are reproducible as RFLP. Therefore, they are suited for the measurement of genetic variation when genetic similarity is high. Major application of both RAPD and AFLP are thus in establishing identities, in determining parentage, in fingerprinting genotypes and in distinguishing genotypes below the species level (Lu *et al.*, 1996; Sharma *et al.*, 1996). AFLP requires more DNA (0.3-1.0 ug per reaction) and is more technically demanding than RAPD, but their automation and the recent availability of kits means that the technology can be brought in a higher level. Using gel scanners, heterozygotes can be identified; otherwise AFLPs are dominant markers (Karp *et al.* 1997).

C. Site-targeted PCR

The opposite approach to arbitrary amplicom profiling is to design primers to amplify specific regions of the genome. The targeted amplified product can be compared on an agarose gel to the corresponding product from another individual. But only changes that are many base pairs in length will be detected. Sequencing manually, or using an automated DNA sequencer, will potentially resolve all possible differences and data from the aligned

sequences can then be compared. This approach is applicable to extremely small samples, e.g. single pollen grains or tiny leaf fragments (Herrmann and Hummel, 1992).

A number of gel systems are available such as TGGE (thermal gradient gel electrophoresis) (Riesner *et al.*, 1992), DGGE (denaturing gradient gel electrophoresis), single strand conformational polymorphism (SSCP) (Hayashi, 1992) and heteroduplex (HD) formation (White *et a.l*, 1992), which provide sensitive detection of sequence variations that can assist in the detection of sequence differences without the need to sequence all the samples. These detection systems are based on the principle of comparing differences in the stability, or configuration, of the DNA under specific gel conditions (Karp *et al.* 1997). They are quit technically demanding and require highly controlled conditions. In the simple PCR-RFLP, or Cleaved Amplified Polymorphic Sequence (CAPS) procedures the amplified product is digested with a specific restriction enzyme and the products directly visualized on the agarose gel by ethidium bromide staining (Akopyanz *et al.*, 1992; Tragoonrung *et al.*, 1992; Ghareyazie *et al.* 1995).

The advantages of PCR-sequencing approaches are in the quality of the data and the information produced. The fragment in which polymorphisms are studied is of known identity and, this approach revealed information on phylogenetic relations. However, there are also clear disadvantages. Unless the frequency of variants is high enough for detection by PCR-FRLP, or other sensitive gel assay, sequencing of all individuals is required, which is resources intensive. The coverage of the genome is highly restricted, often to only one sequence. Although cpDNA and mtDNA primers are available, there are currently few nuclear genes that can be used at the below-species level and the rate at which sequences vary (and therefore the success of this strategy) also appears to differ between genomes. Because

of the importance of low copy nuclear markers, numerous efforts are currently being expended towards the identification of universally useful primer pairs (Strand *et al.*, 1997). Additional problems, when conserved primers are used for PCR, are contamination by DNA from other organisms and the detection of multiple gene copies and pseudogenes (Karp *et al.*, 1997).

a. Sequence-tagged microsatellite (STMS)

Microsatellites or simple sequence repeats (SSRs) are highly mutable loci which may be present at many sites in all three sources of DNA. Since the flanking sequences at each SSR may be unique, if SSR loci are cloned and sequenced, primers to the flanking regions can be designed to define a sequence-tagged microsatellite (STMS) (Beckmann and Soller, 1990). There are several important advantages of sequence-tagged microsatellites. They are (usually) a single locus which, because of the high mutation rate, is often multi-allelic (Saghai-Maroof *et al.* 1994). They are co-dominant markers and can be detected by PCR (non-hybridization based) assay. They are very robust tools that can be exchanged between laboratories and their data are highly informative and reproducible (Morgante and Oliveri, 1993). Although some changes can be resolved on agarose gels, it is common to distinguish STMS on polyacrylamide sequencing gels where single repeat differences can be resolved and all possible alleles detected. The assay is relatively quick and throughput can be increased by selecting a small number of different STMS with alleles on non-overlapping size ranges and multiplexing either the PCR reactions, or, more easily, the products of the separate reactions, so that all the alleles of the different loci can be run in a single lane on the gel. Multiplexed STMS have also been automated. Unless the investigator is extremely fortunate, however, STMS will not be available for their species of study. Retrieval of microsatellites

has not been easy in plants because of their relatively low abundance compared with animal genomes. STMS often shows limited cross-transferability to other genera and even to other species within the same genus. An investigator wishing to use microsatellites is thus probably first faced with having to isolate them. Whilst retrieval strategies have now been devised which work with high efficiency (Edwards *et al.*, 1996), STMS development necessitates a considerable investment of time and extra skilled expertise and resources (Karp *et al.*, 1997).

b. Single nucleotide polymorphisms (SNPs)

A new generation molecular markers, called single nucleotide polymorphisms (SNPs) is developed and used. These polymorphisms are single-base substitutions between sequences (Gupta *at al.,* 2001). SNPs do not always need these gel-based assays, they are also the most abundant of all marker systems known so far, both in animal and plant genomes (Gupta *at al.,* 2001). SNPs occur more frequently than any other type of marker, and are very near to or even within the gene of interest (Wang *et al.*, 1998). More importantly, SNPs allow the unification of the candidate gene approach and association-based fine mapping to identify gene(s) of interest, they also aid in the association of linkage analysis to the phenotypic and genotypic data (Lai, 2001). SNPs have been developed which are based on single base changes within the genome (Landegren *et al.*, 1998; Lindblad-Toh *et al.*, 2000).. SNPs have become popular tools for identifying genetic loci that contribute to phenotypic variation based on linkage disequilibrium (Wang *et al.*, 1998). Compared with other genetic markers, SNPs are more abundant in the genome and are much more stably inherited (Wang *et al.*, 1998). Another advantage of SNP-based genotyping is that SNP detection does not involve gel electrophoresis, which is relatively slow and labor intensive (Osman *et al.*, 2003). Many different strategies have been developed for high throughput detection of SNPs including

high-density oligonucleotide hybridization arrays (Wang *et al.*, 1998), dynamic allele-specific hybridization (Pennisi, 1998), and the Taqman assay (Livak *et al.*, 1995).

The frequency and nature of SNPs in plants is beginning to receive considerable attention; several studies used SNPs: in Soybean (Zhu *et al.*, 1995b), *Arabidopsis thaliana* (Cho *et al.*, 1999)*, Zea mays* (Tenaillon *et al.*, 2001), Rice (Nasu *et al.*, 2002; Hayashi *et al.*, 2004), and *Eurycoma longifolia* (Osman *et al.*, 2003), all previous studies have provided estimates of SNP diversity in these species. SNPs can be identified on using microarrays or denaturing high-performance liquid chromatography (DHPLC), which is used to visualise SNPs (Patil *et al.*, 2001).

The advantages of using SNPs are: the low mutation rate, high abundance of SNPs, easy to type, new analytical approaches are being developed at present, cross-study comparisons are easy and data repositories already exist (Schlotterer, 2004), the main advantage is their high potential for an automated highthroughput analysis at moderate cost (Chen *et al.*, 1998). The disadvantages of using SNPs are: substantial rate heterogeneity among sites, expensive to isolate, ascertainment bias and low information content of a single SNPs (Schlotterer, 2004).

D. Variations or combinations of the basic techniques

The basic molecular techniques described above can be further refined and also combined in several ways. Sequence tagged site, (STS), is the general term given to locus defined by its primer sequences. An STS can be created for any site, provided that the locus can be cloned and sequenced. This may be desirable, when for example RFLP probes are

being used to test large numbers of samples (Livneh *et al.*, 1992), or when a stable, robust and reliable PCR marker linked to genes controlling a trait of interest is required. Sequence characterized amplified regions (SCARs) are derived from individual RAPD markers (Paran and Mitchelmore, 1993). The RAPD fragments (bands) are cloned; the nucleotide sequences of the terminal ends are determined and used to design primers for specific amplification of the desired fragments. There are also many semi-arbitrary PCR methods: In Direct Amplification of Minisatellite-region DNA (DAMD), VNTR core sequences, such as M13, are used as primers in PCR reactions (Heath *et al.*, 1993). In Single Primers Amplification Reaction (SPARs), the principle is similar but primers are based on the core motifs of microsatellites (Gupta *et al.*, 1994). Again, polymorphic banding patterns are produced. Inter-simple sequence repeat amplification (ISSR) is similar to SPARs but involves the anchoring of designed primers to a subset of SSRs and results in the amplification of the regions between two closely spaced oppositely oriented SSRs (Kanety *et al.*, 1995). Microsatellite primers can also be used in conjunction with AFLPs in techniques referred to as SAMPLE (Morgante and Vogel, 1994)

- **Analysis of molecular data**

Usually, spatial analyses are conducted on independent diallelic loci and the diploid genotype at each location is converted into the values 0, 0.5 and 1 according to the frequency (none, one, and two) of a particular allele. Phenetic analyses of individuals using binary data from allozymes or microsatellites can be also undertaken (Ayres and Ryan, 1999) so that construction of genetic distance matrices or use of raw data for further analyses is feasible in both cases. When the spatial approach is at a larger scale, allele frequencies are used (Adrian *et al.*, 2003).

In contrast, RAPD, ISSR and AFLP segregate as dominant markers, and must be treated as phenotypic characters (presence/absence data). In this case, genetic matrices, composed of ‘‘1s’’ and ‘‘0s’’, are used where each row shows the data of a particular individual and each column shows the presence or absence of a particular band. These data are usually converted into similarity matrices for calculation of genetic distances. They can also be explored using summary measures, which has the advantage that only a reduced number of statistics are necessary (Bertorelle and Barbujani, 1995) but the clear disadvantage that they can only rarely be related to very informative genetic coefficients (Epperson *et al.*, 1999). Allele frequencies can also be indirectly estimated from these markers and results can be used to obtain the classical parameters of population genetic studies. Nevertheless, in order to do this, several assumptions must be made that do not always coincide with reality (Lynch and Milligan, 1994). This technique has been superseded by molecular techniques that reveal variation directly at the DNA level.

It is essential to understand the different ways that the data derived by molecular techniques can be analyzed before considering their application to diversity studies (Hillis and Mortiz, 1990; Soltis *et al.*, 1992; Avis 1994; Weir, 1996; Adrian *et al.* 2003). Two main types of analysis will be relevant:

1. Analysis of genetic relationships among samples
2. Calculation of population genetic parameters, in particular diversity and its partitioning at different levels.

The analysis of genetic relationships among samples starts with the construction of matrix specifying the character-state of each marker for each DNA sample from individuals, but could consist of DNA bulked from a number of individuals. Marker states may be binary, as

in the presence or absence of RAPD bands or restriction sites (as revealed by RFLPs and related techniques), or multi-state, as the nucleotide (A, T, C or G) present at the particular position in a DNA sequence (Karp *et al.*, 1997).

The DNA sample marker matrix of character-states is then commonly used to construct a DNA sample matrix of pair-wise genetic distance (or similarities) (Adrian *et al.* 2003). There are different ways to calculate the genetic distance (or similarity) between samples on the basis of differences between them in the states of a set genetic markers (e.g. Hendrick, 1974), but a commonly used index is Nei's genetic distance (D) (Nei, 1973).

There are two main approaches to analyze the resulting distance (or similarity) matrix and displaying the results: One is to use Principal Coordinate Analysis (PCA) to produce 2- or 3-dimensional scatter plot of the samples such that the geometrical distances among them with a minimum of distortion. Aggregations of samples in such a plot will reveal sets of genetically similar material. The other approach is to produce a dendogram (or tree-diagram) linking together in clusters samples that are more genetically similar to each other than the samples in other clusters. Clusters are linked to each other at progressively lower levels of similarity until all the samples being analyzed are included in a single cluster. Such Cluster Analysis may proceed according to a range of different algorithms, but some of the more widely used ones include Unweighted Pair Group Method with Arithmetic Average (UPGMA), Neighbour-Joining Method and Ward's Method. Different combinations of genetic distance/similarity index and clustering algorithm may give rise to somewhat different dendograms (Karp *et al.*, 1997).

Both PCA and cluster analysis are called 'phenetic' methods, they are based on measures of overall distance or similarity between samples.

However, there is another, philosophically quite distinct approach to the analysis of genetic relationships, referred to as 'cladistics'.

Cladistic analysis begins with a sample marker character-state matrix, and also results in dendograms, though these are sometimes called cladograms to distinguish them from the phenograms of cluster analysis. The difference is that two samples are placed together in the same cluster (or clade) of a cladograms not on the basis of high genetic similarity between them calculated from all markers taken together, but because they share a particular state of a given marker (or markers). The two approaches are also sometimes distinguished as 'distance' and 'character-state' respectively. Because it is possible to generate many cladograms from a single dataset, due to conflicts among characters, so-called parsimony approaches are used to choose among them. A most-parsimonious cladogram is one that requires the least number of character-state changes. There is a wide range of parsimony algorithms, each with its own data requirements and assumptions. Some require that the polarity of character changes be known, i.e. which character changes are ancestral and which are derived. Cladograms are reconstruction of phylogenies. RAPD data, because of uncertainty over the identity of bands, is not usually suitable for this kind of analysis (Karp *et al.*, 1997).

To measure the genetic diversity and genetic structure (between and within populations), the F-statistics of Wright (1965, 1978) and the G-statistics of Nei (1973) are commonly employed. Estimates of these statistics are based on allele frequencies, and the most appropriate molecular data for such statistical analysis are clearly those in which allele frequencies can be determined directly, such as RFLPs, STMS and sequence haplotypes. Of these, sequences and restriction site data are unique between molecular markers in providing

both frequency and phylogenetic information. Nevertheless, suitable statistical treatments are also available for dominant markers such as RAPDs, though in at least one case population differentiation coefficients based on indirectly estimated RAPD frequencies were not concordant with those based on RAPD frequencies directly estimated from haploid macrogametophytes (Szmidt *et al.*, 1996). Careful treatment also needs to be given to difficulties arising from the occurrence of a large numbers of alleles at one locus in STMS, and for various sources of sampling error within and between populations (Weir and Cockeram, 1984). There are several software packages which may be used to calculate genetic parameters and distances and others for general statistical analysis (Karp *et al.*, 1997).

Methods based on variable polymerase chain reaction (PCR) amplification such as amplified fragment length polymorphisms (AFLPs) can provide a rapid and affordable approach to collecting polymorphism data on a genomic scale (Campbell *et al*. 2003; Luikart *et al*. 2003). However, these markers are typically ambiguous about the genotypes that underlie them. In particular, in diploids, a band will be obtained if either or both of the homologous chromosomes contain an amplifiable sequence. In polyploids, there can be ambiguity even with codominant markers. Even when it is possible to determine which alleles are present, it might be difficult to determine the number of each. These ambiguities need to be addressed in any analysis (e.g.Holsinger *et al*. 2002; Hardy 2003; Hill & Weir 2004; Hollingsworth & Ennos 2004; Kosman & Leonard 2005).

STRUCTURE, is a simple approach for accounting for genotypic ambiguity in studies of population structure. Pritchard *et al.*, in 2000, assumed a model in which there are *K* populations (where *K* may be unknown), each of which is characterized by a set of allele frequencies at each locus (Pritchard *et al.*, 2000). Individuals in the sample are assigned (probabilistically) to populations, or jointly to two or more populations if their genotypes

indicate that they are admixed. The model does not assume a particular mutation process, and it can be applied to most of the commonly used genetic markers, provided that they are not closely linked.

The program *structure* is a software package for using multi-locus genotype data to investigate population structure. Its uses include inferring the presence of distinct populations, assigning individuals to populations, studying hybrid zones, identifying migrants and admixed individuals, and estimating population allele frequencies in situations where many individuals are migrants or admixed. It can be applied to most of the commonly-used genetic markers, including SNPs, microsatellites, RFLPs and AFLPs. The basic algorithm was described by Pritchard, Stephens & Donnelly (2000). Extensions to the method were published by Falush, Stephens and Pritchard (2003b), and (2007) and (Hubisz *et al.,* 2009).

The method can produce highly accurate assignments using modest numbers of loci—*e.g.*, seven microsatellite loci in an example using genotype data from an endangered bird species (Pritchard *et al.*, 2000)

STRUCTURE uses a Markov chain Monte Carlo (MCMC) algorithm to cluster individuals into populations on the basis of multilocus genotype data (Pritchard *et al* . 2000; Falush *et al* . 2003b), and it has been applied to problems such as identifying cryptic population structure detecting migrants or admixed individuals, and inferring historical population admixture (e.g. Rosenberg *et al*. 2002; Falush *et al*. 2003a; Albert *et al*. 2006; Lecis *et al*. 2006; Ostrowski *et al*. 2006).

- **Application for techniques**

RFLPs are co-dominant markers and allele frequencies, and therefore population statistics can be calculated directly for single copy loci, they are useful markers for population

studies and diversity classification, provided that sufficient polymorphisms can be detected in the species under study. Unless they are recorded as a combination of probe and restriction site data, RFLPs need to be converted into frequency data which have some limitations. When VNTRs are used as probes in RFLPs, multi-locus profiles are produced which share the same feature, and thus applications, described for arbitrary (or semi-arbitrary) primed/or multi-locus profiling techniques. This is also true for RFLPs in which the probes used are homologous to highly-repeated sequence families where several bands will also occur on a gel with a single probe enzyme combination (Karp *et al.*, 1997).

The derived data from arbitrary primed AFLP and multi-locus fingerprinting approaches have their strength in distinguishing individuals. Major applications of these approaches are thus in establishing identities, in determining parentage, in fingerprinting genotypes and in distinguishing genotypes below the species level. (Lu *et al.*, 1996; Sharma *et al.*, 1996; and Tohme *et al.*, 1996). The difficulty of achieving robust, repeatable, profiles in arbitrary primed approaches such as RAPDs does, however, make their reliability for 'typing/fingerprinting' questionable.

RAPDs have been used in all kind of diversity studies at all taxonomic levels, including population and phylogenetic studies, but RAPDs provide limited data (Karp *et al.*, 1997). The arbitrary (or semi-arbitrary) primed/or multi-locus profiling techniques produce multi-band profiles, in which the number and placement of bands generated and many and depending upon the technique and the primers used. These techniques compare different genomes at several points but the identity of these points is not known. Using data from such multi-band profiling procedures is extremely important to recognize that:

i. They are usually dominant markers;

ii. In the absence of pedigree analysis, the identity of individual bands is not known and there may be uncertainty in assigning markers to specific loci;

iii. The presence of a band of apparently identical molecular weight in different individuals is not evidence that the two individuals share the same homologous fragment, and

iv. Single bands can sometimes be comprised of several co-migrating amplification products.

These limitations in data quality are important because they reduce the efficiency of the analytical methods described previously, as assumptions, such as independence. (i.e. that the markers do not represent the same or linked mutation), known mutational models, neutrality, non-recombination, etc. are essential facets of the models used. In using RAPDs for populations studies, for example, these limitations do not prevent the estimation of allele frequencies necessary for population genetic analysis, but they do reduce the accuracy of such estimation relative to codominant markers such as RFLPs. To achieve the same degree of statistical power using RAPDs (or any other codominant marker system), compared with codominant markers, 2-10 times more individuals need to be sampled per locus (Lynch and Milligan, 1994). In the use of RAPDs for phylogeny more criteria need to be satisfied to give credence to the analysis (Clark and Lanigan, 1993).

Site-targeted PCR sequence markers are containing a comprehensive record of their own history. In addition to revealing the grouping of individuals into different classes, appropriate analysis based on sequence data (or restriction site data) can provide hypothesis on the relationship between different categories clustered together. In contrast, frequency data from RAPDs and AFLPS only provide the means to classify individuals into nominal genotypic categories. It is argued by many that technologies that yield sequence data are the

only appropriate methods for taxonomic studies and for any study in which phylogenetic information is important (Karp *et al.*, 1997). This is an important point to grasp for population studies, particularly when the diversity data are used for conservation,

Sequencing will allow the determination of which gene sequences, in samples taken from within or between populations, are the most closely related and hence share a most recent common ancestor. For such genealogical relationships (which may be separated from the genealogy of the individuals carrying genes) the influences of genetic factors, such as population size, whereas in the case of markers that provide only frequency information these factors are confound (Milligan *et al.*, 1994). This differences is of particular relevance to conservation, where demography (the description and prediction of population growth and age structure) is considered to be as, or more, important than genetic factors (Lande, 1988).

The STMS is a PCR-based assay of a single locus with, potentially, an infinite number of alleles. Identity and assignment of alleles are thus not a problem. The markers are codominant so allele frequencies can be determined directly and their rate of change renders them particularly suitable for below-species studies. STMS therefore provide ideal tools for population studies and for assessing diversity among genotypes within species (Karp *et al.*, 1997). The problem with STMS concerns the mutational mechanisms by which alleles arise and the occurrence of large numbers of allelic variants. The accuracy with which true homology can be inferred for different genotypes diminishes as genetic distance becomes greater, because of the increasing possibility that different forward and back mutation events may result in alleles of the same size. Phylogenetic inferences are therefore problematic with STMS. Similarly, some population genetics estimates require careful treatment to account for the large numbers of alleles. In both cases, appropriate statistical procedures are being

developed. Another problem with STMS is the occurrence of null alleles, as a result of mutation in the primer site. These will not produce a band on a gel and heterozygotes with null alleles can therefore be misclassified as homozygotes (Karp *et al.*, 1997).

It is very important for researchers and investigators to select the most appropriate technique for their studies, given the constraints of time, money or other resources they face. It should be understood that the process outlined is flexible regarding which techniques are most appropriate for which purposes. The aim is to provide a logical framework in which the different methodologies can be assessed. It is important to appreciate that molecular genetics is a rapidly developing field and frequently technologies are advancing faster than our understanding of their full potential or limitations. New techniques are continually being described and new information about pre-existing techniques is continually altering our understanding and interpretation of data obtained from them. Furthermore, molecular geneticists often disagree about which techniques should be chosen in a given experiment. It is not the case, therefore, that there is only one technique that should be chosen, but rather that there are clear reasons why it is better to choose some techniques compared with others and the limitations of any chosen technique should be recognized.

Weising *et al.*, (1998) briefed the following suggestion to select the molecular marker. ‘A molecular marker can be derived from any kind of molecular data which provides a screenable polymorphism between two organisms that are to be compared. Various techniques to visualize such polymorphisms have been or are being developed (Winter and Kahl, 1995). An ideal marker system would have to meet a number of criteria, no markers are yet available which fulfill all of the desirable criteria; one can already choose between varieties of marker systems each of combines at least some of the following properties:

- High level of polymorphism;
- Codominant inheritance (discrimination of homo- and heterozygotic states;
- Unambiguous designation of alleles;
- Frequent occurrence in the genome;
- Even distribution throughout the genome;
- Selectively neutral behavior (no pleiotropic effects);
- Easy access (no cloning);
- Easy and fast assay (e.g. by procedures amenable to automation);
- High producibility;
- Easy exchange of data between laboratories;
- Development at reasonable costs.'

➢ **Application for genetic diversity and phylogeny studies of *Lathyrus***

Genetic diversity assessments of numerous crop species have been conducted with DNA markers alone or in cycle with morphological analyses (Noli *et al.*, 1997; Paul *et al.*, 1997; Yee *et al.*, 1999). However, molecular techniques have not been widely used to examine genetic variation or interspecific relationships in the genus *Lathyrus* (Chtourou-Ghorbel *et al.*, 2001). An attempt to study the usefulness of RFLP and RAPD for examining the levels of genetic variation within and between populations from a wide range of geographical origins, and, representing species of the genus *Lathyrus* and also for estimating genetic relationships between these populations concluded that RAPDs are equivalent to RFLPs in the estimation of genetic diversity in populations of *Lathyrus*; moreover, because of

their relative simplicity and lower cost, RAPDs are considered more practical than RFLPs for studies on germplasm organization and characterization (Chtourou-Ghorbel *et al.*, 2001).

Morphologic homoplasy is rendering difficult the taxonomic classification of some *Lathyrus* species. In addition to botanic identification of species, chromosome homology and homeology using different cytogenetic techniques are used to support the phylogenic relationships between species. Schifino-Wittmann (2001) used isozymes patterns on 18 accessions of five *Lathyrus* species allowed an unexpected grouping between *L. pubescens* and *L. sativus* and found that some bands were specific to some species. DNA molecular techniques provide powerful tools to understand the systematics of *Lathyrus* genus. Asmussen and Liston (1998) conducted the largest molecular investigation of *Lathyrus* to date which allowed reviewing the classification done by Kupicha (1983). Kenicer *et al.* (2005) used nuclear ribosomal and chloroplast DNA to study the systematics and biogeography of 53 *Lathyrus* species. The results supported generally the recent classification based on morphologic traits, resolved the clades between *Lathyrus* and *Lathyrostylis* sections, but questioned the monophyly of the section *Orobus sensu*. The study also brought some suggestions of the geographic origin of different species.

2.2 Ecogeography of genus *Lathyrus* L.

2.2.1Introduction to ecogeographic study

To make the most efficient use of limited resources, plant germplasm collectors and conservationists must have a clearly defined set of target taxa, and must know as much as possible its geographic distribution, ecology, phenology and diversity (Maxted *et al.* 1995). The ecogeographic study is defined by Maxted *et al.,* (1995) as "An ecological, geographic and taxonomic information gathering and synthesis process. The results are predictive and can be used to assist in the formulation of collection and conservation priorities".

There is a difference between 'study' and 'survey', the ecogeographic study involves a more detailed data analysis and interpretation phase than survey. A study will involve detailed collation of fresh environmental data and multivariate analysis of the patterns of distribution and may take several years to complete. A survey will focus on collating data recorded by other plant collectors, rather than collecting fresh data, and may be restricted to a media search and collating passport data from herbarium specimens or germplasm accessions Maxted *et al.* (1997).

The current threats to plant genetic resource call for more efficient and effective actions for conservation of plant genetic resources (Maxted & Kell, 1996). This need is underlined in Article 8 of the Convention on Biological Diversity (CBD). Particularly in the field of *in situ* conservation, the CBD calls on nations to:

"Develop where necessary, guidelines for the selection, establishment and management of protected areas or areas where special measures need to be taken to conserve biological diversity." Article 8 -CBD (UNCED, 1992)

Ecogeographic techniques provide a partial means of fulfilling the objectives of this article. In practice, all conservation activities, whether *in situ* or *ex situ*, are necessarily preceded by some form of geographic data collection and analysis (Maxted & Kell, 1996). The results of an ecogeographic survey help clarification of the priorities and the appropriate strategy that should be applied to conserve the target gene pool as a whole (Maxted *et al.,* 1995). Localities inhabited by a species will be characterized by more or less specific environmental constraints. The passport data associated with herbarium specimens, germplasm accessions and other plant records, as well as data from media sources (i.e. literature, computer database and the internet) can be used to identify these constraints (Maxted *et al,* 1995). The analysis of the large and complex data sets resulting from ecogeographic surveys, will ultimately lead to better understanding of taxon:

1. distribution in particular regions and ecosystems (i.e. Geography)
2. patterns of intraspecific diversity (i.e. Taxonomic and genetic diversity)
3. relationships between ecological conditions and the survival or frequency of variants (i.e. Ecology)

The data can be synthesized to produce three basic products:

a) the database, which contains the raw data for each taxon;

b) the conspectus, which summarizes the data for each taxon; and

c) the report, which discusses the contents of the database and conspectus, as well as proposing future collection and conservation strategies (Maxted *et al.,* 1995).

This methodology for ecogeographic survey is composed of three key phases:

- Project Design

- Data Collection and analysis
- Production

The data collection and analysis phase involves the gathering and collation of ecological, taxonomic and geographical data to produce an accurate and useful dataset. The dataset is analysed using various techniques to create the products: Ecogeographic database, Ecogeographic conspectus and Ecogeographic report which are essential for creating a foundation strategy for both *in situ* and *ex situ* conservation (Maxted *et al.*, 1997a).

The utilization of Geographical Information Systems (GIS) computer programs is vital for producing reliable ecogeographic information easily and relatively quickly. GIS software requires geo-referenced data from germplasm or herbarium specimens to create accurate and usable products for use in conservation. They produce useful visual products such as maps and graphs and also have strong statistical and algorithmic capabilities. The tools within GIS package are very useful in terms of predictive abilities, with many programs allowing the user to make assumptions about the distribution of a species based upon accession data already entered into the system combined with climate data which comes with the GIS software. Other tools in these programs allow the user to map species richness, observation richness, and also allocate genetic reserves in areas of optimum species richness. Ultimately, the quality of data entered into a GIS will affect the quality of the final output. If poorly geo-referenced data is the basis of a GIS analysis then the results will be inaccurate and any conservation strategies based upon this would be of little use. Several other softwares including DIVA-GIS software provided by Hijmans *et al.* (2001) (http://www.cipotato. org/diva/) can be used to identify areas for potential establishment of natural reserves for conservation of species.

2.2.1.1 Project design

Project design encompasses several processes that are concerned with the project initiation and establishment of clear project objectives. It requires the collating information for accurate identification of taxon using proper floras, herbaria, monographs, taxonomic databases and ecological works to be recommended by experts which can be found in *Index Herbariorum* (Holmgren *et al.*, 1990) which also contains major international and national herbaria where important dried plant collections are held. The right classification provides connections to other taxonomic literature: lists of accepted taxa, taxon descriptions, synonymized lists, distribution maps, identification aids and techniques, ecological studies, bibliographies and taxonomic notes. Also, many databases and networks are available to provide any particular information as distribution and nomenclature, such as *Kew Index.* Then, it is very important to define the target areas using all the available resources such as Floras and previous collection missions. The international herbaria are usually richer and have a broader taxonomic coverage, and most of the materials conserved are used to produce taxonomic revisions and monographs, also it has broader geographical coverage. Local herbaria are good for a regional coverage of the target area, and have better documented materials if adequate taxonomic expertise is available. Passport data of the previous collecting missions or the major germplasm collections held by the national or international genebanks and botanical institutions can be accessed from several sources including Bioversity International (former IPGRI) international directories of germplasm collections and the International Directory of Botanic Gardens *(*Heywood *et al.,* 1990*).*

It is recommended to design and build a database to record all the ecogeographical information associated with herbarium and germplasm specimens. There are many database softwares which can be used to structure the ecogeographical study database (Excel, Access,

FoxPro, etc.) provided that the data are standardized and codified. The code used should be accepted standards and used consistently such as those included in the International Union of Biological Sciences Commissions on Taxonomic Database (TDWG), the IUCN system for describing the conservation status of species (IUCN, 1994a) and the Bioversity International (former IPGRI) plant descriptor list developed for most economic species.

2.2.1.2 Data collation and analysis

There are many sources to collate data related to an ecogeographic survey including Catalogues, directories and databases of major local and international herbaria, botanical gardens and genebanks, in addition to publications taxonomic notes or reports made by taxonomists. Geographic, ecological and taxonomic data could also be obtained from soil, vegetation and climatic maps, atlases etc. most of which can be now accessed via internet. Current conservation activities for the target taxon can be accessed through catalogues and databases of botanical gardens, genebanks and *in situ* conservation areas. International directories of germplasm collections (Bioversity International production), Germplasm Databases such as SINGER (System-wide Information Network of Genetic Resources) or the other institutional websites are the major resources to review the conservation status. It is important to select reliable herbarium specimen having detailed ecogeographic passport data or showing features of particular taxonomic, ecological or geographical interest. Maxted, 1995, recommended that third of the examined specimens are enough to be included in the database. These various sources can provide information on: accepted taxon name, local names, areas of distribution, habitat preference, genetic and phenotypic variation, breeding efforts, biotic constraints, archaeological and ethnobotanical evidence, in addition to conservation status. Passport data can provide additional information on locations of specimens and accessions, phenological data, vegetative type, use and/or agricultural practice,

competitive ability, palatability, ability to stand grazing, and plant uses (Guarino and Maxted, 1996). Upon compilation, the database should be examined and all errors to be corrected before analysis. Indexing the database fields and using GIS tools will help in minimizing errors. Checking for duplication particularly for the herbarium specimens and minimize the duplication will be useful.

The ecological and geographical data can be analyzed to help to identify the particular geographical locations and habitats favored by the target taxa. One of the simplest means of ecogeographic data analysis is to use frequency distribution which could identify the particular and preferred niche and can be used to relocate previous collection areas and indicate other areas where the taxon is likely to be found. Correlation of the occurrence of specimens along environmental gradients correlation of morphological characters with particular environmental conditions will help indicate possible ecotypic adaptation, in both wild and cultivated materials. Ecogeographic data can also be mapped and taxon distribution maps can be used in conjunction with topographical, vegetation, rainfall, geological, soil and other thematic maps to predict where else the target taxon might be found (Stace, 1989). Enclosing line maps can be used to indicate concentrations of species. These maps, known as Isoflor maps, do not show actual species distribution, but each line is a contour delimitating a greater or lesser concentration of species.

The analysis of ecogeographic database can be much facilitated by the use of the Geographical Information System (GIS), a database management system dedicated to the simultaneous handling of spatial data in graphics from and of related, logically attached non-spatial data (Burrough, 1986).

There are many GIS tools software to analyze and produce too many features to predict the presence of a particular taxon. All of the above methods of analysis are based on consideration of a few environmental factors at a time, or a single morphological variable. Maxted *et al.,* in 2005, produced their publication on "*An ecogeographic survey:* African *Vigna*. Systematic and Ecogeographic Studies of Crop Genepools" as as examples of the previous mentioned technique. (Maxted *et al.*, 2005). Ecogeographic data, however, is multivariate, in that many items of data are available for each record (e.g. collecting site, germplasm accession or herbarium specimen). Where an ecogeographic study is undertaken, the data collated during the study is likely to be much higher quality than that collected solely from herbarium specimen passport data during a survey. One of the most thoroughly statistically tested ecogeographic data sets is that reported by Cocks and Ehrman (1987), Ehrman and Cocks (1990), and Ehrman and Maxted (1990) for the annual legumes in Syria. These authors undertook comprehensive field work over several years, during which time they gathered extensive ecogeographic data and were able to use this data to predict potential areas of conservation. For example, Ehrman and Cocks (1990) used various methods of cluster analysis on their environmental data to classify the collecting sites into groups or classes (clusters) the members of which had climates which were more similar overall (rather than as regards any one single variable) to one another than they were to members of any other class.

It should be taken into consideration that the resources required to undertake a study are relatively expensive. Therefore, if an ecogeographic investigation is to be used as a routine part of collection and conservation activities then the quicker, less expensive option is likely to be favored. As surveys focus on collating passport data from herbarium specimens or

germplasm accessions, the data is unlikely to be sufficiently robust to permit detailed multivariate analysis.

2.2.1.3 Production phase

The final production phase of the project commences with the synthesis of all the data collected during the study. The ecogeographic database, conspectus and report should be seen as the three essential products of an ecogeographic study. The ecogeographic database contains the raw data of the project. The conspectus summarizes the available ecological, geographical and taxonomic information for the target taxon through part or the whole of its range. The report interprets the data held in the other products and will aid the conservationist in selecting conservation priorities. The report discusses the contents of the database and conspectus and must draw general conclusions concerning the group's ecogeography and presents a concise list of conservation priorities; If possible, the following points should be covered:

a. The delimitation of the target taxon;

b. The classification of the target taxon that has been used;

c. The mode of selection of the representatives specimens;

d. The choice of hardware and software;

e. The ecogeographic database file structures and inter-relationships;

f. Discussion of the database contents;

g. Discussion of the target taxon ecology;

h. Discussion of the target taxon phytogeography, discussion of the distribution patterns and a summary of the distribution in tabular forms;

i. Discussion of any interesting taxonomic variants encountered during the study;

j. Discussion of the current and potential uses of the target taxon;

k. Discussion of the relationship between the cultivated species and their wild relatives;

l. Discussion of any particular identification associated with the group, presentation of any particular aids to vegetative, floral and fruiting specimens;

m. Discussion of *in situ* and *ex situ* conservation activities associated with the target taxon, including the extent of diversity already conserved;

n. Discussion of the genetic erosion threats facing the group;

o. Discussion of priorities and suggested strategy for future conservation of the target taxon;

p. The ecogeographic conspectus may be included within the report as an appendix or as separate entity.

q. Conservation status of the target species.

The various products of the ecogeographic survey provide a basis to formulate future conservation priorities and strategies for the target taxon and to select areas of particular interest e.g. areas with high concentrations of diverse taxa or adaptation to harsh conditions such as drought, salinity, etc.

The ecogeographic survey or study must conclude with a clear, concise statement of the proposed conservation strategy for the target taxon and proposed conservation priorities. This will answer questions such as: what part can local workers play in the

conservation activities, should population level be closely monitored to assess the threat of genetic erosion, should a national or international collecting team be directed to collect the priority target taxa, it is possible to conserve that taxa *in situ*, is *in vitro* conservation requires or is a more detailed study required before these questions can be answered? Once specific areas of genetic variation have been highlighted, a route that covers the maximum number of sites in the minimum time or location for an *in situ* reserve can be suggested.

2.3 Identification and field guides

The inability of many to use contemporary identification aids has led to confusion of specimen identity and has undoubtedly resulted in the application of poor conservation strategies and the poor utilization of much valuable material. The growing out and re-identification of several large germplasm collections (Maxted 1989, 1992; Maxted & Bisby 1986, 1987) has established that a significant proportion of *ex situ* conserved material currently held in national and international gene banks is either wrongly identified or is not identified at all. There are four basic methods of identification: (1) expert determination, (2) recognition, (3) comparison, and (4) the use of keys and related methods such as synopses, outlines, and tables of characters (Radford *et al.*, 1976). Of these, expert determination is the most reliable method (Stevenson *et al.*, 2003), but experts are few and even if specimens are collected and sent to them the time delay before the identification is achieved makes this approach not practical. Species recognition based on experience is also of limited application because as concluded by Morse (1971), recognition depends on either being self-taught or learned from an expert, which again emphasizes the shortage of taxonomic expertise. In contrast, comparison covers a broad array of approaches, including searching through museum specimens for matching specimen, reading descriptions, reviewing illustrations and flicking

through named photographs. This approach does work but is time-consuming and requires access to named comparative materials. By far the most practical and efficient means of identification is the use of keys or related identification aids. Keys offer a step-by-step approach to identify a species commonly employing a dichotomous hierarchical tree in which the user follows a sequential path to the end of the branch, at which time the species of interest is identified. Despite their widespread use by the taxonomic community (Fortuner, 1989; Thompson, 1999), keys have serious limitations due to the amount of technical botanical terminology used to describe plant parts and their stylized format, also writing keys is highly skilled and badly written keys abound. The use of these keys remains a seriously limiting problem for those who lack formal biological training.

The conservation and other non-taxonomic communities needs to benefit from recent but well-established developments in computer science to apply innovative methods of plant identification and computer-aided-learning programs, which can be used by professional and amateur communities alike.

2.3.1 Plant identification

Identification is the naming of an organism by reference to an already existing classification (Stace, 1989). Identification is usually achieved by using different aids, such as dichotomous keys, multi-access keys, illustrations and interactive identification programs. Identification or "determination" of a plant specimen involves two steps; firstly, the decision as to which taxon (e.g. genus, species or subspecies) the specimen represents, and secondly, the decision as to what is the "accepted" name to use for it, if more than one name has been used for that taxon (Maxted and Crust, 1995). The second step is largely related to establishing the accepted name for a taxon; distinguishing between this name and numerous synonyms of

misapplied names that have been used for the taxon. Establishing which names are accepted is achieved by discussion with an appropriate taxon expert or taxon network (such as the International Legume Database and Information Service), or simply using the most recent or most commonly applied name.

Specimens are commonly identified to species, but if lower taxonomic entities have been described they could be named to subspecies, variety, etc. The correct identification of the specimen is achieved by comparing its characteristics to the sets of "key" characteristics possessed by each species. If the specimen's characteristics fall within the range of a species' "key" characteristics, then the specimen is identified as a representative of that species, the range of the "key" characteristics for each species having been previously determined by a detailed study of a broad range of specimens representing that species.

There are basically two forms of identification, matching and elimination. Matching involves the comparison of the specimen to taxon descriptive data or some form of exemplar, such as a named herbarium sheet. Clearly, trying to match a specimen to one of a large number of possible taxa could be time-consuming and some method is needed to narrow down the possibilities. Identification by elimination involves the user in comparing a specimen to a set of mutually exclusive short descriptions and making a decision as to which fits the specimen better, repeating the process for another set of descriptions until only one taxon remains. Often, identification will begin by elimination, and proceed by matching when the range of possible taxa has been narrowed down to manageable proportions.

The identification process using the field guide relies on a combination of simple keys as the user scans the illustrations for a match and carefully compares what is known about the specimen in view or in hand with pertinent text and graphical information provided in the

guide. The keys help people focus their search in a section of the book in which the number of choices is relatively small. Plant guides are much more likely to use two or three different characteristics to help users narrow their scanning efforts (Stevenson *et al.*, 2003). The best guides are the ones that give references to similar species in each species account. Users can make direct comparisons with these to increase the confidence of a positive identification. Sometimes, one single taxon-specific character among all of those given is enough to identify the species (e.g. a leaf, a flower, a twig, a fruit, or a piece of bark for trees, etc.) (Stevenson *et al.*, 2003).

Given that identification is often an unconscious process, software tools should try to mimic the way we identify objects naturally and help reduce the user's frustration when the process becomes more explicit. Stevenson *et al*. (2003) suggested that software should: (1) provide training tools and games to let people become familiar with the "cast of characters" slowly, instead of being overwhelmed and confused by having to learn a lot of new things at once; (2) work to reduce the time necessary to identify a species by choosing likely possibilities from a line-up approach; and (3) suggest further queries that will aid in making the final positive identification. Field guides are a way for people to connect with the environment by putting a specific face on the term "biodiversity." The eco-informatics revolution is helping professional and amateur biologists take advantage of the rapid advances in digital technologies to share their knowledge about biodiversity with non-specialists. Non-specialists, in turn, through citizen science projects, are showing that their knowledge of species can be used to help monitor ecological changes as they relate to evolutionary dynamics and more pressing issues such as biodiversity loss, invasive species, and global climate change (Lubchencho *et al.*, 1991).

2.3.2 Field guides

There is no firm definition of what constitutes a field guide, normally they cover a particular taxon throughout its range or the breadth of taxa found in a particular geographic area, and they commonly presents taxonomic background, morphological descriptions, habitats, behaviour, ecology, distribution maps, uses, conservation notes and simple dichotomous keys suitable for field use, possibly annotated with line drawings, photographs or paintings.

This field guide for Grass pea and Chicklings (*Lathyrus* L.) includes all of these components, plus notes on the conservation status and threat assessment, and will include a taxon statement for each taxon, as follows:

Page A	Page B
	a. Accepted name
	b. Synonyms
	c. Description
Line drawing	d. Habitat
	e. Chromosome number
	f. Uses
	g. Geographical distribution
Photographs	

As well as the field guide component outlined above, this guide, in an attempt to specifically address the problem of non-expert identification, also this guide is accompanied by a CD with an interactive identification system, Lucid, and the *Lathyrus* data set, to help the non-expert identify *Lathyrus* taxa.

Lucid was developed specifically as a means of helping taxonomists communicate with non-specialists who wish to identify animals, plants and other organisms. Once constructed, the keys are freely available via the Lucid website (www.lucidcentral.com). Lucid key are accessed via the Lucid Player which provides the interface for users to load and interact with Lucid keys, using text, images, videos and sounds to help select those taxonomic, diagnostic or other features that best describe the particular case being

investigated. As the user selects character states, Lucid narrows down the particular options, such as specific taxa. When the user of a Lucid key is deciding which character states or symptoms best describe the particular specimen or problem of concern, multimedia material, such as line drawings, photographs, videos or sounds, can be used to help make the right decision. Once a specimen has been identified to a particular taxon the user is provided with a full range of multimedia fact sheets, sub-keys or links to websites for further information or recommendations.

Lucid keys can be built in various languages and use terminology familiar to the user, allowing the package to be used internationally and across a wide range of capabilities. Potential users range from biologists, geologists, agriculturists, veterinary and medical scientists to university and high-school students and the public at large. Details on operation of the Lucid key are provided on the accompanying CD.

2.4 Conservation status of *Lathyrus* L.

The genus *Lathyrus* L. is a member of the legume tribe ***Vicieae*** of the *Papilionoideae* along with *Vicia* L.; *Lens* Mill.; *Pisum* L. and *Vavilovia* A. Fedorov. The precise generic boundaries between these genera have been much debated which has led to an abundant and complex synonymy, but the oroboid species appear to form a bridge between *Lathyrus* and *Vicia* (Kupicha, 1981). *Lathyrus* is a large genus containing around 160 species ((Lewis *et al*., 2005; ILDIS, 2010), mainly located in Europe, Asia and North America, extending to temperate South America and tropical East Africa, but the genus has its centre of diversity primarily located in the Mediterranean and Irano-Turanian regions (Kupicha, 1981). It is adapted to temperate regions but can also be found at high altitudes in tropical Africa. Endemic species are present on all continents, except Australia and Antarctica.

Lathyrus species have been found in many different habitats, open, disturbed open habitats; such as field margins and roadsides, and in closed habitats such as woodlands and steps (Sarker *et al.,* 2001). The species considered more advanced are generally those found in the more disturbed, open communities (Sarker *et al.,* 2001). The cultivated species have mostly evolved from disturbed habitats; they were originally the wild and weedy floras of agricultural fields (Vavilov, 1926). Farming systems have therefore had a great influence on the recent evolution of the genus. Their weedy nature would explain the widespread distribution of many species (Sarker *et al.,* 2001).

The genus contains many restricted endemic species, for which only very few sites have been documented or which are bound by specific soil types and climatic regimes (Maxted and Goyder, 1988; Ehrman and Maxted, 1990; Maxted *et al.*, 1990; Maxted, 1993c; Maxted *et al.*, 1993e; Francis *et al.*, 1995; Bennett *et al.*, 1998). The ecogeographic distribution of all but a few *Lathyrus* species is poorly understood, particularly those in section *Notolathyrus* that are endemic to South America. There is a need for a detailed ecogeographic study of the whole genus if it is to be effectively and efficiently conserved and utilized (Sarker *et al.* 2001).

L. sativus L., *L. cicera* L. and *L. ochrus* (L.) DC. provide important human food, animal feed and fodder sources. *L. sativus* is widely cultivated for human consumption, as well as for fodder and green manure. Its primary centres of cultivation are in Southern Asia, particularly in Bangladesh, China, India, Nepal, and Pakistan, and Ethiopia (Asthana, 1996), with more limited production in southern Europe and West Asia. It is an important low risk aversion crop because it has relatively good tolerance to water-logging (in the case of flooding), good

ability to grow on residual moisture after the end of the rains or in drought, and because it requires low production costs (Tadesse *et al*., 1997).

An ecogeographic survey was undertaken to draw up draft conservation strategies for the genus, both *in situ* and *ex situ*. The survey revealed that conservation efforts need to be focused on *L. sativus, L. cicera and L. ochrus* and other species over the whole of their native distribution (GCDT, 2009).

2.4.1 Global *ex situ* conservation

Relatively large *ex situ* seed collections exist of cultivated and wild *Lathyrus* species. However, the collections are not comprehensive in terms of species diversity and there remain numerous gaps in conserved materials, particularly for the South America species and those species of less immediate utilization potential. The most diverse range of species has been collected by Maxted and co-workers, in conjunction with Bioversity Internatioanl (Former IBPGR) and ICARDA, in addition, with Centre for Legumes in Mediterranean Agriculture (CLIMA), Australia, which have engaged in 17 forage legume collection missions to the North Eastern Mediterranean region since 1986. The Genetic Resource Section (GRS) of ICARDA has made four collection missions with national collaborators in 1981, 1989, 1990 and 1993. All of the material collected by Maxted and co-workers and the GRU is held in the national genebanks of the country of collection, as well as being duplicated at the ICARDA genebank. Each of these collecting expeditions reported varying levels of genetic erosion occurred in the genus, especially in species such as *L. ochrus*, *L. gorgoni* and *L. cicera.* Detailed information about the current conservation status of *Lathyrus* species is documented by WIEWS (World Information and Early Warning System on Plant Genetic Resources), which contains information on national PGR holdings

(www.fao.org/ag/agp/pgr/wiews/) and SINGER (System-wide Information Network for Genetic Resources), which contains information on CGIAR holdings (http://www.cgiar.org/singer).

Turkey has the richest diversity of *Lathyrus* species genetic diversity. Davis (1970) reported the presence of 58 species in Turkey, some of them endemic at local or regional level and many of these are held in the gene bank of the national program. Prior to 1987, Turkish collection missions were targeted on forage grasses, and legume genetic resources and *Lathyrus* species were not given a high priority. Targeted expedition were launched specifically to collect forage legumes in 1987, 1988, 1995, 1996 and 1997 from nine different agricultural regions (Sabanci, 1996) and this material is held at the Aegean Agricultural Research Institute in Menemen and the majority of accessions are duplicated at ICARDA. The number of species collected is over half of those found by Davis, including a new species, *L. belinensis* Maxted and Goyder, which is closely related to *L. odoratus*, first discovered during the 1987 mission. These expeditions focused on collecting material from areas of Turkey with a Mediterranean climate (Aegean and Southern Turkey) and they did not attempt to systematically collect representative collections from throughout the country. They also concentrated at the lower altitudes favored by annual species. Therefore, some endemic species, particularly perennial species, were not encountered and are not currently conserved. Undoubtedly, the environment in Turkey is being changed rapidly by human intervention through building dams, constructing recreational areas along the coast and overgrazing (Tan, 1998). The flora is obviously suffering genetic erosion as a result and there is a need to give priority to the collection of *Lathyrus* germplasm from throughout the country, particularly in the under-collected areas of the North, central and South East.

Table 2.3 lists the main collections of *Lathyrus* around the world, together with an indication of the composition of each collection in terms on the percentages of accessions of wild relatives, landraces and breeding materials (breeders advanced lines etc.), as well as the percentage of the collection that originated in the country concerned (GCDT, 2009).

Table 2.3. Major *Lathyrus* collections in the world (data collected from 2005 and 2007)

No	Country	Genebank / institutes	TOTAL No of *acces.*	Wild relatives	Land races	Breeding material	Origin collected in country
1	GLOBAL	ICARDA	3327	45%	54%	0.1%	17%
2	France	Université de Pau, IBEAS	4477	n.a.	n.a.	n.a.	34%
3	India	NBPGR	2619	3%	85%	12%	94%5
4	Bangladesh ***	GRC Bangladesh Agric. Res. Inst.	1841	-	100%	-	100%
5	Chile	Centro Reg. de Inv. Carillanca	1424	n.a.	n.a.	n.a.	n.a.
6	Australia ***	Australian Temp. Field Crops Coll.	986	28%	39%	19%	0.6%
7	Russia ***	VIR	848	43%	30%	18%	40%
8	Canada	PGRC, Canada	840	10%	90%	-	n.a.
9	USA	Western Regional Plant Introduction Station, USDA, Pullman, Washington	669	n.a.	n.a.	n.a.	7%
10	Ethiopia ***	BCRI	588	2%	75%	25%	98%
11	Germany***	IPK	568	40%	n.a.	n.a.	5%
12	Spain ***	Fernando Franco Jubete	543	n.a.	n.a.	n.a.	n.a.
13	Algeria	Institute National Agronomique	437	n.a.	n.a.	n.a.	n.a.
14	Hungary ***	Research Centre for Agrobotany	394	1%	22%	n.a.	22%
15	Spain ***	INIA	377	14%	86%	-	100%
16	Bulgaria***	Institute for PGR "K.Malkov"	368	1.6%	80%.	n.a.	5.4%
17	Turkey	AARI	363	94%	n.a.	n.a.	100%
18	Nepal***	Nepal Agricultural Research Council	164	-	100%	-	100%
19	Armenia ***	Institute of Botany, National Academy of Sciences of Armenia	157	98%.	1%	1%	99%
20	Pakistan	Plant genetic Resources Institute	130	n.a.	n.a.	n.a.	n.a.
21	Portugal***	Genebank,, Braga	199	5%.	30%	n.a.	45%
22	China	CAAS	80	n.a.	n.a.	n.a.	100%

23	Azerbaijan ***	Genetic Resource Institute, National Academy of Science	66	47%	33%.	20%.	100%.
24	Czech Republic ***	Research Institute of Crop Production	52	75%	-	25%	56%.
25	Greece ***	Greek Genebank, Agricultural Center of Mecedonia and Thrace	47	-	2%	98%	100%
26	Slovakia ***	Research Institute of Plant Production	47	-	87%	13%	87%.
27	Cyprus ***	Agricultural Research Institute	31	-	100%	-	100%
28	Poland ***	PGR Laboratory, Research Institute of Vegetable Crops	10	-	-	100%	30%.
TOTAL			**21652**				

n.a. Detailed information not available

* From ECPGR/Pau database

** From EURISCO database

*** From accession-level data sent to ICARDA in April 2007

University of Pau in France with 4,477 accessions and ICARDA in Syria with 3,327 accessions hold the largest collections, with the Indian, Bangladeshi and Russian collections coming next with 2,619, 2,432 and 1,835 accessions respectively. Nearly all (98%) of the material held by NBPGR in India and in Ethiopia national genebank, and 100% of the accessions held in Portugal, Turkey and Nepal are indigenous material.

Table 2.4 gives a breakdown of the collections in terms of species richness. The two largest collections (Pau, France and ICARDA) both comprise about 50% *L. sativus.* The collections in the main grass pea producing countries all have high percentages of *L. sativus*: those in Bangladesh, Ethiopia, India and Nepal all comprise at least 70% *L. sativus*.

Table 2.4. Number of accessions for the three major *Lathyrus* species

Country	Genebank / institutes	No of acc. *L. sativus*	No of acc. *L. ochrus*	No of acc. *L. cicera*	Total No of acc. All *Lathyrus*
GLOBAL	ICARDA	1756	137	208	3327
France	Université de Pau, IBEAS	2382	0	789	4477
India	NBPGR	2561	0	1	2619
Bangladesh ***	GRC Bangladesh Agric. Res. Inst.	1841	0	0	1841
Russia ***	VIR	632	21	195	848
Canada	PGRC, Canda	781	0	0	840
Chile	Centro Reg. de Inv. Carillanca				1424
Australia***	Aus. Temp. Field Crops Coll.	592	51	302	985
USA	Western Regional Plant Introduction Station, USDA	242	25	33	669
Ethiopia***	BCRI	435	151	0	588
Spain *	Fernando Franco Jubete	108	0	328	543
Germany***	IPK	254	48	266	568
Algeria	Institute National Agronomique	10	0	16	437
Hungary***	Research Centre for Agrobotany	296	3	58	394
Spain***	INIA	157	7	179	377
Bulgaria***	Institute for PGR "K.Malkov"	213	38	44	368
Turkey	AARI	22	0	35	363
Greece *	Agricultural Center of Mecedonia and Thrace	208	0	112	320
Portugal	Genebank, Braga	136	0	116	256
Nepal***	Nepal Agricultural Research Council	164	0	0	164
Pakistan	Plant genetic Resources Institute	11	0	0	130
Armenia ***	Institute of Botany, National Academy of Sciences	3	0	154	157
China	CAAS				80
Czech Republic	Research Institute of Crop Production	3	0	0	52
Slovakia **	Research Institute of Plant Production	47	0	0	47
Cyprus *	Agricultural Research Institute	44	0	0	44

Azerbaijan ***	Genetic Resource Institute, National Academy of Science	29	0	37	66
Cyprus **	Agricultural Research Institute	19	12	0	31
Poland **	Plant genetic Resource Laboratory, Research Institute of Vegetable Crops	16	0	0	16
TOTAL					**21652**

* From ECPGR/Pau database

** From EURISCO database

*** From accession-level data sent to ICARDA in April 2007

The analysis of the status of *Lathyrus* collections showed that many collections have high regeneration needs which in some cases may be urgent. Substantial arrangements are needed to ensure a safety duplication of genetic resources of some rich and unique collections, ideally in a genebank in a second country or at ICARDA genebank. Genebanks are encouraged to send copies of their holding for long-term conservation at Svalbard Seed Vault, Norway. Most collections have passport data well documented, but only a small portion of the collections are characterized and evaluated and few databases are accessible via internet. During the workshop held by the Trust and ICARDA in Aleppo, April, 2007. It was proposed that Bioversity International and ICARDA take a joint lead on the global management of information and databases on *Lathyrus*, with Bioversity continuing to concentrate on building up the South Asia regional database, and for ICARDA to tie this in with its own database and those of other collections (reference and others) in the WANA region and elsewhere, so as to develop a crop registry as the central feature of truly integrated global information system for *Lathyrus*.

Based on all accumulated information, important gaps were identified in the coverage of global genetic diversity in existing collections (Table 2.5). But a more detailed analysis are needed on the conservation status, species richness coverage, geographic coverage, arrangements made for regeneration. The efforts of collection, conservation and characterization should be extended to the associated *Rhizobium* strains.

Table 2.5. Possible gaps in global *Lathyrus ex situ* conservation (GCDT, 2008)

Country	*L. sativus*	*L. cicera*	*L. ochrus*
Egypt	+	+	
Iraq	+	+	
Iran	+	+	
Tunisia		+	+
Greece			+
Turkey			+
Russia	Black Sea Coast and Volga-Kama region		
Iraq	Kurdish area		
Bangladesh	Syleth area (high altitude)		
India	Northeast and Eastern parts		
Ethiopia	High altitude areas, recently opened area by roads.		
Afghanistan	Northeast and Central part		
Spain	Almeria (Andalucía) and Murcia		

Blank: No gaps identified in the collections
\+ : Gaps identified

2.4.2 ICARDA genebank holdings of *Lathyrus*

ICARDA has the second largest collection of *Lathyrus* germplasm for the Mediterranean region after Pau-France. At present ICARDA is concerned with collection and conservation for *Lathyrus* species in the Mediterranean region and other *Lathyrus*-growing areas of the world. ICARDA holds in-trust 3327 *Lathyrus* germplasm from 50 countries under the auspices of the International Treaty on Plant Genetic Resources for Food and

Agriculture of the United Nations Food and Agriculture Organization (FAO) (Table 2.6). While the emphasis at ICARDA for genetic resources and improvement of *Lathyrus* is for three species (*L. sativus*, *L. cicera* and *L. ochrus*), a sizeable collection of 50 other species is being maintained (Table 2.6). The majority of accessions of all species of *Lathyrus* held in the ICARDA genebank, except *L. sativus*, are from the West Asia and North African region. The collections have been collected from cultivated or from naturally occurring populations, found mostly in disturbed habitats such as roadsides, crop fields and orchards. The *L. sativus* accessions in the ICARDA collection are from Ethiopia and the Indian sub-continent and are local landraces. Besides, expeditions within the Mediterranean, expeditions from ICARDA have been made to Bangladesh, Ethiopia, India, Nepal and Pakistan, primarily to collect genetic resources of *L. sativus*.

Table 2.6. ICARDA holdings of *Lathyrus* species in *ex-situ* conservation by origin

Country of Origin	Number of species	Number of priority species*	Accessions of priority species*/Total number of accessions
Afghanistan	2	0	0/25
Algeria	9	6	25/32
Armenia	11	5	25/52
Australia	3	2	2/3
Azerbaijan	13	4	20/57
Bangladesh	3	2	2/1114
Bulgaria	2	0	0/19
Canada	1	0	0/5
Cyprus	3	2	25/46
Czech Republic	2	1	1/3
Denmark	1	1	1/1

Ecuador	2	1	1/2
Egypt	2	1	1/2
Ethiopia	1	0	0/176
France	4	1	1/4
Georgia	8	2	8/27
Germany	7	3	3/11
Greece	7	4	78/110
Hungary	1	0	0/5
India	3	2	3/10
Iran	6	3	3/22
Iraq	5	4	6/9
Italy	3	3	6/6
Jordan	9	5	21/39
Kazakhstan	1	0	0/1
Lebanon	8	6	14/23
Moldova, Republic of	2	1	1/2
Morocco	10	5	50/148
Nepal	2	0	0/86
Norway	1	0	0/1
Pakistan	5	2	2/89
Palestine	3	2	3/6
Portugal	5	3	16/28
Russian Federation	4	3	4/69
Slovakia	1	0	0/9
Spain	5	4	7/9

Switzerland	1	0	0/1
Syrian Arab Republic	27	16	327/560
Tajikistan	7	2	2/36
Tunisia	6	4	32/38
Turkey	21	13	170/364
Turkmenistan	7	3	9/25
Ukraine	1	0	0/32
United Kingdom	1	1	1/1
United States of America	1	0	0/1
Unknown	4	4	7/7
Uruguay	1	0	0/1
Uzbekistan	3	1	1/12
Total			**878/3327**

* Priority species which included only those species in crop gene pool GP1B and GP2 or taxon groups TG1b and TG2 (Maxted *et al.*, 2009)

The majority of accessions of all species of *Lathyrus* held in the ICARDA genebank, except *L. sativus*, are from the West Asian and North African as well as Central Asian regions (CWANA). ICARDA focused on three species of *Lathyrus* (*L. sativus*, *L. cicera* and *L. ochrus*) but holds also around 50 other species (Table 2.7).

Table 2.7. ICARDA's holdings by *Lathyrus* species

Taxa_name	Countries represented	Number of accessions
Lathyrus amphicarpos	2	3
Lathyrus annuus	11	79
Lathyrus aphaca	18	304

Lathyrus articulatus	9	101
Lathyrus basalticus	1	5
Lathyrus belinensis	1	1
Lathyrus blepharicarpus	6	47
Lathyrus cassius	4	11
Lathyrus chloranthus	3	8
Lathyrus chrysanthus	1	7
Lathyrus cicera	24	208
Lathyrus cilicicus	1	6
Lathyrus ciliolatus	1	2
Lathyrus clymenum	7	17
Lathyrus cyaneus	1	2
Lathyrus digitatus	1	1
Lathyrus gloeospermus	1	2
Lathyrus gorgoni	5	67
Lathyrus hirticarpus	1	1
Lathyrus hierosolymitanus	4	112
Lathyrus hirsutus	9	43
Lathyrus inconspicuus	16	191
Lathyrus laxiflorus	2	2
Lathyrus marmoratus	5	28
Lathyrus nissolia	7	15
Lathyrus occidentalis	1	1
Lathyrus ochrus	16	137

Lathyrus odoratus	2	4
Lathyrus pallescens	1	1
Lathyrus pseudocicera	7	77
Lathyrus rotundifolius subsp. *miniatus*	1	2
Lathyrus sativus	33	1756
Lathyrus setifolius	4	8
Lathyrus sp.	8	30
Lathyrus sphaericus	7	27
Lathyrus stenophyllus	1	2
Lathyrus sylvestris	1	1
Lathyrus tingitanus	7	13
Lathyrus tuberosus	2	2
Lathyrus vinealis	1	4
Total		**3327**

Around 60 % of these accessions are georeferenced, 65% are characterized and about 30% are evaluated for at least ß-ODAP content. Some accessions are characterized using DNA molecular techniques.

2.4.3 Global *in situ*/on-farm conservation of *Lathyrus*

It is undoubtedly true that there is currently serious genetic erosion of *Lathyrus* diversity, particularly in the Mediterranean (IBPGR, 1985), largely as a result of intensification of agriculture, overgrazing, decline of permanent pastures and disappearance of sclerophyll evergreen trees, as well as maquis and garrigue shrubs vegetation in the

Mediterranean Basin. Many weedy *Lathyrus* species are associated with traditional farming systems which are also disappearing rapidly throughout the region. Most of the drylands of CWANA region are also subject to the adverse effects of climate change which is amplifying the loss of biodiversity. There has been, however, a systematic attempt to conserve *Lathyrus* diversity in the Eastern Mediterranean.

There has been a growing interest among genetic conservationists in the *in situ* conservation of plant genetic resources, because of the urgent need to protect natural and agro-ecosystems threatened with imminent change and the need to decrease the reliance of plant genetic resource conservationists on a single technique, seed conservation. *In-situ* conservation, whether in a genetic reserves or on-farm, has so far not been adopted for *Lathyrus* species, except for an initial attempt in Turkey (Ertug Firat and Tan, 1997) at Kaz Dag (Aegean Anatolia), Amanos, (Southern Turkey) and Ceylan Pínner (in Southeast Turkey. Maxted (1995) proposed the establishment of sites for reserves for *Vicieae* species in Syria and Turkey, but these ideas have not yet been initiated. There is an urgent need to make positive steps to establish both reserves for the wild species of *Lathyrus* and on farm projects to conserve the ancient landraces of cultivated *Lathyrus* species (Sarker *et al*., 2001; Maxted *et al.*, 2010).

The GEF-ICARDA regional project on "Conservation and sustainable use of dryland agrobiodiversity" implemented in Jordan, Lebanon, Palestine and Syria during 199-2010 concluded that natural habitats in most of the monitoring areas surveyed are under severe threats by overgrazing and habitat destruction (Amri *et al*., 2005). Areas for *in situ* conservation of wild relatives of cereals and food legumes and species of forage legumes including *Lathyrus* were recommended to the four countries. In Syria, Qal'at Al Hosn and

Qual'at Sala Hadeen, initially it might be felt that these areas are too dominated by human intervention to be sustainable. However, that human intervention may be exactly the factor that aids sustainability of the target taxon. Both Qal'at Al Hosn and Qual'at Sala Hadeen are major tourist attractions in Syria. There would be scope to encourage ecotourism associated with a reserve near the castle and as many tourists visit the site anyway it could provide a sustainable source of income for the conservation project. A reserve site, like Mimas in Southern Syria, was selected because it contains abundant and hopefully genetically diverse populations of the target taxon. Therefore, the first step in formulating the management plan is to observe the anthropogenic, biotic and abiotic dynamics of the site, as was done by the "Conservation and Sustainable Use of Dryland Agrobiodiversity" project. It was and still is being surveyed so that the species present in the ecosystem are known, the ecological interactions within the reserve are understood, a clear conservation goal is decided and a means of management implementation agreed. Many annual *Lathyrus* species are weedy species of disturbed land. This by definition makes them very vulnerable to changes in human activity, such as changes in agricultural practice, increased or decreased stocking levels, application of herbicide. Therefore it is not a case of simply allowing the site to reach a climax community, as the majority of *Lathyrus* species are not found in climax communities. A detailed assessment of the current management regime was required and the application of the management plan has required extensive experimentation to ensure the most appropriate environment for the target taxa is supplied.

With the limited release and adoption of new varieties, it is believed that landraces are still widely used by farmers living under harsh conditions. This is supported by the results of farming systems surveys in Eritrea, Ethiopia and Bangladesh which indicated the limited use of inputs and mainly seeds of improved varieties. Primarily three candidate species for on-

farm conservation are grass pea (*L. sativus* L.), chickling-vetch (*L. cicera* L.) and Cyprus-vetch (*L. ochrus* (L.) DC.), which are socio-economically important as a human or animal feed or as a source of fodder. *L. sativus* is widely cultivated for human consumption, as well fodder and green manure, primarily in Bangladesh, China, India, Nepal, Pakistan and Ethiopia (Asthana, 1996). *L. cicera* is cultivated in Greece, Cyprus, Iran, Iraq, Jordan, Spain and Syria and *L. ochrus* in Cyprus, Greece, Syria and Turkey (Saxena *et al*., 1993). Traditional cultivation of *L. cicera* is disappearing rapidly in the Mediterranean Basin, but one area where cultivation is maintained is in the Djebel Al-Arab in Southern Syria. Coincidentally, this region has been designated as an area for active plant conservation by the General Commission for Scientific Agricultural Research, as part of their Global Environment Facility funded "Conservation and Sustainable Use of Dryland Agrobiodiversity" project and so the region is potentially open for the establishment of an on-farm project. Several other species within the genus are cultivated as ornamental species, particularly sweet pea (*L. odoratus* L.), everlasting pea (*L. latifolius* L.) and narrow leaf everlasting pea (*L. sylvestris* L.), but for these their genetic diversity is held either by commercial breeding companies or hobby specialist breeders. As such commercial breeding companies would normally conserve their breeding material using *ex situ* techniques (commonly using seed storage) or by amateurs in home gardens. Thus in this particular exemplar genus it is unlikely that ornamental *Lathyrus* species will be conserved on-farm.

2.4.4 Major conclusions of the *Lathyrus* L. global conservation strategy

Because of its inherent adaptation to harsh conditions and its importance as a survival food for some of the poorest people in the world and as a potential crop for adapting to climate change, yet recognizing the dangers that its excessive consumption can cause, grass

pea was listed in 1991 among the crops included in the multilateral system of access and benefit sharing under the International Treaty on Plant Genetic Resources for food and Agriculture (ITPGRFA). In 2005, the Global Crop Diversity Trust (GCDT) launched the development of regional and global conservation strategies for crops of global importance (species in Annex 1 of ITPGRFA). The Trust in collaboration with ICARDA developed the long-term conservation strategy for major food legumes including lentil, faba bean and *Lathyrus.* In the regional strategies, *Lathyrus* and other food legumes were given lower regional priorities compared to crops such as cereals (rice, wheat, maize, barley, sorghum etc.) and other staple crops such as banana, coconut, yam, potato, and cassava. Europe, West Asia, North Africa, South Asia and Eastern Africa recognized the crop as being of secondary importance with focus for use as forage. In South Asia it ranked 22nd of the top 24 highest priority crops and in Ethiopia 19th of the 21 highest priority crops. In the rest of the world it was ranked as being of only negligible or no priority at the regional level.

The process of development of *Lathyrus* conservation strategy included two consultation meetings in India (2005) and ICARDA (2007), a questionnaire sent to 36 genebanks to seek comprehensive information on the status of *Lathyrus* collections, in addition to reviewing additional sources of information such as:

- *Lathyrus* Genetic Resources in Asia, Proceedings of a Regional Workshop, Raipur, India, 1995, edited by R.K. Arora, P.N.Mathur, K.W. Riley and Y. Adham. IPGRI, 1996
- *Lathyrus* Genetic Resources Network, Proceedings of a IPGRI-ICARDA-ICAR Regional Working Group meeting, New Delhi, India, edited by P.N. Mathur, V. Ramanatha Rao and R.K. Arora. IPGRI, 1998.
- Grass pea. *Lathyrus sativus* L. by C. Campbell, IPK/IPGRI, 1997.

- *Lathyrus* Germplasm Collections Directory, complied by P.N. Mathur, A. Alercia and C. Jain, IPGRI, 2005
- The regional crop conservation strategies for Asia, West Asia and North Africa, Central Asia, and East Africa

Various databases and information sources available on the internet were also consulted including:

- The Consultative Group on International Agricultural Research (CGIAR) System-wide Information Network on Genetic Resources (SINGER) database: http://singer.grinfo.net/
- USDA – Genetic Resources Information Network (GRIN) database: http://www.ars-grin.gov/npgs/
- European PGR collection catalogue - EURISCO - http://eurisco.ecpgr.org/
- ECPGR: http://www.ecpgr.cgiar.org/databases/Crops/*Lathyrus*.htm
- FAO – World Information and Early Warning System on PGRFA (WIEWS): http://apps3.fao.org/wiews/wiews.jsp
- Bioversity International Directory of Germplasm Collections: http://www.bioversityinternational.org/Themes/Genebanks/Germplasm_Collection_Directory/index.asp
- Central Asia and Caucasus Regional Database (available on CD, contact at ICARDA: j.konopka@cgiar.org)

The final document included pertinent recommendations for more coordinated efforts for effective conservation and sustainable use of *Lathyrus* genetic resources through strengthening partnerships and networking among all genebanks conserving *Lathyrus*

collections. The needs for regeneration and for applying best practices are recommended for reliable conservation, documentation and utilization of important and threatened collections. Human and institutional capacity development needs are expressed. Due attention should also given to promoting *in situ*/on-farm conservation of *Lathyrus* landraces and wild relatives. The parties to the ITPGRFA and others are encouraged to facilitate the access to *Lathyrus* genetic resources under the terms of the multilateral system for access and benefit-sharing of the Treaty. However, clarification is needed about the coverage of the use of *Lathyrus* species other than as for forage breeding and research purposes. Proper documentation of all passport and characterization/evaluation information needs to be improved through development of *Lathyrus* registry to avoid duplicates and to ensure easy use of genetic resources.

2.5 Crop improvement of *Lathyrus* L.

Despite the advantages of adaptation to harsh conditions, relatively little research efforts have been directed to improve grass pea. Sporadic efforts on its improvement through genetic and agronomic manipulations were initiated in India, Canada, Bangladesh, Ethiopia and Nepal during the late seventies and by the International Center for Agricultural Research in the Dry Areas (ICARDA) in 1989. Under climate changes with serious concerns about sustainability of agricultural production and food security worldwide, interest in the underutilized crops such as grass pea has been renewed in many countries (Crino *et al*., 2004; Falco and Pardo, 2000; Grela *et al.,* 2010; Hanbury *et al.,* 1999; Mera *et al.,* 2000; Milczak *et al.,* 2001; Polignano *et al.,* 2009; Siddique *et al.,* 1996; Yang and Zhang, 2005; Vaz-Patto *et al.,* 2006).The limited breeding efforts around the world are focusing on three main pulse species which are grown and used for human consumption: *L. sativus, L. cicera*, *L. ochrus* and to a lesser extent *L. clymenum.* Their aim is to improve its yield, resistance to biotic and

abiotic stresses and, most importantly, to reduce the percentage, or ideally eliminate, of the neurotoxin from the seed. The most widely cultivated of these three species is *L. sativus* known as the poor person's insurance crop (Tadesse *et al.,* 1997). It is nutritionally on a pair with other grain legume species, containing up to 30% crud protein (which is high in lysine), about 60% carbohydrates and 0.6% fat (Hartman *et al.,* 1974). However, the seed may contain 0.1-2.5% of the water soluble non-protein amino acid ODAP (β-N-oxalyl-α, β diaminopropionic acid) or OAP (l-3-oxalylamino-2-amino propionic acid), neurotoxins which can cause lathyrism leading to crippling and paralysis of the lower limbs (Barrow *et al.*, 1974; Kaul and Combes, 1986). In order to reduce neurotoxin to a safe level for human consumption, several attempts have been made in the past to develop grass pea varieties with low ODAP (Abd-El-Moneim *et al.,* 2000; Addis and Narayan, 2000; Campbell *et al.,* 1994; Crino *et al.,* 2004; Hanbury *et al.,* 2000b; Mehta and Santha, 1996; Vaz-Patto *et al.,* 2006) in addition to low cost agronomic practices and post-harvest processing. The potential role of Belgium (Ghent University), Canada (Morden, Manitoba), India (Indira Gandhi Agricultural University, Raipur), and ICARDA in neurotoxin screening and breeding were especially recognized. At present, several grass pea breeding programs are involved in the development of very low or toxin-free *L. sativus* varieties (Malek *et al.*, 1996) with good indications of success. *L. cicera* and *L. ochrus* also have similar problems of neurotoxins, preliminary screening at ICARDA indicated that none of the *Lathyrus* species tested were ODAP free, although the ODAP content was very low in several lines; *L. cicera* had low mean β-ODAP content (0.16%) followed by *L. sativus* (0.48%) and *L. ochrus* (0.57%). On average, ODAP levels in *L. ochrus* and *L. sativus* were about four to five times higher than those in *L. cicera* (Sarker *et al.*, 2001), which makes it a priority for more active conservation. Several studies reported a wide range of variation for ODAP content: 0.04 to 0.76% ODAP content in a set

of 503 grass pea accessions procured from ICARDA (Hanbury *et al*., 1999); 0.128 to 0.872% ODAP content among 1,187 accessions (Pandey *et al*., 1997a). At ICARDA, 1128 of the cultivated *Lathyrus* species chosen from ICARDA holdings and screened for ODAP content and the preliminary results showed a range of 0.073 to 0.952%. Eleven accessions of *L. cicera* and two of *L. sativus* showed ODAP content lower than the critical level of 0.20 % (unpublished data, 2009). Multi-environment evaluation of grass pea germplasm at ICARDA indicated that Ethiopian germplasm has maximum variability for ODAP content. Germplasm from Ethiopia and Indian Subcontinent is generally high in ODAP (0.7-2.4%) as compared to 0.02-1.2% in germplasm from the Near East (Abd-El-Moneim *et al.,* 2000).

ICARDA has characterized more than 50% of the accessions for main descriptors (ICARDA-GRS database with more than 1,082 accessions belonging to 30 species evaluated for 21 descriptors and agronomic traits at ICARDA (Robertson and Abd-El-Moneim, 1997). A detailed catalogue on grass pea germplasm comprising characterization and evaluation information on 63 traits for 1,963 accessions has recently been published in India (Pandey *et al.,* 2008). A wide range of variability has been observed for all the traits of breeders' interest such as crop duration, plant height, pods per plant, seeds per pod, seed weight, biomass, seed yield, and ODAP content (0.067 to 0.712%). Starting 2009, all *Lathyrus* accessions planted in the field for regeneration, multiplication and characterization at ICARDA were evaluated for grain and dry forage yields.

ODAP content is a polygenic trait and is highly influenced by genotype, environment and their interactions (Dahiya and Jeswani, 1974; Hanbury *et al.,* 1999; Ramanujam *et al.,* 1980; Sharma *et al.,* 1997). Conventional breeding efforts have resulted in development of high yielding varieties with low ODAP. In India, Pusa 24 with 0.2% ODAP content (Dahiya

and Jeswani, 1974) and recently Prateek and Mahateora have been developed through hybridization. In Bangladesh, two varieties, BARI Khesari 1 and BARI Khesari 2 have been developed (Malek *et al.,* 1996). At ICARDA, several grass pea breeding lines with <0.1% ODAP concentration which led to the releases of Wasie variety in Ethiopia, and Ali-Bar cultivar has been in Kazakhstan. In Canada, a low ODAP (0.03%) line, LS 8246 has been released for fodder and feed purpose (Campbell and Briggs, 1987). However, maintenance of genetic purity and low ODAP content is rendered difficult in some grass pea varieties due to outcrossing by bees. Mutation breeding has been employed to create additional genetic variability for ODAP content in order to develop zero/low ODAP varieties (Lal *et al.,* 1986; Nerkar, 1972; 1976; 1989; Prasad and Das, 1980; Rybinski, 2003; Rybinski *et al.,* 2006; Swaminathan *et al.,* 1970; Talukdar, 2009). Two varieties of grass pea, namely Poltavskaya in the former USSR and Bina Khesari-1 in Bangladesh have been developed through mutation breeding using EMS (0.01%) and gamma rays (250 Gy), respectively. Somaclonal variation can also contribute to development of mutant with low ODAP (Malik *et al.,* 1993; Mehta, 1997; Mehta and Santha, 1996; Mehta *et al.,* 1994; Roy *et al.,* 1991; 1992; 1993; Santha and Mehta, 2001; van-Dorrestein *et al.,* 1998). At ICARDA, the existing protocols for explants culture have been used to create somaclonal variation (van Dorrestein *et al.,* 1998) have allowed the identification of lines with consistently <0.1% ODAP and high yield under multi-location evaluation (Abd-El-Moneim *et al.,* 2000). Some researchers have attempted to transform grass pea but without evidence for inheritance of transgene (Barna and Mehta, 1995; Datta, 1995; Mehta, 1997). More efforts are needed to exploit the genetic diversity existing within species of grass pea genepools.

The effects of ODAP can be reduced through supplementation grass pea flour with methionine rich cereals. As an alternative approach of enhancing methionine levels in grass

pea is through manipulating its biosynthesis by making the key enzyme aspartate kinase insensitive to feedback inhibition (Karchi *et al*., 1993). Agronomic practices such as the application of zinc sulphate can reduce ODAP concentration (Abd-El-Moneim *et al*., 2010).

Various food processing methods such as soaking in water, boiling or steeping have been used to reduce ODAP content in grass pea seeds but not sufficiently enough for safe consumption (Getahun *et al*., 2005; Tekle-Haimanot *et al*., 1993; Ganpathy and Dwivedi, 1961; Geda *et al*., 1995). Cooking and processing of grass pea have resulted in up to 40% reduction of ODAP (Padmajaprasad *et al*., 1997). Similarly, roasting of grains at 150^{o}C for 1 hour reduces ODAP content by 82%, while dry autoclaving of seeds for 30 min reduces ODAP content by 39% (Akalu *et al*., 1998; Rao *et al*., 1969). Fermentation has been found to reduce ODAP by 80-90% (Kuo *et al*., 1995) and degerming the cotyledons by 70% (Prakash *et al*., 1977).

In conclusion, grass pea and other important *Lathyrus* crops continue to provide the basis for the livelihoods of poor communities in some countries of South Asia and Sub-Saharan Africa and provide opportunities for diversification of cereal-based farming systems and for adapting to climate change. Genetic resources of both cultivated and wild species are needed for the genetic improvement using both conventional approaches and biotechnological tools, mainly to solve the problem of ODAP content. Therefore, global attention is needed to conserve the remaining diversity of the *Lathyrus* genus and this work is contributing to this.

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2.7 Appendices

Appendix 2.1. Classification of *Lathyrus* species according to Kupicha (1983) with more recently described species indicated with as asterisk

Section 1. *Orobus*

Old World members

L. davidii Hance
L. gmelinii Fritsch
L. krylovii C. Serg.
L. emodi Fritsch
L. vaniotii Leveille
L. aureus (Steven) Brandza
L. incurvus (Roth) Willd.
L. occidentalis (Fischer & Meyer) Fritsch
L. laevigatus (Waldst. & Kit.) Gren.
L. transsilvanicus (Sprengel) Reichb. f
L. humilis (Ser.) Sprengel
L. linifolius (Reichard) Bassler
L. dominianus Litv.
L. frolovii Rupr.
L. komarovii Ohwi
L. venetus (Miller) Wohlf.
L. alpestris (Waldst. & Kit.) Kit.
L. libani Fritsch
L. niger (L.) Bernh.
L. japonicus Willd.
L. pisiformis L.
L. palustris L.
L. wilosonii Craib
L. quinquenervius (Miq.) Litv.
L. vernus (L.) Bernh.
L. dielsianus Harms

New World members

L. arizonicus Britton
L. bijugatus T. White
L. brachycayx Rydb.
L. delnorticus C. Hitchc.
L. eucosmus Butters & St. John
L. polymorphus Nutt.
L. graminifolius (S. Watson) T. White
L. hitckcockianus Barneby & Reveal
L. holochlorus (Piper) C. Hitchc.
L. jepsonii E. Greene
L. littoralis (Nutt.) Endl.
L. nevadensis S. Watson
L. ochroleucus Hook. F.
L. parvifolius S. Watson
L. pauciflorus Fern
L. polyphyllus Nutt.
L. rigidus T. White
L. splendens Kellogg
L. sulphureus Brewer
L. torreyi A. Gray

L. tracyi Bradshaw
L. laetiflorus E. Greene
L. lanszwertii Kellogg
L. leucanthus Rydb.
L. venosus Mulhlenb.
L. vestitus Nutt.
L. whitei Kupicha
L. zionis Hitchc.

Section 2. *Lathyrostylis*

L. ledebouri Trautv.
L. pannonicus (Jackq.) Gracke
L. nivalis Hand.-Mazz.
L. atropatanus (Grossh.) Sirj.
L. tukhtensis Czeczott
L. variabilis (Boiss. & Kotschv) Celak
L. satdaghensis P.H. Davis
L. karsianus P.H. Davis
L. cyaneus (Steven) K. Koch
L. digitatus (M. Bieb.) Fiori
L. armenus (Boiss. & Huet) Celak
L. pallescens (M. Bieb.) K. Koch
L. pancicii (Jurrisic) *Adamovic*
L. brachypterus Celak
L. bauhinii Genty
L. filiformis (Lam.) Gay
L. spathulatus Celak
L. elongatus (Bornm.) Sirj.
L. cilicicus Hayek & Siehe
L. boissieri Sirj.

Section 3. *Lathyrus*

L. mulkak Lipsky
L. cirrhosus Ser.
L. grandiflorus Sibth. & Smith
L. rotundifolius Willd.
L. tuberosus L.
*L. pygmaeus** Gomblaut
L. sativus L.
L. amphicarpos L.
L. cicera L.
L. tingitanus L.
L. marmoratus Boiss. & Blanche
L. blepharicarpus Boiss.
L. ciliolatus Rech. F.
L. hirticarpus Mattatia & Heyn
L. chrysanthus Boiss.
L. trachycharpus (Boiss.) Boiss.
L. lycicus Boiss.
L. phaselitanus Huber-Mor & P.H. Davis
L. undulatus Boiss.
L. heterophyllus L.
L. latifolius L.
L. sylestris L.
L. stenophyllus Boiss. & Heldr.
L. tremolsianus Pau
L. annuus L.
L. hierosolymitanus Boiss.
L. cassius Boiss.
L. odoratus L.

L. basalticus Rech. F.
L. lentiformis Plitm.
L. gorgoni Parl.
L. pseudo-cicera Pampan.
*L. belinensis** N. Maxted & D.J. Goyder
L. hirsutus L.
L. chloranthus Boiss.

Section 4. *Orobon*

L. roseus Steven

Section 5. *Pratensis*

L. binatus Pancic
L. czeczottianus Bassler
L. hallersteinii Baumg.
L. laxiflorus (Desf.) Kuntze
L. layardii Ball ex Boiss.
L. pratensis L.

Section 6. *Aphaca*

L. aphaca L.
L. stenolobus Boiss.

Section 7. *Clymenum*

L. clymenum L.
L. gloeospermus Warb. & Eig
L. ochrus L.

Section 8. *Orobastrum*

L. setifolius L.

Section 9. *Viciopsis*

L. saxatilis (Vent.) Vis.

Section 10. *Linearicarpus*

L. angulatus L.
L. hygrophilus Taubert
L. inconspicuus L.
L. sphaericus Retz.
L. tauricola P.H. Davis
L. vinealis Bioss. & Noe.
L. woronowii Bornm.

Section 11. *Nissolia*

L. nissolia L.

Section 12. *Neurolobus*

L. neurolobus Boiss. & Heldr.

Section 13. *Notolathyrus*

L. berterianus Colla
L. cabrerianus Burkat
L. campestris Philippi
L. hasslerianus Burkat
L. hookeri G. Don
L. linearifolius Vogel
L. lomanus I.M. Johnston
L. longipes Philippi
L. macropus Gillies
L. macrostachys Vogel
L. magellanicus Lam.
L. multiceps D. Clos
L. nervosus Lam.
L. nigrivalvis Burkat
L. paraguayensis Hassler
L. paranensis Burkat
L. parodii Burkat
L. pubescens Hook. & Arn.
L. pusillus Elliott
L. subandinus Philippi
L. subulatus Lam.
L. tomentosus Lam.
L. tropicalandinus Burkat

Appendix 2.2. General Key to *Lathyrus* taxa of the Mediterranean Basin and Caucasus, Central and West Asia Regions

1(0) Annual 2

Biennial 22

Perennial..... 23

2(1) Stipule base hastate 3

Stipule base semi-hastate 4

Stipule base sagittate 7

Stipule base semi-sagittate 8

3(2) Leaf rachis ends in tendril; Plant slender; Leaflets reduced; Stipule broader than the leaflet; Style straight ….....……..…… *L. aphaca*

Leaf rachis ends in arista; Plant sturdy; Leaflets present; Stipule 1 mm wide;

Style twisted …….…….. *L. nissolia*

4(2) Leaflets paripinate ……..… *L. odoratus*

Leaflets pinnate ……..……5

5(4) Legume broadly-linear …… *L. clymenum*

Legume linear-sublanceolate …….. *L. pygmaeus*

Legume oblong-linear …….. 6

Legume oblong …….…….. *L. belinensis*

Legume broadly-oblong …….…….*L. basalticus*

6(5) Growth habit ascending; apex mucronate; Corolla cream; Legume valve hairy;

Stipule margin incised …….…….. *L. saxatilis*

Growth habit decumbent; apex obtuse; Corolla pink; Legume valve glandular-

verrucose; Stipule margin entire ……..*L. lycicus*

7(2) Corolla white; Legume valves hairy; Seed viscose ……..…….... *L. gloeosperma*

Corolla yellow; Legume valves not hairy; Seed smooth ...…… *L. stenolobus*

Corolla brick-red; Legume valves reticulate-nerved; Seed reticulate …….. *L. ciliolatus*

8(2) Calyx teeth equal ……...9

Calyx teeth unequal …… 12

9(8) Legume linear ……. 10

Legume oblong ……... 11

Legume broadly elliptic-oblong. …….. *L. blepharicarpus*

Legume narrowly oblong ……... *L. stenophyllus*

Legume elliptic-oblong ……. *L. trachycarpus*

10(9) Leaflet apex mucronate; Stipule 1-15 mm wide; Corolla yellow; Style twisted; Stem ridged …….. *L. tauricola*

Leaflet apex acute; Stipule 1 mm wide; Corolla pale-lavender; Style straight; Stem terete ……. *L. inconspicuus*

11(9) Calyx teeth straight; Style linear straight; Legume valves not hairy; Stipule lanceolate …….. *L. marmoratus*

Calyx teeth reflexed; Style spathulate twisted; Legume valves tuberculate; Stipule lanceolate-ovate ……..... *L. hirticarpus*

12(8) Plant sturdy ……..... *L. chrysanthus*

Plant slender to sturdy …….…… 13

Plant slender …….……... 14

13(12) Leaf rachis laminate; Growth habit decumbent; Upper legume suture narrowly-winged; Seed sub-globose …….. *L. ochrus*

Leaf rachis not laminate; Growth habit ascending; Legume upper suture keeled; Seed sphaerical …….…… *L. cassius*

14(12) Stem winged …….…….. 15

Stem terete …….. 19

15(14) Upper legume suture broadly winged ……..… *L. sativus*

Upper legume suture narrowly-winged ……...16

Upper legume suture narrow ……...17

Upper legume suture keeled …….. *L. gorgoni*

Upper legume suture canaliculated …… 18

16(15) Legume valves reticulate-nerved; Stipule glabrous ……..…….. *L. cicera*

Legume valves longitudinally-nerved; Stipule pubescent …… *L. pseudo-cicera*

17(15) Leaflets linear; apex acute; Stipule glabrous; 1-15 X as broad as stem; Style straight ……...……...……. *L. setifolius*

Leaflets linear-elliptic; apex shape obtuse; Stipule pubescent; 1-3 mm wide, Style twisted ……..…… …… *L. chloranthus*

18(15) Leaflets paripinnate; Stipule width 05-15 mm; Style linear; Seed coarsely-tuberculate …….. *L. annuus*

Leaflets pinnate; Stipule 0.5-5 mm wide; Style linear-spathulate; Seed ruminate-rugulose ……..... *L. hierosolymitanus*

19(14) Stipules subulate …… 20

Stipules lanceolate-subulate ……..…….21

20(19) Leaflets linear; Growth habit erect; Legume linear; Legume valve reticulate-nerved; Legume glabrous ……... *L. vinealis*

Leaflets linear-elliptic; Growth habit decumbent; Legume oblong-linear; Legume valves glandular-verrucose; Legume pilose ……..……..... *L. phaselitanus*

21(19) Leaf rachis ends in murco …….. *L. woronowii*

Leaf rachis ends in tendril …….. *L. sphaericus*

Leaf rachis ensd in aristate ..…….. *L. sphaericus*

22(1) Plants sturdy; Growth habit erect; Leaf rachis ends in Murco; Leaflets elliptic; apex shape obtuse ……..… *L. trachycarpus*

Plants slender; Growth habit decumbent; Leaf rachis ends in tendril; Leaflet linear-elliptic; apex shape mucronate ……...…… *L. hirsutus*

23(1) Calyx teeth equal …… 24

Calyx teeth unequal …… 27

24(23) Leaflets subdigitate; Style obovate-spathulate ……. *L. cilicicus*

Leaflets pinnate; Style linear ……..…… 25

25(24) Legume valves hairy ……..……. 26

Legume valves gland-dotted …… *L. laxiflorus*

26(25) Leaflets elliptic …...…….. *L. laxiflorus* subsp *laxiflorus*

Leaflets lanceolate ……..……*L. laxiflorus*

Leaflets ovate ……*L. laxiflorus* subsp *laxiflorus*

27(23) Leaf rachis laminate …… *L. pratensis*

Leaf rachis not laminate ……. 28

28(27) Calyx teeth straight …… 29

Calyx teeth reflexed …….. *L. libani*

29(28) Stem winged …….. 30

Stem terete …….. 32

Stem angled …… 47

30(29) Corolla purplish-pink ……. *L. sylvestris*

Corolla pink 31

Corolla purple ..... *L. palustris*

31(30) Leaflet apex obtuse; Plants sturdy; Style oblong; Upper legume suture keeled; Calyx not gibbous …….. *L. rotundifolius*

Leaflet apex undulate-margined; Plants lender; Style linear; Upper legume suture narrowly-winged; Calyx gibbous …… *L. undulatus*

32(29) Leaflets paripinate ...…….. 33

Leaflets subdigitate ...……...37

Leaflets pinnate ……...…….43

Leaflets sub-sessile ...…….*L. digitatus*

33(32) Leaflet apex mucronate …….. *L. pallescens*

Leaflet apex acute …… 34

Leaflet apex acuminate ……..*L. vernus*

Leaflet apex subobtuse ……*L. niger*

34(33) Legume valves not hairy ……...……..35

Legume valves reticulate-nerved ……...……...36

Legume valves gland-dotted …….. *L. venetus*

35(34) Stipule base sagittate; Vegetative parts pubescent..... *L. satdaghensis*

Stipule base semi-sagittate; Vegetative parts glabrous …….. *L. karsianus*

36(34) Stipules glabrous; Plants slender to sturdy; Style linear; Legume linear; Growth habit erect ……..…….. *L. brachypteras*

Stipules pubescent; Plants sturdy; Style linear-spathulate; Legume oblong-linear; Growth habit ascending …….……... *L. nivalis*

37(32) Plants slender to sturdy ……..…… 38

Plants slender ……...…… 39

38(37) Growth habit erect; leaflet apex acute; Stipule base semi-sagittate; Stipules as broad as stem ……..…….. *L. brachypteras*

Growth habit ascending; leaflets apex obtuse; Stipule base sagittate; Stipule 1 mm wide ……. ……..……..…… *L. variabilis*

39(37) Legume linear …….. 40

Legume linear-sub-lanceolate …….. *L. digitatus*

40(39) Style linear ……... 41

Style linear-spathulate …… *L. cyaneus*

Style spathulate ……... 42

41(40) Calyx teeth shorter than tube ……. *L. armenu*s

Calyx teeth equal the tube length. …… *L. cyaneus*

42(40) Stipules subulate ..……... *L. tukhtensis*

Stipules lanceolate-subulate …..……... *L. spathulatus*

Stipules lanceolate …..…… *L. tukhtensis*

43(32) Stipule base semi-hastate ……... *L. japonicus*

Stipule base sagittate 44

Stipule base semi-sagittate 45

44(43) Leaf rachis ends in murco; Leaflets lanceolate; Stipules lanceolate-accuminate; Legume broadly-linear; densely-pilose …….……..…….. *L. czeczottianus*

Leaf rachis ends in tendril; Leaflets elliptic-lanceolate; Stipules lanceolate-ovate; Legume oblong-linear; pilose ……. *L. layardii*

45(43) Stipules 1-3 mm wide …….. *L. tuberosus*

Stipule width 1-2 X as broad as stem ……..……...……...… *L. incurvus*

Stipules as broad as stem …….……... 46

Stipules broader than stem ……... *L. aureus*

46(45) Leaflet apex acute; Stipules lanceolate-subulate; Plants slender; Style straight; Legume valves not hairy …….. *L. cyaneus*

Leaflet apex subobtuse; Stipule lanceolate; Plants slender to sturdy; Style twisted; Legume valves reticulate-nerved …….. *L. roseus*

47(29) Leaflets subdigitate; Leaf rachis ends in murco; Leaflets linear-elliptic; Stipules lanceolate; Stipule base semi-sagittate …….. *L. boissieri*

Leaflets pinnate; Leaf rachis ends in tendril; Leaflets elliptic-lanceolate; Stipules lanceolate-ovate; Stipule base sagittate …….. *L. layardii*

CHAPTER THREE

CLARIFICATION OF INFRA-GENERIC *LATHYRUS* CLASSIFICATION USING MORPHOLOGIC CHARACTERS AND AFLP MARKERS.

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3.1 Abstract

Lathyrus species are important economical crops providing food and animal feed for poor communities living under harsh conditions of some Sub-Saharan Africa and South Asia countries. Further exploitation of *Lathyrus* genetic resources is needed to adapt to harsher environments and to lower levels of ODAP (β-N-oxalyl-α,β-diaminopropionic acid) neurotoxin present in *Lathyrus* species causing Lathyrism paralysis. Morphological homoplasy is rendering difficult the taxonomic classification of some *Lathyrus* species. In addition, the botanic classifications need to be supported by other means such as chromosome morphology and homeology, and molecular techniques to gain insights on the phylogenic relationships between species. Amplified Fragment Length Polymorphism (AFLPs), along with the morphologic characters were used to clarify the taxonomic and phylogenetic relationships within and between the sections and the species of the genus *Lathyrus*. A total of 184 accessions belonging to 38 predefined taxa belonging to nine predefined sections and

originating from the Mediterranean Basin and Caucasus, Central and West Asia Regions were evaluated using 47 morphologic characters and six AFLP polymorphic primers combinations. Distance-based and Bayesian-based methods and Principal Coordinate Analysis were used to cluster the accessions at species and sections levels. The results showed that the Sect. *Aphaca, Clymenum, Lathyrostylis* and large part of *Lathyrus* section could be differentiated either by using morphological characters or AFLP markers. In addition, the Sect. *Orobus, Pratensis* and *Orobastrum* could also be separated when using model-based clustering analysis. The Sect. *Linearicarpus* and *Nissolia* stayed grouped when applying different clustering methods to morphological characters and AFLP markers. Both morphological characters and polymorphic markers were able to assign efficiently the species and sub-species to their respective sections, however, morphological characters allowed the discrimination of all species compared to AFLP markers. But, some AFLP markers were unique and specific to individual sections and species. The used of STRUCTURE program improved further the classification of sections, but most importantly could highlight the genetic relationships among species. Further research is needed to expand on the use of DNA molecular markers in species identification and their assignment to different genepools of cultivated crops.

Keywords: *Lathyrus*, taxonomy, morphological characters, AFLP, clustering analysis.

3.2 Introduction

Lathyrus is a large genus containing around 170 species (ILDIS, 2010; Smartt, 1990), mainly located in Europe, Asia and North America, extending to temperate South America and tropical East Africa, but the genus has its centre of diversity primarily located in the Mediterranean and Irano-Turanian regions (Kupicha, 1981). Several *Lathyrus* species are cultivated for human consumption, animal feed, and fodder, as well as for ornamental

purposes in addition to their importance as soil nitrifiers and as dune stabilizers. Of these, *L. sativus* L., *L. cicera* L. and *L. ochrus* (L.) DC. provide important human food, animal feed and fodder sources. *L. clymenum* to a lesser extent and *L. tuberosus* occasionally are grown for human consumption (Hanelt, 1992; Kearney, 1993; Kearney and Smartt, 1995; Asthana, 1996).

The majority of the cultivated species are placed in *Lathyrus* Sect. *Lathyrus*, possibly explaining why this section has received more taxonomic interest. Grass pea, *Lathyrus sativus* L., the most widely cultivated *Lathyrus* species, because of its adaptation to harsh conditions, is a pulse crop contributing significantly to sustain the livelihoods of the poorest people mainly in South Asia and Sub-Saharan countries, despite the Lathyrism problem arising from the over-consumption of ODAP neurotoxin (Agrawal *et al*., 2011; GCDT, 2009). There is a potential for further exploitation of the *Lathyrus* gene pool particularly, to adapt to the adverse effects of climate change (Deulvot et al., 2010) and to reduce ODAP content of consumed species to safer levels (GCDT, 2009; Agrawal *et al*., 2011). Grass pea has been recognized as an important crop for which there is a high degree of international inter-dependence with respect to its genetic resources and as such included in the Annex 1 of the International Treaty on Plant Genetic Resources for food and Agriculture (ITPGFA, 2004).

The genus *Lathyrus* L. is a member of the legume tribe *Vicieae* of the *Papilionoideae* (*Vicia* L.; *Lens* Mill.; *Pisum* L. and *Vavilovia* A. Fedorov), with debated generic boundaries between these genera and complex synonymy, but the oroboid species appear to form a bridge between *Lathyrus* and *Vicia* (Kupicha, 1981). *Lathyrus* genus has undergone more than 20 major classifications since Linnaeus's work. The genus *Lathyrus* has the same size as genus *Vicia* and it is easier to identify, with more clear vegetative characters than *Vicia* (Kupicha, 1983). The morphology and taxonomy of *Lathyrus* have been studied by several

scientists (Bassler 1966, 1973 & 1981; Davis, 1970; Czefranova, 1971; Kupicha, 1983). Kupicha (1983) published her study "The infrageneric structure of *Lathyrus*" and showed that the Eurasian species have been classified in a broadly similar manner by all authors. The five groups *Clymenum*, *Aphaca*, *Nissolia*, *Cicercula* and *Lathyrus* (which, except the last, are composed entirely of annuals) are generally accepted, while the remaining species, mostly perennials, have been assigned to progressively smaller, more numerous and better-defined sections. Asmussen and Liston (1998) summarized the evolution of the taxonomic identification of *Lathyrus* genus based on morphological characters. Although the use of these characters has led to a great improvement in the infra-generic classification of *Lathyrus,* there is still a need to study this genus in more details and to solve several unresolved taxonomic issues (Kupicha, 1983). The classification proposed by Kupicha (1983) dividing the species into 13 sections has been generally accepted but does not clearly reflect the phylogenetic relationships among the sections and species, needed to elucidate further the genepools for cultivated species. Townsend and Guest (1974) reported that the primary gene pool of grass pea is poorly differentiated in terms of morphological characters, as there are no clear-cut differences between the cultivated and wild forms. Within *L. sativus* species, the white flowered, white seeded varieties are tentatively included in GP1A and the blue flowered and small speckled seeded forms are in GP1B, the latter is considered as primitive forms of cultivated grass pea (Jackson and Yunus; 1984; Smartt; 1984). GP2 includes: *L. chrysanthus*, *L. gorgoni*, *L. marmoratus* and *L. pseudocicera*, with which *L. sativus* can cross and produce ovules, and possibly more remotely *L. amphicarpos*, *L. blepharicarpus*, *L. chloranthus*, *L. cicera*, *L. hierosolymitanus* and *L. hirsutus*, with which *L. sativus* can cross and with which pods are formed (Sarker *et al.,* 2001).

Several other methods were used to study the phylogeny and relationships among different *Lathyrus* species including karyotype analysis (Murray *et al*., 1992; Battistin and Fernandez, 1994; Schifino-Wittmann, 2001), chromosome banding and *in situ* hybridization (Lavania and Sharma, 1980; Unal *et al*. 1995; Murray *et al*., 1992), and DNA content and sequencing (Narayan, 1991; Ceccarelli *et al*. 2010). The majority of *Lathyrus* species are diploid 2n=2x=14 chromosomes with some variation in karyotype, but the majority of chromosomes are sub-metacentric, indicating that some chromosome structural differentiation and translocations have occurred (Yunus, 1990; Campbell, 1997; Sarker *et al.* 2001). Klamt and Schifino-Wittmann (2000) showed that all *Lathyrus* species originating from Southern Brazil have conserved chromosome morphology and differed by as much as 20% in total complement DNA size which could result from changes in chromosome size during evolution. Ali *et al*. (2000) concluded that karyotype features reflect well the phylogenetic relationships among *Lathyrus* species belonging to different sections. Genetic diversity and taxonomic/phylogenetic relationships among grass pea and its close relatives in the Sect. *Lathyrus* were studied using inter-specific hybridizations (Yunus, 1990; Kearney, 1993). Yunus (1990) crossed 11 species in Sect. *Lathyrus* with *L. sativus*, and found that *L. cicera* and *L. amphicarpos* gave viable seeds. Other species formed pods but these did not form fully developed viable seeds. *L. cicera* is thought morphologically to be the closest relative of *L. sativus* (Yunus & Jackson, 1984). *L. cicera* and *L. amphicarpos* can be intercrossed and any other genetic transfer involving other species will have to be assisted by biotechnology tools (McCutchan *et al*., 1999; Durieu & Ochatt, 2000; Ochatt *et al*., 2001). It is possible to apply Harlan and De Wet's gene pool concept to this crossability information for *L. sativus* with cultivated and wild species of *L. sativus* included in the primary gene pool. The secondary gene pool included other biological species that will cross with some difficulty with the crop

species: *L. chrysanthus*, *L. gorgoni*, *L. marmoratus* and *L. pseudocicera*, with which *L. sativus* can cross and produce ovules, and possibly more remotely *L. amphicarpos*, *L. blepharicarpus*, *L. chloranthus*, *L. cicera*, *L. hierosolymitanus* and *L. hirsutus*, with which *L. sativus* can cross and with which pods are formed. The remaining species of the genus can be considered members of the tertiary gene pool (GP3) requiring the production of transgenic (Yunus, 1990; Sarker *et al.* 2001). The progenitor of *L. sativus* remains unknown, but several Mediterranean candidate species have been identified and they resemble the cultigen's morphologically, namely *L. cicera*, *L. marmoratus* Boiss., *L. blepharicarpus* Boiss. and *L. pseudocicera* Pampan.

Genetic diversity assessments of numerous crop species within *Lathyrus* have been conducted with DNA markers alone or in addition to morphological analyses (Noli *et al.*, 1997, Paul *et al.*, 1997; Yee *et al.*, 1999). A broad range of molecular techniques have been developed to study the genetic diversity, and species relationships, and to assist in selection during the breeding process (Haig, 1998; Brauner *et al.*, 1992; Nguyen and Wu, 2005). The use of DNA techniques has increased significantly, especially those techniques based on the polymerase chain reaction (PCR) such as Single Sequence Repeats (SSR), Random Amplified Polymorphic DNA (RAPD), Inter-simple Sequence Repeats Amplification (ISSR) and Amplified Fragment Length Polymorphism (AFLP), because they provide a large number of potentially polymorphic loci (Heun *et al.*, 1994). AFLP were used successfully to study the taxonomic relationship of *Vicia* species (van de Wouw *et al.,* 2001). The chloroplast genome (cpDNA) is often used to assess variation at the taxonomic level as it appeared highly conserved across species, while mitochondrial DNA (mtDNA) is particularly suited to intra-specific and population level studies (Karp *et al.*, 1996; Demesure *et al.* 1995). Isozymes (Ben Brahim *et al*., 2002), RFLPs (Chtourou-Ghorbel *et al*., 2001), RAPDs (Croft *et al*., 1999), chloroplast DNA restriction sites (Asmussen & Liston, 1998) and AFLPs (Bard *et al*.,

2002) have been used to study the interspecific diversity and phylogeny relationship of species of the genus *Lathyrus*. Different levels of diversity have been detected in the different species reflecting their different perenniality and breeding systems. Schifino-Wittmann (2001) used isozymes patterns on 18 accessions of five *Lathyrus* species allowed an unexpected grouping between *L. pubescens* and *L. sativus* and found that some bands were specific to some species. By using convicilin storage protein gene sequences, de Miera *et al*. (2008) showed that *L. sativus, L. annuus, L. cicera* and *L. tingitanus*, all belonging to Sect. *Lathyrus* formed a monophyletic group, while *L. latifolius* of the same section is included in the group formed by *L. clymenum* and *L. ochrus* of the Sect. *Clymenum*. Ceccarelli *et al*. (2010) used satellite DNA to show the close phylogenetic relationship between *L. sylvestris* and *L. latifolius* confirming the results of Asmussen et Liston (1998) using chloroplast DNA. Chtourou-Ghorbel *et al*. (2001) concluded that RAPDs are equivalent to RFLPs in assessing the genetic diversity of five *Lathyrus* species belonging to the Sect. *Lathyrus* and *Clymenum*, in addition to their simplicity and low costs. Asmussen and Liston (1998) conducted the largest molecular investigation of *Lathyrus* to date which allowed a review of the classification proposed by Kupicha (1983). Kenicer *et al.* (2005) used nuclear ribosomal and chloroplast DNA to study the systematics and biogeography of 53 *Lathyrus* species. The results supported generally the recent classification based on morphologic characters, resolved the clades between *Lathyrus* and *Lathyrostylis* sections, but questioned the monophyly of the Sect. *Orobus sensu* (Kupicha, 1983). These studies have also brought some suggestions of the geographic origin of different species. The molecular diversity analysis supported the close phylogenetic proximity between *L. sativus* and *L. cicera* based previously on morphological and hybridization studies (Kupicha, 1983; Jackson & Yunus, 1984; Yunus *et al*., 1991).

The present study aims at examining the genetic diversity and relationships of *Lathyrus* species at the section and species levels based on morphological characters and using AFLP molecular markers and at assessing the pertinence of the different clustering approaches.

3.3 Material and Methods

3.3.1 Choice of materials:

A total of 184 accessions of 38 taxa belonging to 9 sections from genus *Lathyrus* and originated from different countries around the Mediterranean Basin and Central and West Asia and North Africa region (CWANA) and Denmark were used in this study. The numbers of accessions per taxa and their geographic distribution are indicated in Table 3.1 and Figure 3. 1. The accessions used were obtained from the ICARDA genebank. Accessions of priority species were chosen to represent the geographic distribution of the genus, and based on the number of accessions available at the ICARDA genebank (details of accessions in Appendix 1).

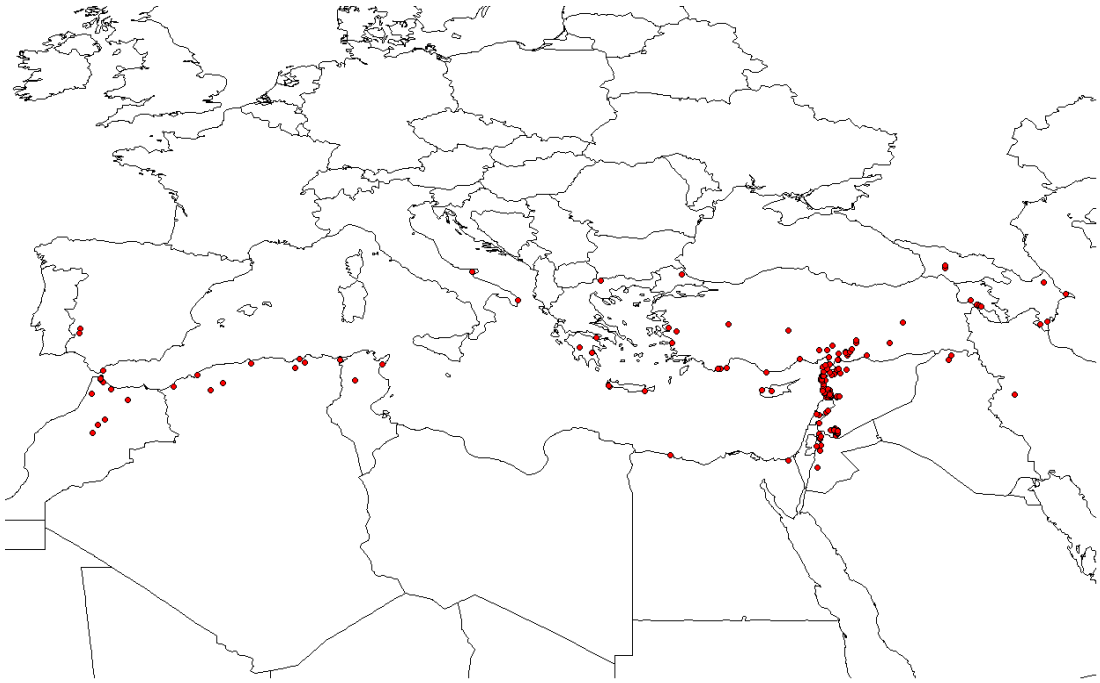

Figure 3.1. Geographic distribution of the *Lathyrus* accessions used in the study.

Table 3.1. Number of accessions for different species and sections of *Lathyrus* used in the study and their countries of origin.

Section	Species	Number of accessions	Country of origin
Aphaca	*L. aphaca*	6	Algeria (1), Greece (1), Jordan (2), Morocco (1) and Syria (1)
	L. aphaca var. *aphaca*	5	Jordan (1) and Syria (4).
	L. aphaca var. *affinis*	6	Algeria (1), Jordan (1), Syria (3) and Turkey (1)
	L. aphaca var. *bifloras*	4	Syria (4)
	L. aphaca var. *floribundus*	4	Syria (2) and Turkey (2)
	L. aphaca var. *modestus*	5	Syria (4) and Turkey (1)
	L. aphaca var. *pseudoaphca*	2	Turkey (2)
Clymenum	*L. clymenum* var. *articulatus*	5	France (1), Greece (1) and Morocco (3)
	L. gleospermus	5	Syria (5)
	L. ochrus	7	Algeria (1), Cyprus (1), Greece (1), Italy (1), Morocco (1), Spain (1), and Syria (1)

Lathyrostylis	*L. cilicicus*	2	Syria (2)
	L. cyaneus	2	Azerbaijan (2)
	L. digitatus	2	Syria (2)
	L. pallescens	1	Turkey (1)
	L. tingitanus	3	Algeria (2) and Tunisia (1)
Lathyrus	*L. amphicarpos*	2	Syria (1) and Unknown (1)
	L. annuus	8	Algeria (1), Palestine (1), Spain (1), Syria (4) and Turkey (1)
	L. basalticus	3	Syria (3)
	L. belinensis	1	Turkey (1)
	L. blepharicarpus	5	Jordan (1), Lebanon (1), Syria (1) and Turkey (2)
	L. cassius	7	Greece (1), Iraq (1), Syria (3) and Turkey (2)
	L. chloranthus	4	Armenia (3) and Iran (1)
	L. chrysanthus	5	Syria (5)
	L. cicera	12	Algeria (1), Greece (3), Jordan (1), Syria (5), Turkey (1) and Unknown (1)
	L. ciliolatus	2	Syria (2)
	L. gorgoni	6	Jordan (1), Lebanon (1), Syria (1) and Turkey (3)
	L. hierosolymitanus	10	Jordan (1), Lebanon (1), Syria (5) and Turkey (3)
	L. hirsutus	6	Azerbaijan (2), Georgia (1), Tunisia (2) and Turkey (1)
	L. marmoratus	8	Egypt (1), Iraq (1), Syria (3), and Turkey (3)
	L. odoratus	2	Italy (1) and Unknown (1)
	L. pseudo-cicera	4	Jordan (1), Syria (1) and Turkey (2)
	L. rotundifolius	2	Armenia (2)
	L. sativus	9	Algeria (1), Cyprus (1), Egypt (1), France (1), Greece (1), Morocco (3) and Turkey (1)
	L. stenophyllus	2	Turkey (2)

	L. sylvestris	1	Denmark (1)
	L. tuberosus	1	Tajikistan (1)
Linearicarpus	*L. inconspicuus*	5	Algeria (1), Syria (2) and Turkey (2)
	L. sphaericus	2	Syria (1) and Turkey (1)
	L. vinealis	4	Turkey (4)
Nissolia	*L. nissolia*	2	Syria (2)
Orobastrum	*L. setifolius*	2	Syria (1) and Turkey (1)
Orobus	*L. occidentalis*	1	Syria (1)
Pratense	*L. laxiflorus* subsp. *laxiflorus*	1	Georgia (1)

3.3.2 Morphological characters

Ten seeds of each accession were germinated in Jiffy-seven pots in the plastic house and after emergence and good establishment the plants were transplanted to the field at ICARDA station in Tel Hadya, Syria. The taxa were identified using accepted species recognized by Davis (1970) and Kupicha (1983) and more recent described species (e.g. *L. belinensis*). Characterization of some taxa which did not produce reproductive parts (pods or seeds) at Tel Hadya (*L. cilicicus, L. cyaneus, L. digitatus, L. pallescens, L. tuberosus, L. vinealis, L. setifolius, L. occidentalis* and *L. laxiflorus*) were done using the herbarium specimens to score the missing characters. A set of 75 characters was selected for this study. The characters used were those previously used by Davis (1970), Kupicha (1988) and Maxted (1990). Additional characters of species not studied by previous taxonomists were included, according to the observed variations in the field (Appendix 2). Vegetative characters of stem and leaflets were measured at the stage of production of the first node which bears a flower or a pod. Leaflets were also measured at the median part of the stem to differentiate the leaflet

shape in the production stage. Pod and seed characterization were made after collecting the matured pods. Observations were made for the characters in different stages of the growing. The colour of flowers, pods and seeds was assessed using Munsell colour charts (Munsell, 1977), which gives reliable description of the colour and uses a scale starting with 1 and having an increment of 1 for each additional class. For this study, only 47 qualitative characters with a total of 174 characters states were used for the morphological classification of species and were used also to check their relevance in differentiating the sections.

3.3.3 Molecular techniques

Five seeds of each accession of the same set of 184 accessions were planted in Jiffy-seven pots in the plastic house. Total DNA was extracted according to the CTAB-method, described by Rogers *et al.* (1985). Fresh leaf material from seedlings was frozen in liquid nitrogen and grounded into a fine powder that was subsequently paced in a 50 ml centrifuge tube with 20 ml pre-warmed 2× CTAB buffer (2 % CTAB, 0.1 M Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA). The suspension was mixed and incubated at 65°C for 30 minutes (min). After the suspension was cooled at room temperature (RT) for 5 min, 14 ml chloroform-isoamyl alcohol (24:1) were added to the tube and the suspension was gently mixed via shaking for 10 min. Centrifugation was performed at 4500 rpm (Beckmann YA-12) for 20 min at RT and the supernatant was transferred to a new tube. After 30 min of RNase (10 µl, 10 mg/ml) treatment, DNA was precipitated with 15-20 ml cold isopropanol. The DNA was transferred into a micro-centrifuge tube and washed twice with a washing buffer (75 % ethanol and 200 mM sodium acetate) for 20 min. After air-drying for about 10 to 20 min, DNA was dissolved in 500 µl of 1× TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

AFLP analysis was performed following the method of Vos *et al*. (1995), consisting of four steps: digestion, ligation, pre-amplification, and finally selective amplification with

three-nucleotide extensions. More than 17 primer combinations were tested and six most polymorphic were used in this study.

I. Digestion of DNA

Digestion of genomic DNA was performed as follows:

Reagent for one reaction (µl)

DNA (50 ng/µl) 10.0 buffer 10× (OPA) 4.0 *Mse*I (10 U/µl) 0.5 *Pst*I (10 U/µl) 0.5 sd H_2O to 40.0 incubated at 37°C for 3 hours.

Ligation of adapters to digested DNA

The ligation reaction was carried out as follows:

Reagent for one reaction (µl)

*Mse*I adapter (50 pmol/µl) 1 *Pst*I adapter (5 pmol/µl) 1 ATP (10 mM) 1 Buffer 10× (OPA) 1 T_4-DNA ligase (1 U/µl) 1 sd H_2O to 10.

This mixture with the digested DNA was incubated for 24 hours at 37°C and then diluted 1:5 using sterilized distilled water (sd H2O) and then stored at −20°C.

AFLP adapters consist of a core sequence and an enzyme-specific sequence.

Adapters

***Mse*I** F**:** 5'-GACGATGAGTCCTGAG-3'

MseI R: 5'-TACTCAGGACTCAT-3'

PstI F: 5'-CTCGTAGACTGCGTACATGCA-3'

PstI R: 5'-TGTACGCAGTCTAC-3'

II. Pre-amplification

The pre-amplification reaction was carried out in a thermocycler (Gene Amp PCR System 9700, PerkinElmer-Applied Biosystems, Weiterstadt). The pre-amplification reactions were prepared as follows:

Reagent for one reaction (µl)

Ligated DNA 2.0

*Mse*I-primer (M00) (50 ng/µl) 1.0

*Pst*I-primer (P00) (50 ng/µl) 1.0

dNTPs (2 mM) 2.5

PCR buffer (10×) 2.5

Taq DNA polymerase (5 U/µl) 0.2

sd H_2O to 25

The PCR profile used consisted of 94°C for 30 sec, 56°C for 30 sec, 72°C for 1 min for 30 cycles. The PCR products were diluted 1:5 with sd H2O and used as templates for selective PCR amplification. Sequences of AFLP primers and the primer combinations used in this study were:

P0-GTA GAC TGC GTA CAT GCA G	**M0**-GAC GAT GAG TCC TGA GTA A

.

III. Selective PCR

Sequences of AFLP primers and the primer combinations used in this study were:

P-GAC TGC GTA CAT GCA G AGG	**M-**GAT GAG TCC TGA GTA A CAG
P-GAC TGC GTA CAT GCA G GGG	**M-**GAT GAG TCC TGA GTA A CAG
P-GAC TGC GTA CAT GCA G AGG	**M-**GAT GAG TCC TGA GTA A CTG
P-GAC TGC GTA CAT GCA G ACT	**M-**GAT GAG TCC TGA GTA A AAG
P-GAC TGC GTA CAT GCA G ACT	**M-**GAT GAG TCC TGA GTA A AAC
P-GAC TGC GTA CAT GCA G CAT	**M-**GAT GAG TCC TGA GTA A CTG

Reagent for one reaction (µl)

Pre-amplified DNA 2.0 Primer M (50 ng/µl) 1.0 Primer P (50 ng/µl) 1.0 PCR buffer (10×) 2.0

dNTPs (2 mM) 2.0 *Taq* DNA polymerase (5 U/µl) 0.2 sd H_2O to 20.

The PCR profile used for selective amplification is: 94°C for 30 sec, 65°C for 30 sec, 72°C for 1 min (one cycle). This program was followed by 11 cycles in which the annealing temperature was decreased by 0.7°C per cycle. Then, the following program was used: 94°C for 30 sec, 56°C for 30 sec, 72°C for 1 min (for 23 cycles)

The products (4µl) were mixed with 4µl of loading buffer then denatured at 94°C for 3 minutes and separated by electrophoresis on a 6 % polyacrylamide gel (PAGE). The bands in the gels were visualized using silver nitrate staining as described in the Promega DNA Silver Staining System Technical Manual.

IV. Polyacrylamide gel electrophoresis (PAGE)

The 6 % polyacrylamide gel mixture was prepared as follows:

Component	**Weight/Volume**	**Final concentration**
Urea	31.50 g	7 M
10× TBE	3.75 ml	0.5×
40 % acrylamide:bisacrylamide (19:1)	11.25 ml	6 %
Add ds H_2O to volume	75.00 ml	

The polyacrylamide gel mixture was filtered through a 0.8-µm filter, and polymerization was initiated using 50 µl of TEMED and 500 µl of 10 % ammonium persulfate (APS). The mixture was quickly and carefully injected between two glass plates to avoid bubble formation. The gel was left to polymerize for about one hour. Before loading, the gel was washed and then preheated at 40°C for 20 min, after which 5 µl of each sample was loaded per well. Electrophoresis proceeded at a constant voltage of 1900 V for about one

hour using 0.5× TBE as the buffer. The bands in the gel were visualized by a silver staining protocol.

10 × TBE buffer

Tris	0.89	M
Boric acid	0.89	M
EDTA	2	mM

Silver staining:

Silver staining protocol is:

Step	Solution	Time
A	fix/stop solution	20 min
B	deionized H_2O	2 min
C	repeat step B twice	2 × 2 min
D	staining solution	30 min
E	deionized H_2O	5-10 seconds
F	developer solution (4-10°C)	2-5 min
G	fix/stop solution	5 min
H	deionized H_2O, twice	2 × 2 min

Solutions used for silver nitrate staining are:

1. ***Developer solution: Final concentration***

Sodium carbonate (anhydrous Na_2CO_3): 280 mM

Formaldehyde 37 % (HCHO): 18.5 mM

Sodium thiosulfate ($Na_2S_2O_3.5\ H_2O$): 8 mM

Dissolved in ds H_2O

2. ***Staining solution***

Silver nitrate ($AgNO_3$): 5.9 mM

Formaldehyde 37 % (HCHO): 18.48 mM

Dissolved in ds H_2O

3. ***Fix/stop solution (10 % acetic acid)***

Glacial acetic acid 100 ml/l

3.3.4 Data analyses

3.3.4.1 Scoring data

Morphological data

The data obtained were transferred to binary matrix with (1) for presence of the character state and (0) for its absence. The final matrix is used to run the similarity between the tested samples.

Molecular data

AFLPs were documented as image files. Polymorphisms were scored visually according to the presence (1) or absence (0) of a band. AFLP bands visually were scored with the aid of digital pictures of the gels and Adobe Photoshop computer software. AFLP possible bands, ranging in size from 70-500bp, across all 184 DNA samples of each of the 6 primer pair combinations were scored as present (1) and absent (0). Only the bands showing polymorphism were considered in the statistical analysis.

3.3.4.2 Statistical analysis

Genetic distance

In order to find the relationships between the different species among the different sections and between the species within the sections, genetic distance is calculated by using Jaccard similarity index (Jaccard, 1908), based on the similarity matrix for both morphological and molecular data. The resulting dendrograms were built using the UPGMA method (Unweighted Pair Group Method with Arithmetic Average) implemented by Darwin software Version 5.0.157 (Perrier and Jacquemoud-Collet, 2006).

In order to determine frequency of distribution of AFLP markers among defined genotypes, a pairwise comparison is performed based on the coefficient of simple matching and the UPGMA algorithm. The number of n(n-1)/2 pairs of comparison is calculate using the Clustering Calculator software (http//www.biology.ualberta.ca/jbrzusto/cluster.php).

The efficiency of AFLP markers used for discrimination was evaluated; a different combination set of primers that discriminate all samples and maximize diversity is tested by using AMaCAID-script available online: http://www.montpellier.inra.fr/BRC-MTR/AMaCAID/. Mantel test (Mantel, 1967), is conducted to evaluate correlation between morphological and molecular characterization Using GENALEX software (Peakall, Smouse, 2006)

Multivariate analysis

Principal Coordinate Analysis (PCA) for both data sets is performed with GENALEX (Peakall and Smouse, 2006) to provide a synthetic representation of distribution and proximity between studied samples according to their morphological taxonomy (at section and species level).

Bayesian analysis

The STRUCTURE algorithm was run using the basic model with admixture and correlated allele frequencies, with the assumed number of genetic K clusters varying from 2 to 16 for section level and K from 30 to 40 for species level, using five replicate runs per K value, a burn-in period length of 50,000, and a post burn-in simulation length of 1000.

To identify the probable number of K clusters explaining the taxonomic classification, posterior probability values for K (log-likelihood) were estimated, assigning K from 2 to 16 and K from 30 to 40. The proportion of membership (qI) of each individual in the remaining presumed clusters was estimated. To find optimal alignments of independent runs, the average pairwise similarity (H') of run results for STRUCTURE was assessed by CLUMPP 1.1.2 program (Jakobsson and Rosenberg, 2007) using greedy algorithm, with 10,000 random input orders and 10,000 repeats. Graphical representation of clustering results was performed with the DISTRUCT software (Rosenberg, 2004).

3.4 Results

3.4.1 Clustering of *Lathyrus* sections and species using morphological characteristics

The morphological characters used in this study were able to discriminate among most of the sections and to assign the species to their respective sections. At section level, nine clusters could be differentiated with a clear distinction between the Sect. *Aphaca, Orobus, Clymenum, Lathyrostylis* and *Pratensis* and with the rest of the sections (Figure 3.2). The Sect. *Linearicarpus* and *Nissolia* are grouped together and linked to *Pratensis* and *Lathyrostylis* and to part of the *Lathyrus* section. *Lathyrus* section showed the largest

variation and can be subdivided into two main sub-clusters, one very diverse group containing the Sect. *Orobastrum* within it, and the other which differed largely from the other *Lathyrus* sub-group and from the other sections by having 1-4 flowers, hairy valves and leaf rachis that never laminates. Large differences are found between species within each section except for the *Aphaca* which is composed of closely related varieties or sub-species.

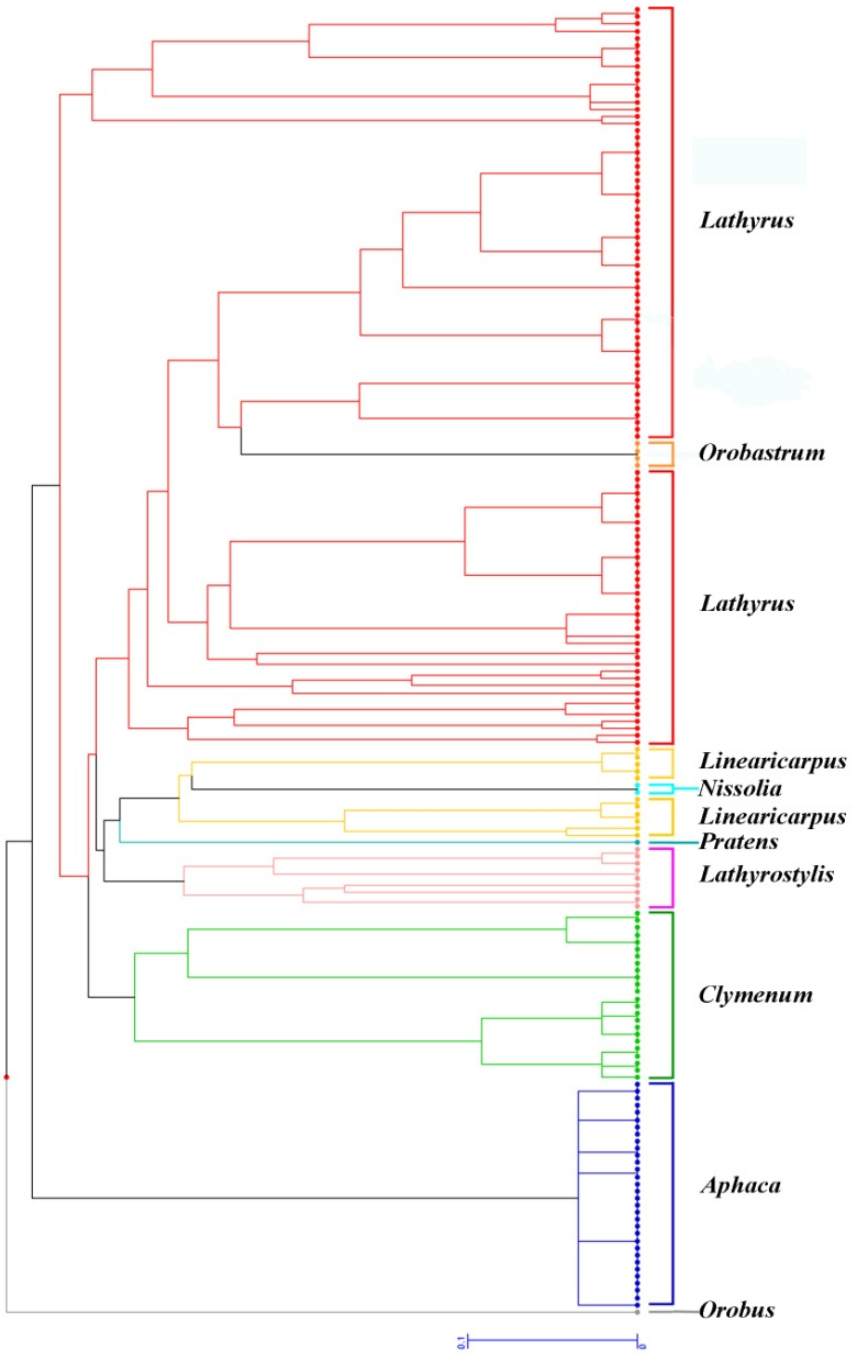

Figure 3.2. Dendrogram of morphological classification at the *Lathyrus* genus sections level using Jaccard similarity and UPGMA algorithm.

At the species level, small variation exists within the species and all the accessions belonging to the same species are grouped together. All the species within the sections and

the sub-clusters in case of *Lathyrus* section were differentiated (Figure 3.3). A total of 38 clusters were identified corresponding to the pre-defined 38 species used in the study. The distance-based clustering method assigned clearly the species to their respective sections except for the species in the Sect. *Linearicarpus* which was separated into two groups, one formed of *L. vinealis* and *L. sphaericus* and the other formed by *L. inconspectus* which is grouped with *L. nissolia.* *Lathyrus occidentalis*, the only species belonging to *Orobus* section was clearly separated from all the other species. Similarly, the sub-species of *L. apahca* were grouped together to form a separate cluster. The species *L. chloranthus*, *L. chrysanthus*, *L. hirsutus* and to some extent *L. odoratus* belonging to *Lathyrus* section formed a very distant group from other *Lathyrus* species and species of the other sections. The four *Clymenum* species were grouped together with *L. clymenum* and *L. articulatus* being close to each other and distant from *L. ochrus* and *L. gleospermus*. Also, the five species of *Lathyrostypis* section were grouped together. The only species of *Nissolia* Sect. *L. Nissolia* was grouped with *L. inconspicuus* belonging to *linearicarpus* section and similarly the only species of *Orobastrum* section (*L. setifolius*) was included with the sub-group of *Lathyrus* section containing *L. gorgoni* and *L. pseudocicera*. *L. sativus* was grouped with *L. blepharicarpus, L. cicera*, and *L. marmoratus* and was closely related to the sub-cluster formed of *L. pseudo-cicera*, *L. gorgoni* and *L. setifolius*. The pairs with most closely related species are (*L. cicera* and *L. marmoratus*), (*L. annuus* and *L. hierosolymitanus*) and (*L. clymenum* and *L. articulatus*).

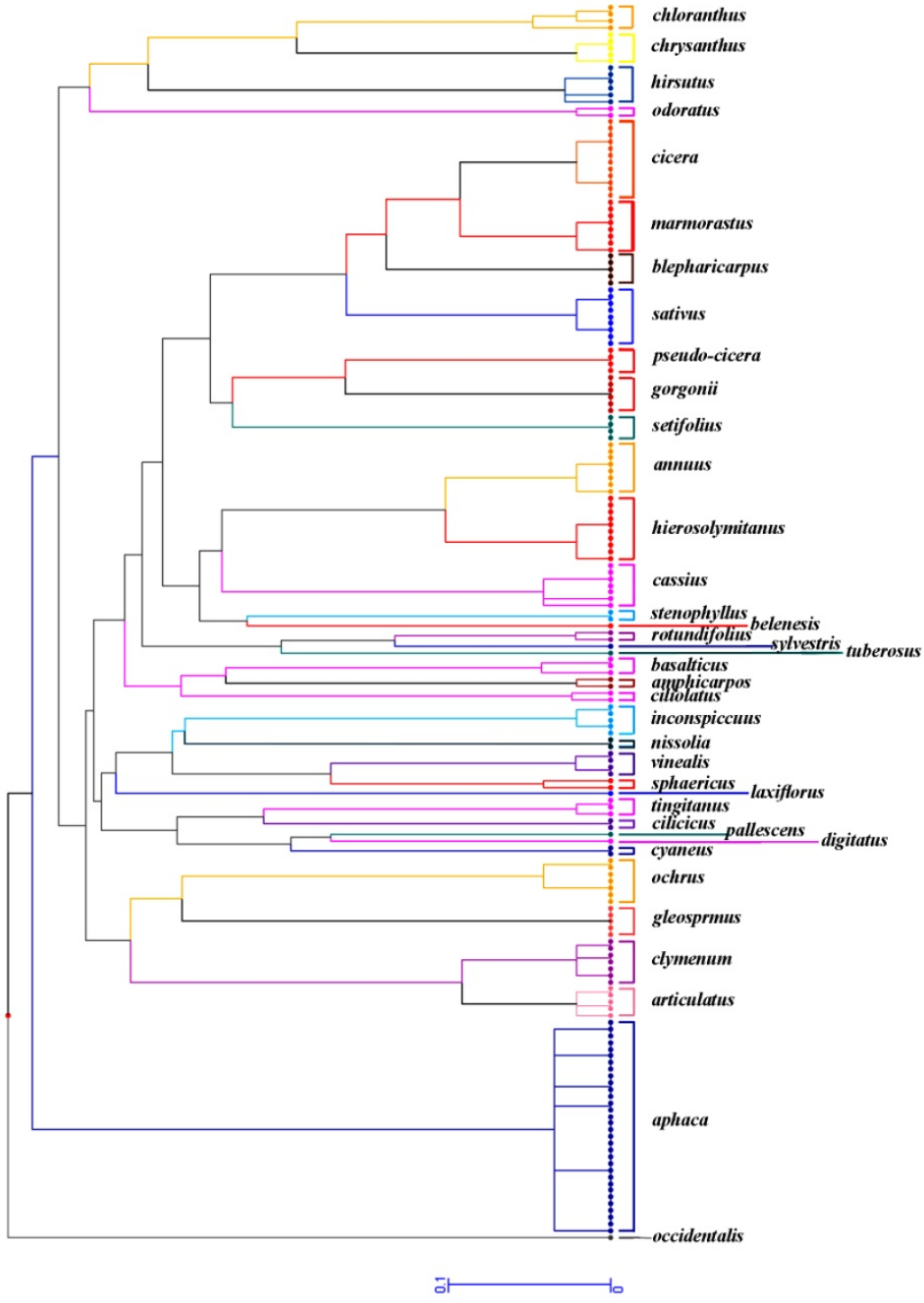

Figure 3.3. Dendrogram of morphological classification for *Lathyrus* genus at the species level using Jaccard similarity and UPGMA algorithm.

Using Principal Coordinate Analysis, only the Sect. *Aphaca* formed a clear and distinct group (Figure 3.4). Some taxa of *Clymenum* section also formed a separate group.

The remaining sections were split into different and composite groups: the species belonging to the Sect. *Orobus, Pratensis* and *Orobastrum* were grouped together with some taxa belonging to the Sect. *Linearicarpus*. Some taxa of *Lathyrostylis* were grouped with some taxa from *Linearicarpus*. The most composite group contained some taxa belonging to the Sect. *Lathyrostylis, Lathyrus, Clymenum and Linearicarpus* which were grouped together and with the species *L. nissolia*. The remaining taxa of the *Lathyrus* section can be assigned to at least five different groups.

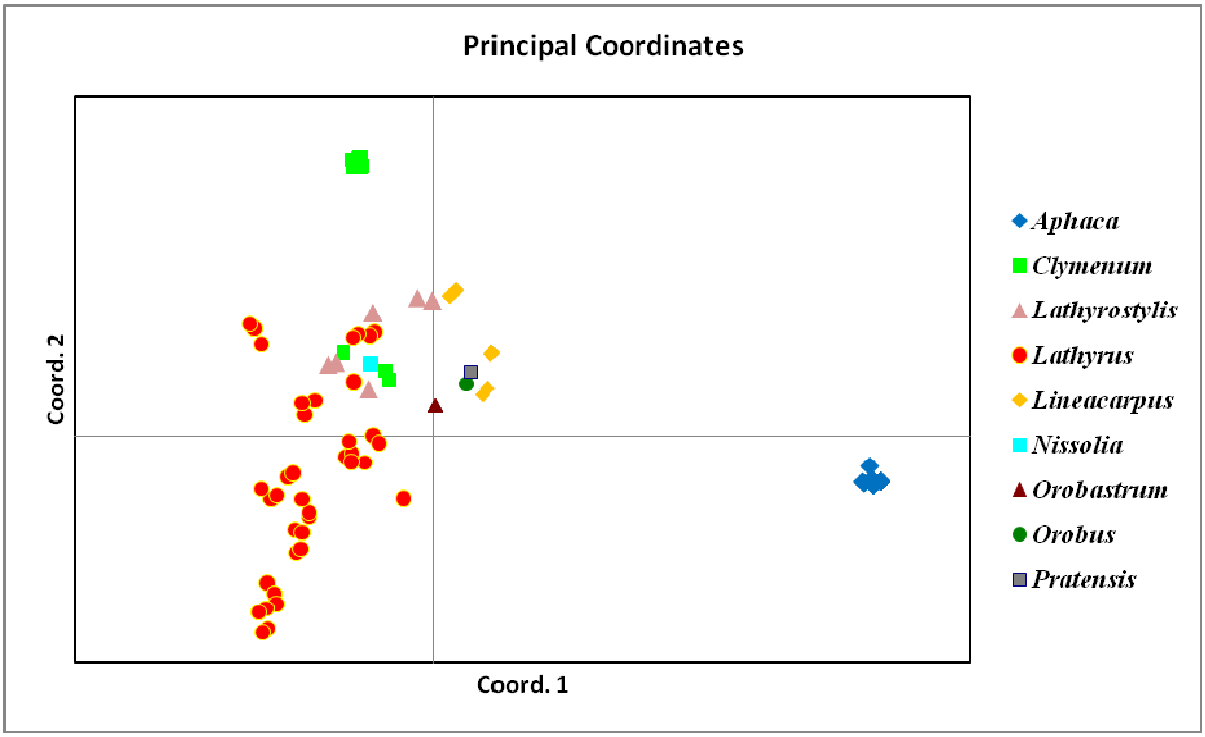

Figure 3.4. Clustering of *Lathyrus* sections using Principal Coordinate Analysis

The use of STRUCTURE program allowed a clear separation of most sections and confirmed the large diversity of the Sect. *Lathyrus* (Fig 5). At K=6, the Sect. *Aphaca, Clymenum, Lathyrostylis* and *Linearicarpus* were differentiated from the large and diverse group formed by *Lathyrus* section. The Sect. *Orobastrum* was differentiated from the other

sections when K=15 or more. At K=9 corresponding to the pre-defined number of sections used in this study, the five sections cited above are clearly differentiated along with the section *Lathyrus* which could be subdivided into at least five distinct sub-groups, the Sect. *Nicossia, Orobus* and *Pratensis* were not differentiated at K=9 and were included with *Lathyrus* group.(Figure 3.5)

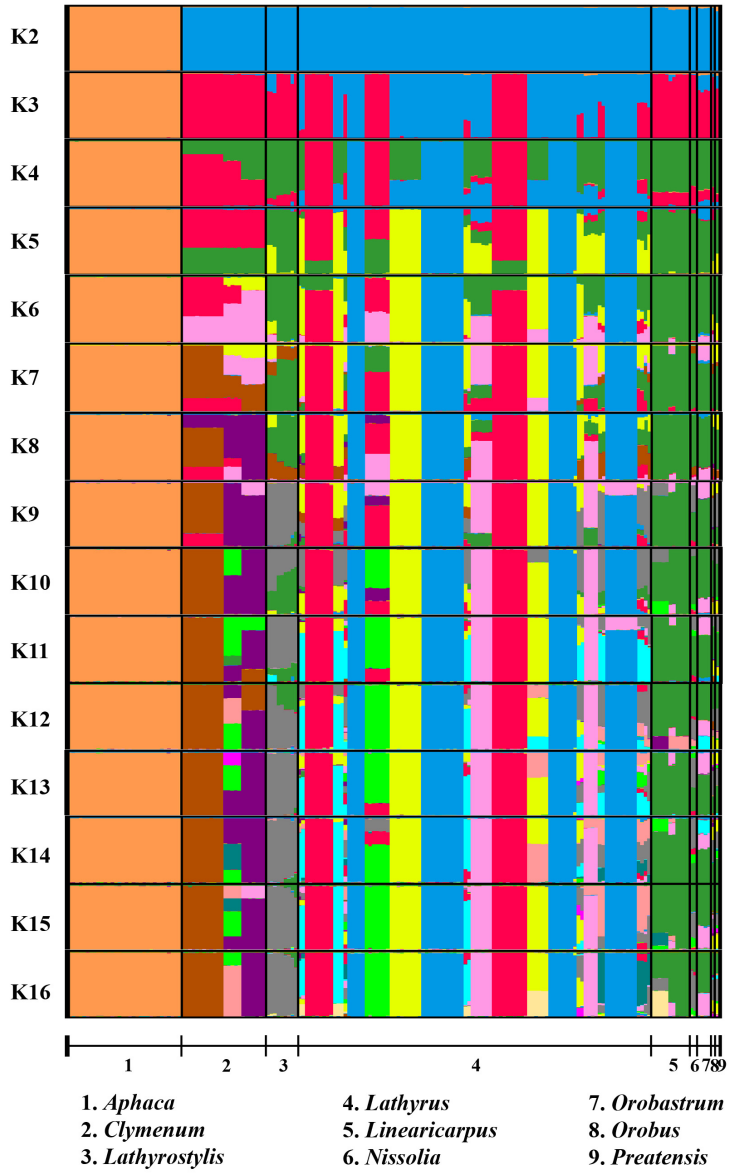

Figure 3.5. Estimated population structure based on morphological characters. Each individual is represented by a thin vertical line, which is partitioned into K coloured segments that represent the individual's estimated membership fractions in K clusters (Black thick lines separate different pre-defined sections)

3.4.2 Clustering of *Lathyrus* sections and species using AFLP Molecular markers

More than 20 AFLP primer-combinations were tested and six combinations which showed very high and replicable polymorphism across 184 accessions were used in this study. High polymorphism is revealed by each of the AFLP primer-combination. A total of 277 clear and polymorphic bands were selected and allowed to discriminate all accessions (Table 3.2). The primer-combinations differed in their discrimination power, which was higher for ACT_AAC, GGG_CAG and AGG_CTG . With the exception of two accessions belonging to *Lathyrostylis* which showed identical profiles for the bands selected, all the accessions of different sections and species were differentiated (Table 3.3).

Table 3.2. Number of polymorphic bands for each AFLP primer-combination and number of discriminated accessions of *Lathyrus* genus

Primer combination	Number of polymorphic bands	Number of accessions discriminated
ACT_AAG	62	107
CAT_CTC	36	136
ACT_AAC	90	142
AGG_CAG	22	115
GGG_CAG	31	138
AGG_CTG	36	150
All	**277**	**182**

Table 3.3. Number of individuals discriminated in each *Lathyrus* genus section using the six AFLP primer combinations

Section name	Total number of accessions	Number of individuals discriminated
Aphaca	32	32
Clymenum	24	24
Lathyrostylis	9	7
Lathyrus	100	100
Linearicarpus	11	11
Nissolia	2	2
Orobastrum	4	4
Orobus	1	1
Pratensis	1	1

Using AMaCAID-script within R software, 151 bands can discriminate all the accessions used in this study. The optimum number of discriminating bands for all samples and for the accessions in different sections are reported in Figures 3.6a to 3.6f.. Table 3.4 shows the number of total and unique bands discriminating the accessions of each section; there are 14 unique bands for the Sect. *Aphaca*, 20 for the Sect. *Clymenum*, 12 for the Sect. *Lathyrostylis*, 72 for the Sect. *Lathyrus* and 15 for the Sect. *Linearicarpus.* The primer-combination GGG_CAG has unique and specific bands for separating the Sect. *Aphaca, Clymenum* and *Linearicarpus*.

Table 3.4. Total and unique number of AFLP bands discriminating the accessions of five *Lathyrus* sections

	Aphaca		*Clymenum*		*Lathyrostylis*		*Lathyrus*		*Linearicarpus*	
	Unique bands	Total discriminating bands	Unique bands	Total discriminating bands	Unique bands	Total discriminating bands	Unique bands	Total discriminating bands	Unique bands	Total discriminating bands
ACT_AAG	2	7	1	6	6	16	23	35	2	8
CAT_CTC	1	4	1	8	2	4	13	17	0	3
ACT_AAC	3	6	6	14	6	14	14	26	7	18
AGG-CAG	4	4	5	6	0	0	5	5	2	3
GGG_CAG	4	4	2	2	0	0	0	0	2	2

AGG_CTG	0	2	5	10	0	0	17	22	2	6
Total	14	27	20	46	12	34	72	105	15	40

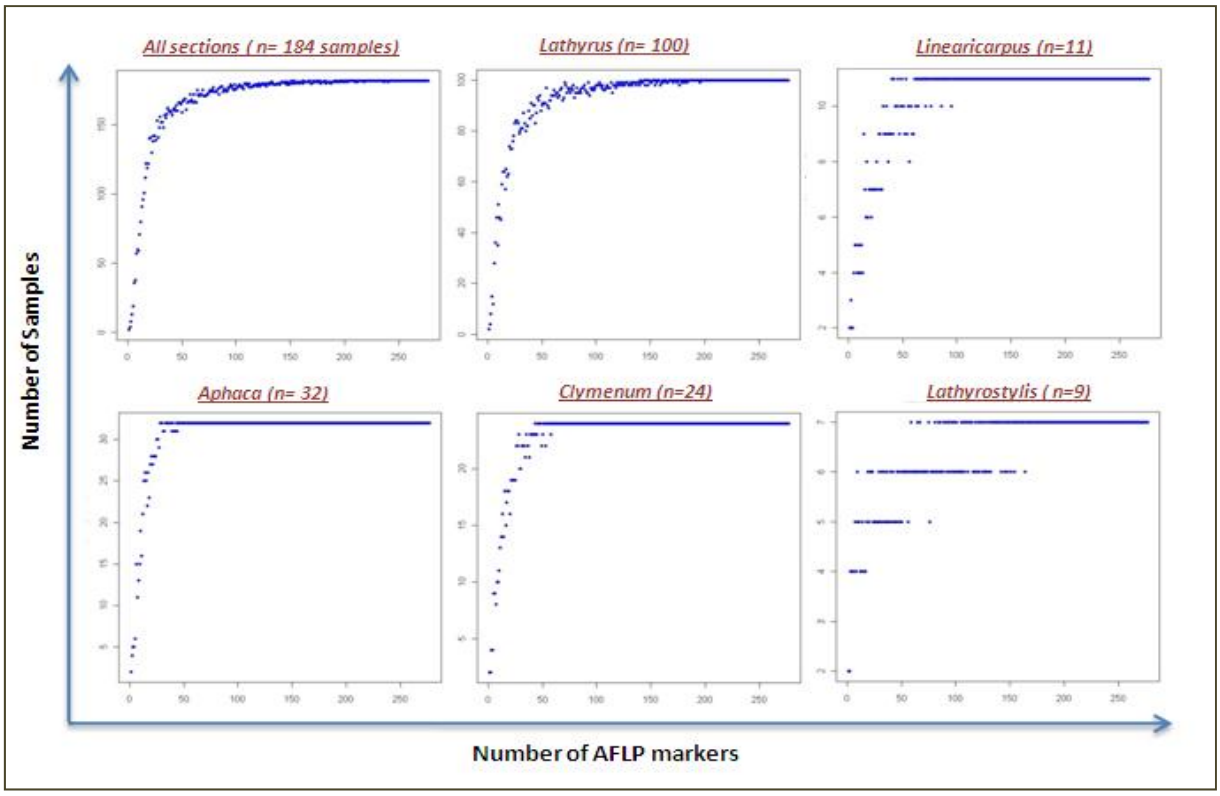

Figure 3.6a to 3.6f. Maximum number of samples discriminated based on the number of markers used for all samples and by observed sections.

To study the relationship among 182 accessions identified, a pairwise dissimilarity is tested using a sample matching coefficient among 16,371 pair comparisons (Figure 3.7). The accessions belonging to the same species and to the monophylitic and closely related species were differentiated with few bands, while the most distant species were different by a total of 133 bands. The majority of accessions were different by 85 to 101 bands showing the large diversity among the accessions and the taxa used.

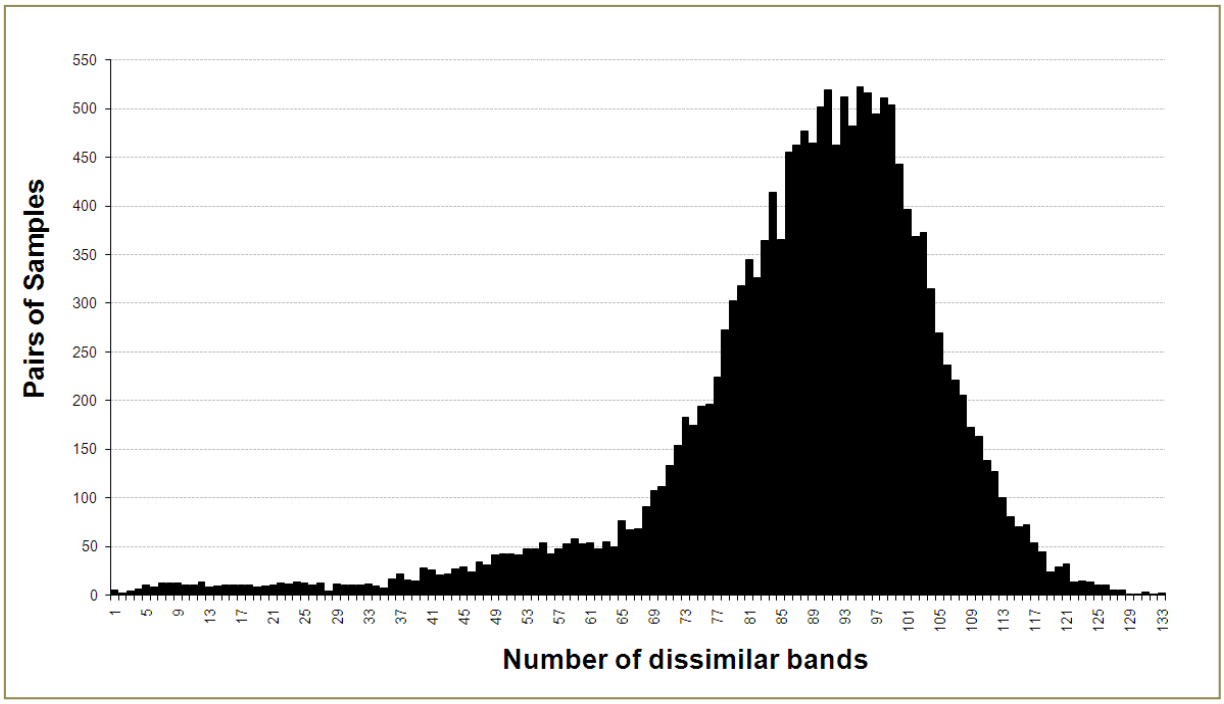

Figure 3.7. Frequency distribution of genetic similarity for all pairwise comparison between 182 accessions belonging to different *Lathyrus* taxa.

The distance-based dendogram at the section level showed a total of seven clusters at the separation line where *Aphaca*, *Lathyrostylis* and *Clymenum* sections were classified in clear and separate clusters(Figure 3.8). At this level, *Lathyrus* section is the most diverse and could be subdivided into at least five groups with one being very distant from the others. Two of these groups are clustered with the Sect. *Clymenum* and *Lathyrostylis* at higher level. The remaining sections were grouped together in a distant cluster formed of two different subgroups: *Linearicarpus* and *Nissolia* subgroup and *Orobastrum, Orobus* and *Pratensis* subgroup.

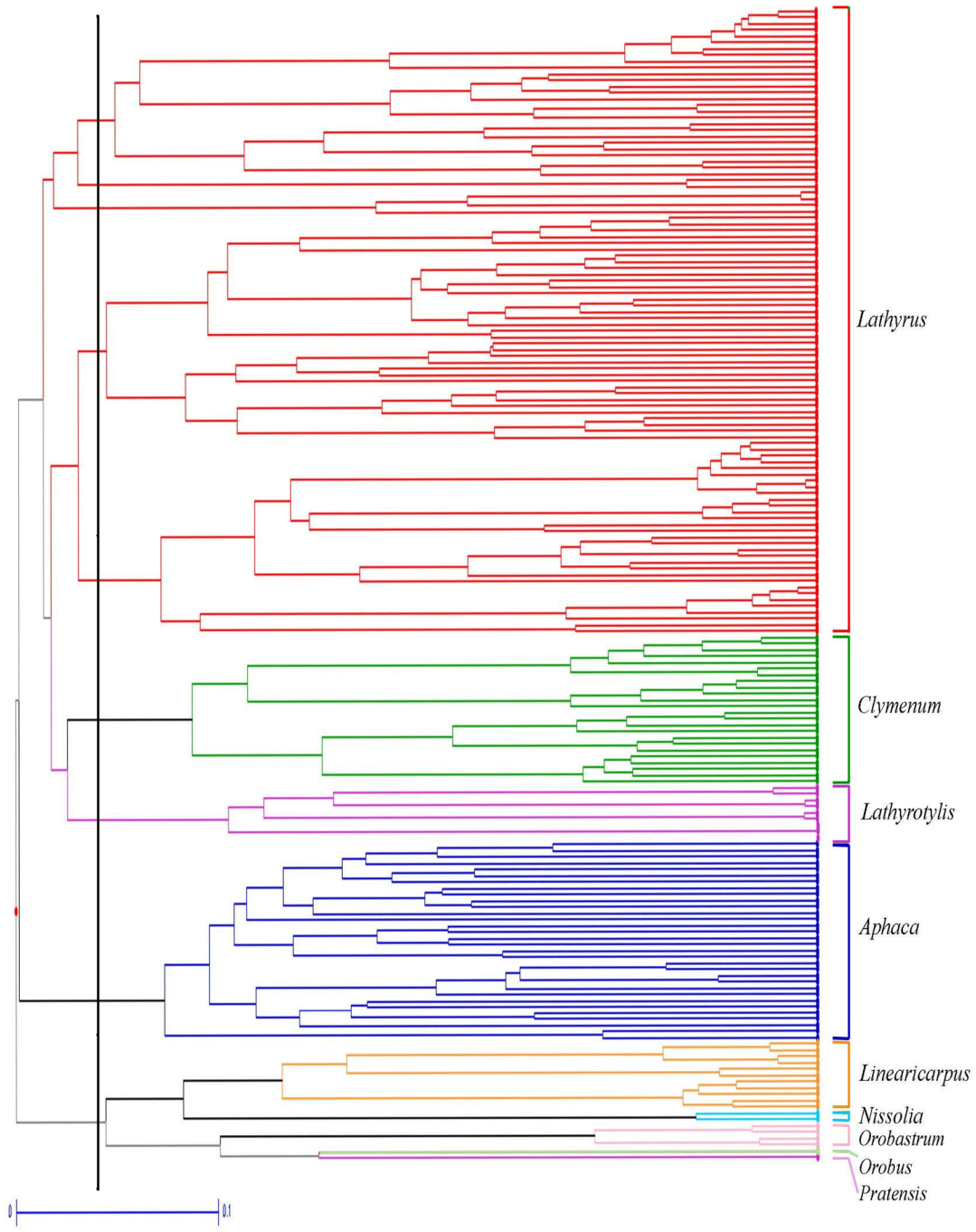

Figure 3.8. Clustering of 184 *Lathyrus* accessions at the section level using 6 AFLP primer-combinations, and based on Jaccard similarity and UPGMA algorithm.

The clustering at the level of species showed that the accessions can be grouped into 13 clusters at the horizontal cutting line where *L. Aphaca* subspecies formed the same cluster (Figure 3.9). The choice of this level is based on the fact that *L. aphaca* is among the sections where there are fewer subspecies based on taxonomic classification. This choice shows also some phylogenic relationships among species. At this point, the species *L. aphaca, L. amphicarpos, L. annuus,* and *L. hierosolymitanus* formed each a separate cluster. The remaining clusters contained several species each. When considering species with more than 2 accessions each, the high within species diversity is found among the sub-species belonging to *Aphaca* section followed by the accessions of *L. cassius, L. gorgoni* and *L. marmoratus* and the lowest within species diversity was found in case of *L. sativus, L. vinealis, L. inconspicuus* and *L. setifolius.* Excluding the highly variable taxa (*L. aphaca*, *L. cassius, L. gorgoni* and *L. marmoratus*) and at the separation horizontal line where *L. marmoratus* formed a separate cluster, 31 clusters can be identified, 29 of which included a separate species each, and the remaining had two sub-clusters which included respectively two closely related species (*L. pallescens* and *L. digitatus*) and three species (*L. stenophyllus*, *L. sylvestris* and *L. tuberosus*). At this separation horizontal line, two accessions of *L. blepharicarpus* were grouped with the accessions of the only accession belonging to *L. belinensis*. This classification allowed to assign most of the species to their respective sections and allowed to highlight the affinities among different species which are grouped in similar clusters. *L. sativus* is grouped with the species *L. pseudo-cicera*, *L. marmoratus* and *L. rotundifolius* and with another sub-cluster containing *L. hirsutus* and *L. odoratus*. The pairs of closely associated species are: (*L. ochrus* with *L. gloeospermus*), (*L. clymenum* and *L. articulatus*), (*L. gorgoni* and *L. cicera*), (*L. chrysanthus* and *L. chloranthus*), (*L. belinensis* and *L. blepharicarpus*), (*L. vinealis* and *L. sphaericus*) and (*L. laxiflorus* and *L. occidentalis*).

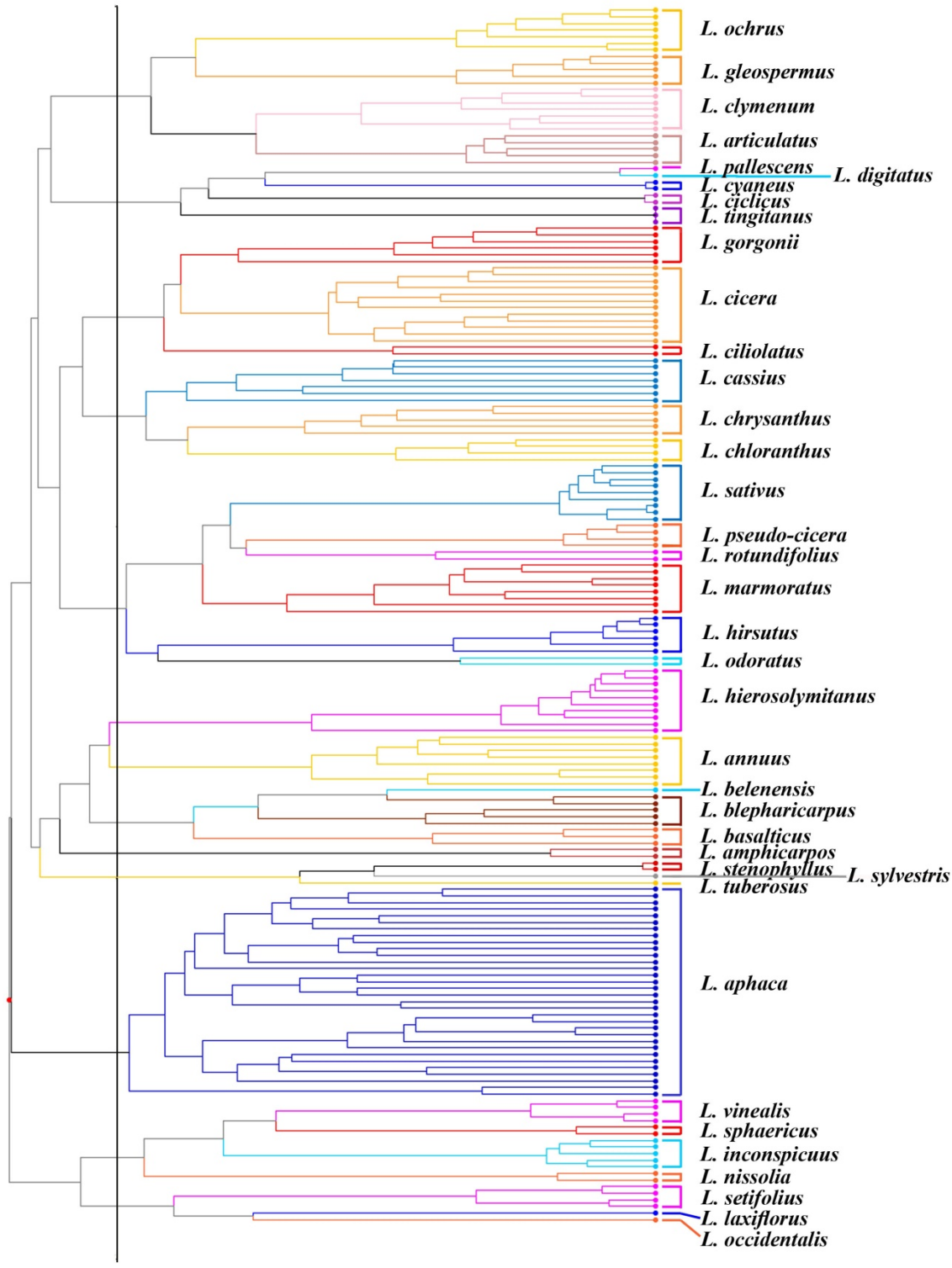

Figure 3.9. Clustering of *Lathyrus* species using 6 AFLP primer-combinations, and based on Jaccard similarity and UPGMA algorithm.

Using Principal Coordinate Analysis, six different sections (*Aphaca, Clymenum, Orobastrum, Linearicarpus, Lathyrostylis* and *Lathyrus*) can be differentiated with *Lathyrus* section having the largest diversity and can be subdivided into at least four groups (Figure 3.10). The Sect. *Nissolia, Orobus* and *Pratensis* formed one cluster close to *Lathyrostylis* cluster. Large number of the species used in this study were also clearly separated using the PCA (Figure 3.11).

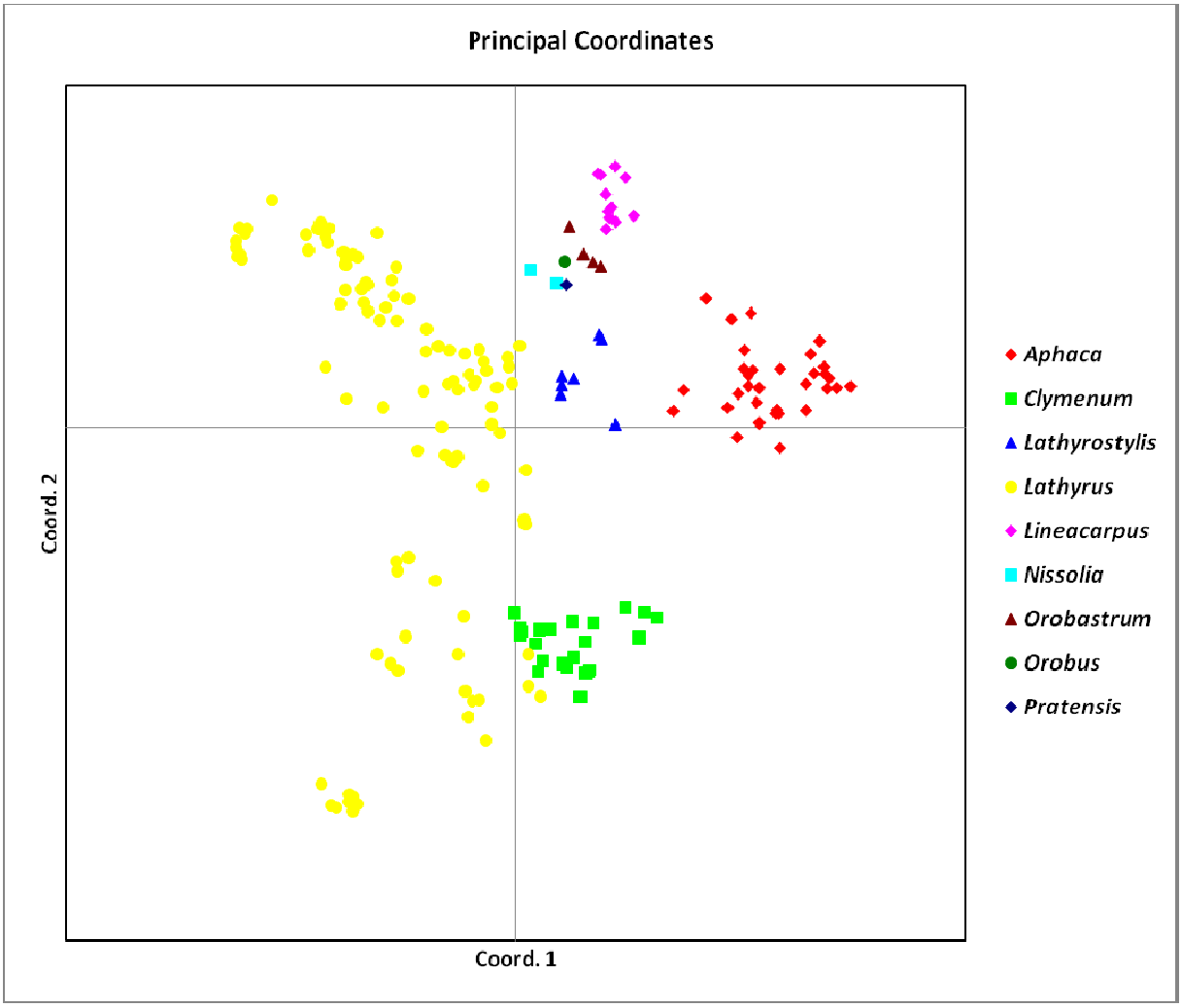

Figure 3.10. Clustering of *Lathyrus* sections using Principal Coordinate Analysis of 6 AFLP primer combinations (computed with GenAlex).

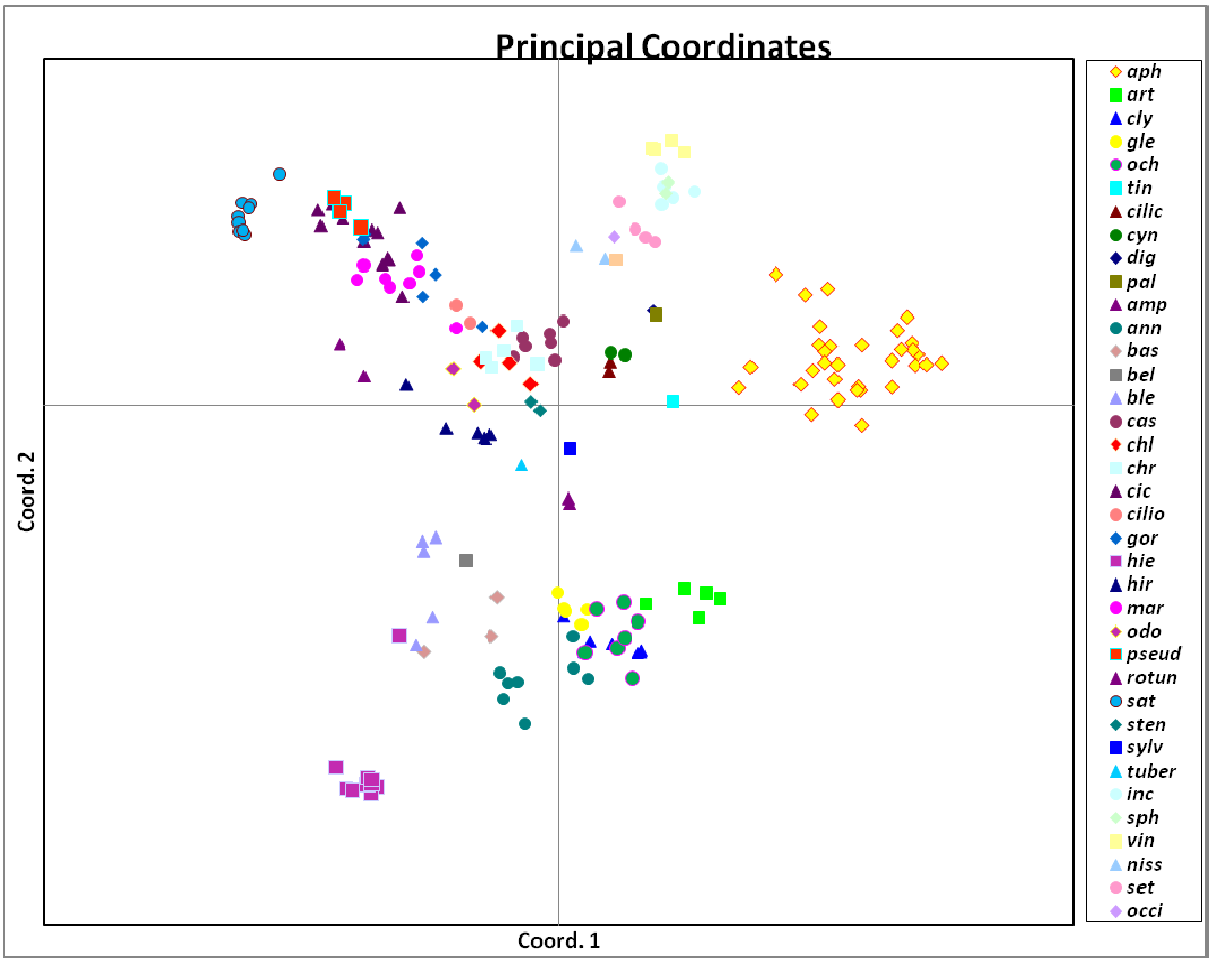

Figure 3.11. Analysis of *Lathyrus* species grouping using Principal Coordinate Analysis of 6 AFLP primer combinations (computed with GenAlex).

The use of STRUCTURE program for AFLP data at the section level allowed for the clear identification of the Sect. *Aphaca, Clymenum, Lathyrostylis* and *Linearicarpus*, and the Sect. *Lathyrus* which showed high diversity with at least six subgroups when K=9 (Figure 3.12). The Sect. *Orobastrum* was distinguished at K=16, while the Sect. *Orobus, Pratensis* and *Nissolia* were not clearly distinguished and were still embedded within the *Lathyrus* sub-groups.

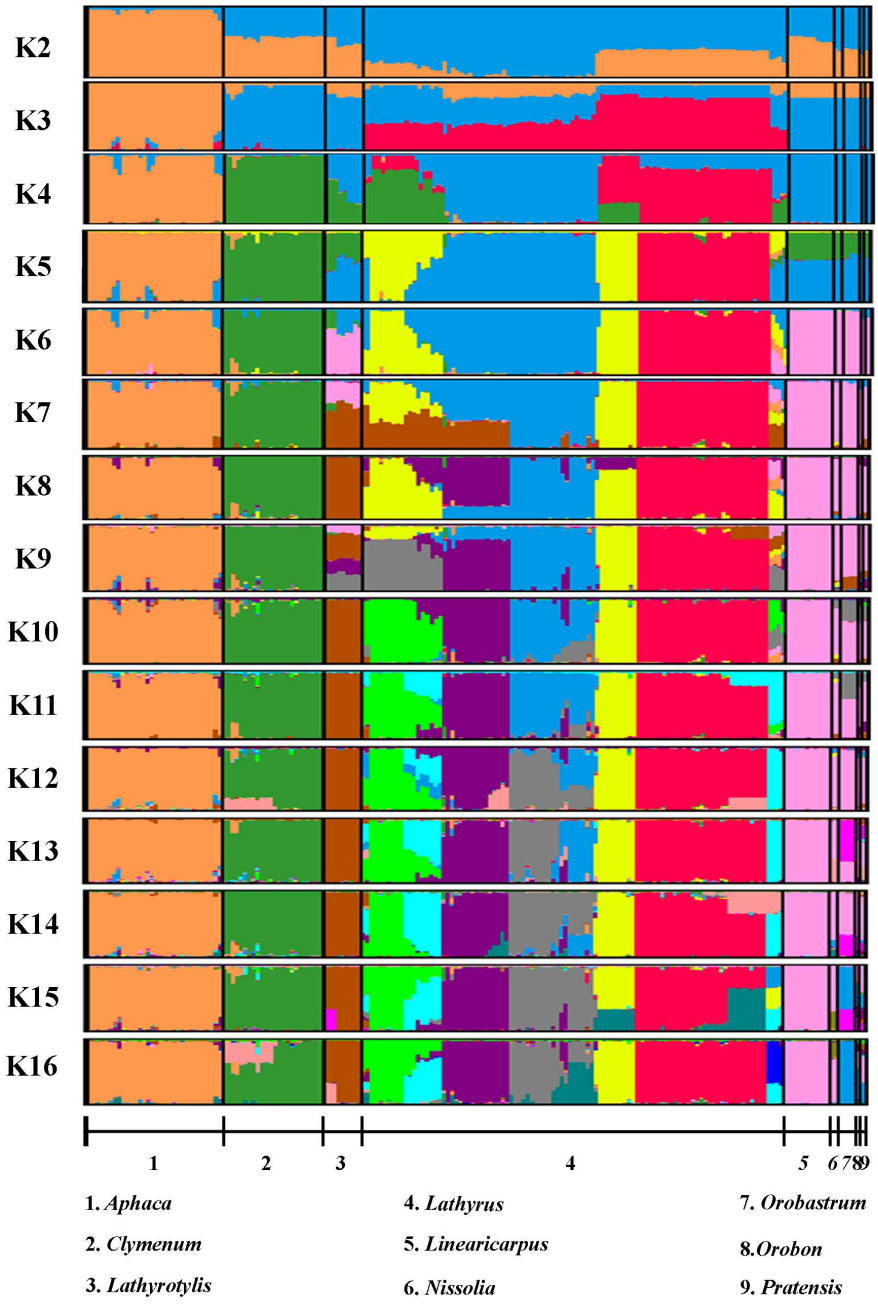

Figure 3.12. Estimated population structure using STRUCTURE program. Each individual is represented by a thin vertical line, which is partitioned into K coloured segments that represent the individual's estimated membership fractions in K clusters. (Black bold lines separate individuals of different sections on the basis of their taxonomic origin)

Based on STRUCTURE and using likelihood analysis (lowest value of Ln P(D)) and the similarity index (high H'), the optimum number of distinct groups were respectively 12

and 6 at the section level. At the species level, an optimum number of 36 species could be differentiated using likelihood analysis, and 34 species using H' similarity index (Table 3.5).

Table 3.5. Number of *Lathyrus* L. genus sections and species clusters assumed using posterior probability values for K (log-likelihood) and average pairwise similarity (H') index.

Number of assumed section	Ln P(D)	H'	Number of assumed species	Ln P(D)	H'
2	-30666.78	0.4772	**30**	-20408.28	**0.8178**
3	-28836.90	0.4557	31	-20289.32	0.6840
4	-27148.18	0.7035	32	-20019.12	0.6002
5	-25625.10	0.7732	33	-19850.26	0.7412
6	-24492.14	**0.9718**	**34**	-19977.68	**0.8150**
7	-23639.64	0.6943	35	-19739.92	0.6696
8	-22618.86	0.8190	**36**	**-19687.50**	0.6574
9	-21737.62	0.6525	37	-20009.94	0.7675
10	-21429.56	0.7296	38	-20382.80	0.7493
11	-20883.40	0.7358	39	-20120.32	0.7283
12	**-20423.06**	0.6890	40	-20351.28	0.7552
13	-20738.94	0.7880			
14	-20606.66	0.7543			
15	-20767.42	0.7160			
16	-20498.80	0.7365			

This STRUCTURE analysis better highlighted the relatedness among the accessions belonging to the same species or to different species of the Sect. *Lathyrus* and *Clymenum* (Figure 3.13). Clear differences exist between the species of *Clymenum* section and those of *Lathyrus* section. Within *Clymenum* section, *L. ochrus* and *L. gleospermus* were distinct from each other and from the other two closely related species *L. clymenum* and *L. articulatus* (the later also considered as a synonym of *L. clymenum* var. *articulatus*). In case of *Lathyrus* section, there are seven different groups:

- First group containing the species *L. amphicarpos* and *L. basalticus* which are producing amphicarpic pods, and *L. annuus* and *L. belinensis*;
- Second group formed of *L. chloranthus* and *L. chrysanthus* closely related and *L. cassius*;
- *L. blepharicarpus* formed a third group showing high within species diversity with two accessions having more affinity with *L. belinensis* accessions and the other three accessions having more affinity with species in the third and fourth groups;
- Fourth group contained *L. cicera, L. ciliolatus* and *L. gorgoni,* all have solitary and brick red flowers;
- Fifth group is formed of *L. hierosolymitanus, L. hirsutus, L. marmoratus, L. odoratus, L. pseudocicera* and *L. rotundifolius* and have their peduncle much longer than the leaf;
- *L. sativus*, the only cultivated species formed a unique group with some affinity with the previous group and to a less extent with *L. chloranthus* and *L. chrysanthus,* all having in common their peduncle much longer than the leaf;
- The last group is formed of the two perennial species *L. tuberosus* and *L. sylvestris* and *L. stenophyllus* all sharing the character of twisted style contortion.

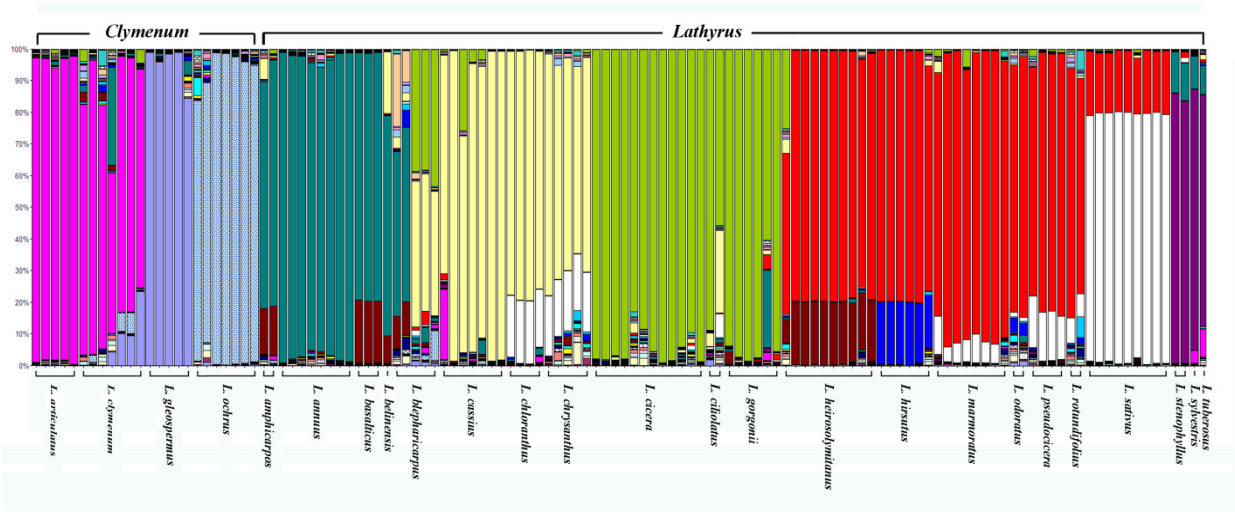

Figure 3.13: Differentiation of species belonging to *Lathyrus* and *Clymenum* sections using STRUCTURE program.

Following the genepool concept and based on the proposed secondary gene pool (GP2) by previous researchers, the species included in the same or closely related sub-clusters with *L. sativus* L. are included in Table 3.6. Using morphological characters, five GP2 species were identified. When using AFLP markers, three GP2 species were identified using distance-based clustering and four when using the population structure analysis. Only *L. marmoratus* and *L. pseudo-cicera* were commonly classified in GP2 of Grass pea by all approaches used in this study. The species *L. setifolius, L. odoratus* and *L. rotundifolius* were added as belonging to GP2 of Grass pea.

Table 3.6. Species that could be potentially included in the secondary genepool of Grass pea (*Lathyrus sativus* L.) based on morphologic characters and AFLP markers affinities

Primary genepool	Species in secondary genepool defined by previous research (Sarker *et al*., 2001)	This study		
		Morphologic characters using distance-based clustering	AFLP markers using distance based clustering	AFLP markers using model-based clustering
L. sativus	*L. chrysanthus* *L. gorgoni*	*L. cicera* *L. marmoratus*	*L. pseudo-cicera* *L. rotundifolius*	*L. pseudo-cicera* *L. rotundifolius*

	L. marmoratus *L. pseudo-cicera* *L. amphicarpos* *L. blepharicarpus* *L. chloranthus* *L. cicera* *L. hierosolymitanus* *L. hirsutus*	*L. blepharicarpus* *L. pseudo-cicera* *L. gorgonii* *L. setifolius*	*L. marmoratus* *L. hirsutus* *L. odoratus*	*L. marmoratus,* *L. hirsutus* *L. odoratus* *L. hierosolymitanus* *L. chloranthus* *L. chrysanthus*

3.5 Discussion

The 47 qualitative taxonomic characters allowed assigning all the accessions to their respective pre-defined sections and species and even to sub-species or varieties within species as in the case for *Aphaca* section confirming the reliability of these characters in the taxonomic classification of genus *Lathyrus* L. These characters, used normally for species identification, were able to discriminate clearly the Sect. *Aphaca*, *Orobus*, *Clymenum, Lathyrostylis* and to some extent *Pratensis*. The Sect. *Linearicarpus and Nissolia* were included in the same cluster while the highly diverse Sect. *Lathyrus* could be subdivided into two main groups, one very distant from all the sections and the other which contained inside it the only species of the Sect. *Orobastrum*. This latter finding was also reported by Kenicer *et al*., (2005) who suggested that *Lathyrus* section should either include the *Orobon* and *Orobastrum* sections or should be redefined to be able to separate the 3 sections as done by Kupisha (1983). These morphologic characters were among 75 characters used by various taxonomists who worked on *Lathyrus* genus (Davis, 1970; Czefranova, 1971; Kupicha, 1983) and the consideration of all the characters, or the use of only the subset of characters adapted to section level differentiation, could improve the distinction among taxa at section level. More research is needed to assign weight to these characters as done with Lucid program used by the authors for developing a user friendly tool to help in taxonomic identification of *Lathyrus* species.

All AFLP primer-combinations used in this study showed high polymorphism with no monomorphic bands across all the species, confirming the usefulness of these markers for studying the genetic diversity and the grouping of taxa at various taxonomic levels. AFLP markers separated individually the Sect. *Aphaca*, *Clymenum* and *Lathyrostypis*, allowed the clustering of *Linearicarpus* with *Nissolia*, and confirmed the large diversity within the Sect.

Lathyrus. This technique grouped the Sect. *Orobus, Orobustrum* and *Pratensis* together when using distance based clustering method. AFLP could successfully be used to support taxonomic identification and phylogenetic relationships of *Lathyrus* confirming the conclusions of previous research on temperate herbaceous tribes of Papilionoid legumes (Wojciechowski *et al*., 2000) and *Vicia* species (van de Wouw *et al*., 2001), and for taxonomic designation of *Eriogonum corymbosum* var. *nilesii* (Ellis *et al*.. 2009). These type of nuclear DNA markers, along with markers of cytoplasmic DNA (cpDNA, rDNA), cytogenetic relationships based on chromosome morphology, banding patterns and *in situ* hybridization, and chromosome pairing following interspecific hybridization can add a significant amount of information to support the botanical classification of *Lathyrus* L. taxa and to elucidate more the phylogenetic and genomic relationships among different taxa. This information is highly needed to define more accurately the species in different genepools of different *Lathyrus* crops. Our study suggested the inclusion of *L. setifolius, L. odoratus* and *L. rotundifolius* as possible species within GP2 of grass pea. This needs to be confirmed by inter-crossing of these species to *L. sativus* L. to determine the homeology and the facility to introgress genes from these species into grass pea. This study showed some specific and unique bands to differentiate among five *Lathyrus* sections (*Aphaca, Clymenum, Lathyrostylis, Lathyrus* and *Linearicarpus*). More research is needed to confirm their uniqueness using more accessions and species from different geographic origins, and to sequence them and turned them into specific DNA markers to be used in the molecular identification of *Lathyrus* sections. Similar analysis should be done at the species and sub-species levels.

In addition to distance-base tree clustering method, Principal Component Analysis (PCA) was used to better highlight the distances between different taxa. In this study, PCA

allowed better visualization of relationships of different *Lathyrus* sections and species compared to the distance-based clustering method when using morphologic characters. In the case of AFLP markers, it has graphically shown clear distinction among six *Lathyrus* sections. This study introduced also a model-based clustering method for the taxonomic classification of *Lathyrus* taxa, using STURUCTURE program suggested by Falush *et al*. (2007). In our study, this version was applied to both morphological characters and AFLP data to assign the accessions to pre-defined sections and species as per Kupicha (1983) classification. The STRUCTURE Bayesian-based method improved further the visualisation of the clusters, but most importantly gave additional information on shared DNA fragments among various accessions at the section and species level. The extended use of this approach with larger number of AFLP and other markers that can saturate the genomes could help in defining homology and homeology among geneomes and chromosomes of closely related species and could therefore help in placing wild species into different genepools of cultivated species.

Our data showed that morphological characters provide clearer distinction among species than when using AFLP primer-combinations used in this study. Both approaches showed a clear distinction of the Sect. *Aphaca, Lathyrostylis* and *Clymenum* confirming the results of Kupicha (1983), Asmussen and Liston (1998) and Kenicer *et al*. (2005). *Pratensis, Orobus* and also *Orobastrum* sections can also be differentiated, when using the model-based clustering approach. The difficulty in discriminating well some of the sections using both morphologic characters and AFLP markers could be due to, 1) the use of all 47 characters at the section level, while there are specific characters used by taxonomists to differentiate among te sections, and 2) the small number of species included in some sections. Similarly, AFLP markers were not able to differentiate some sections such as *Linearicarpus* and

Nissolia, and among *Pratensis, Orobastrum* and *Orobus* and this could be attributes also to the small number of species and accessions belonging to these sections. The high diversity within Sect. *Lathyrus* could be due to the large number of species and accessions used in this study. This high diversity within *Lathyrus* section call for an in-depth analysis of this section as suggested by Kenicer *et al*., (2005) to either combine Sect. *Orobon* and *Orobastrum* with Sect. *Lathyrus* or to redefine them as separate sections. Badr *et al*. (2002) proposed not to subdivide this monophyletic section. Kupicha (1983) also reported on the high diversity within *Lathyrus* section, but advised to keep it as one section. Based on the results from this study, the sections were aligned with the main classifications proposed by various taxonomists for the *Lathyrus* sections as summarized in the following Table 3.7.

Table 3.7. Classification of *Lathyrus* sections using morphological characters and AFLP markers in comparison with previous classification.

Davis 1970	Czefranova (1971)	Kupicha (1983)	Dogan, Kence and Tigin (1992)	Asmussen & Liston (1998)	Kenicer et. al, (2002)	This study	
						Morphological Characters	AFLP Characters
		Notolathyrus		*Orobus*	*Notolathyrus*		
Orobus	*Lathyrobus*	*Orobus*	*Orobus*		*Orobus*	*L. occidentalis*	*L. occidentalis*
Lathyrostylis	*Orobus*	*Lathyrostylis*	*Lathyrostylis*	*Lathyrostylis*	*Lathyrostylis*	*Lathyrostylis*	*Lathyrostylis*
Pratensis	*Pratensis*	*Pratensis*		*Pratensis*	*Pratensis*	*L. Laxiflorus*	*L. laxiflorus*
	Eurytrichon			*Aphaca*	*Aphaca*	*Aphaca*	*Aphaca*
Aphaca	*Aphaca*	*Aphaca*	*Aphaca*				
Neurolobus	*Neurolobus*	*Neurolobus*		*Neurolobus*	*Neurolobus*		
Orobon	*Orobon*	*Orobon*	*Orobon*	*Lathyrus*			
Lathyrus	*Lathyrus*	*Lathyrus*	*Lathyrus*		*Lathyrus*	*Lathyrus*	*Lathyrus*
Cicerula	*Cicerula*		*Gorgonia*				
			Cicerula	Cicerula		*Orobastrum*	
Orobastrum	*Orobastrum*	*Orobastrum*				*Lathyrus*	
							Orobastrum
				Orobastrum			*Lathyrus*
		Linearicarpus		*L. sphaericus*	*L. sphaericus*	*Linearicarpus*	*Linearicarpus*
				L. angulatus	*L. angulatus*	*Nissolia*	
		Viciopsis				*Linearicarpus*	
Nissolia	*Nissolia*	*Nissolia*		*Nissolia*	*Nissolia*		*Nissolia*
Clymenum	*Clymenum*	*Clymenum*	*Clymenum*	*Clymenum*	*Clymenum*	*Clymenum*	*Clymenum*
				L. gleospermus			

This study contributes to gain more insights on the *Lathyrus* L. genus towards better understanding of the taxonomic and phylogenetic relationships among different sections and species. This is highly crucial for better use of *Lathyrus* genetic resources in the genetic improvement of grass pea and other cultivated *Lathyrus* species.

3.6 References

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3.7 Appendices:

Appendix 1. ICARDA genebank identification number (IG) for different accessions of *Lathyrus* taxa included in the study

IG	***Taxa***	***Section***	***Sp_ID***	**ORI**
66149	*Lathyrus aphaca*	*Aphaca*	*aph*	DZA
64768	*Lathyrus aphaca*	*Aphaca*	*aph*	GRC
66075	*Lathyrus aphaca*	*Aphaca*	*aph*	JOR
66077	*Lathyrus aphaca*	*Aphaca*	*aph*	JOR
107534	*Lathyrus aphaca*	*Aphaca*	*aph*	MAR
65293	*Lathyrus aphaca*	*Aphaca*	*aph*	SYR
66142	*Lathyrus aphaca var. affinis*	*Aphaca*	*aph*	DZA
66046	*Lathyrus aphaca var. affinis*	*Aphaca*	*aph*	JOR
65257	*Lathyrus aphaca var. affinis*	*Aphaca*	*aph*	SYR
65298	*Lathyrus aphaca var. affinis*	*Aphaca*	*aph*	SYR
65410	*Lathyrus aphaca var. affinis*	*Aphaca*	*aph*	SYR
65807	*Lathyrus aphaca var. affinis*	*Aphaca*	*aph*	TUR
65216	*Lathyrus aphaca var. aphaca*	*Aphaca*	*aph*	JOR
65383	*Lathyrus aphaca var. aphaca*	*Aphaca*	*aph*	SYR
65509	*Lathyrus aphaca var. aphaca*	*Aphaca*	*aph*	SYR
65558	*Lathyrus aphaca var. aphaca*	*Aphaca*	*aph*	SYR
66037	*Lathyrus aphaca var. aphaca*	*Aphaca*	*aph*	SYR
65337	*Lathyrus aphaca var. biflorus*	*Aphaca*	*aph*	SYR
65388	*Lathyrus aphaca var. biflorus*	*Aphaca*	*aph*	SYR
65398	*Lathyrus aphaca var. biflorus*	*Aphaca*	*aph*	SYR
65564	*Lathyrus aphaca var. biflorus*	*Aphaca*	*aph*	SYR
65260	*Lathyrus aphaca var. floribundus*	*Aphaca*	*aph*	SYR
65641	*Lathyrus aphaca var. floribundus*	*Aphaca*	*aph*	SYR
65012	*Lathyrus aphaca var. floribundus*	*Aphaca*	*aph*	TUR
65722	*Lathyrus aphaca var. floribundus*	*Aphaca*	*aph*	TUR
65256	*Lathyrus aphaca var. modestus*	*Aphaca*	*aph*	SYR
65370	*Lathyrus aphaca var. modestus*	*Aphaca*	*aph*	SYR
65511	*Lathyrus aphaca var. modestus*	*Aphaca*	*aph*	SYR
66001	*Lathyrus aphaca var. modestus*	*Aphaca*	*aph*	SYR
66018	*Lathyrus aphaca var. modestus*	*Aphaca*	*aph*	TUR
65741	*Lathyrus aphaca var. pseudoaphaca*	*Aphaca*	*aph*	TUR
65769	*Lathyrus aphaca var. pseudoaphaca*	*Aphaca*	*aph*	TUR

64732	*Lathyrus articulatus*	*Clymenum*	*art*	FRA
64781	*Lathyrus articulatus*	*Clymenum*	*art*	GRC
107739	*Lathyrus articulatus*	*Clymenum*	*art*	MAR
107851	*Lathyrus articulatus*	*Clymenum*	*art*	MAR
107839	*Lathyrus articulatus*	*Clymenum*	*art*	MAR
66148	*Lathyrus clymenum*	*Clymenum*	*cly*	DZA
119631	*Lathyrus clymenum*	*Clymenum*	*cly*	ESP
64783	*Lathyrus clymenum*	*Clymenum*	*cly*	GRC
107568	*Lathyrus clymenum*	*Clymenum*	*cly*	MAR
107775	*Lathyrus clymenum*	*Clymenum*	*cly*	MAR
111095	*Lathyrus clymenum*	*Clymenum*	*cly*	TUN
65990	*Lathyrus clymenum*	*Clymenum*	*cly*	TUR
65606	*Lathyrus gleospermus*	*Clymenum*	*gle*	SYR
65587	*Lathyrus gleospermus*	*Clymenum*	*gle*	SYR
108326	*Lathyrus gleospermus*	*Clymenum*	*gle*	SYR
136787	*Lathyrus gleospermus*	*Clymenum*	*gle*	SYR
65599	*Lathyrus gleospermus*	*Clymenum*	*gle*	SYR
65226	*Lathyrus ochrus*	*Clymenum*	*och*	CYP
66121	*Lathyrus ochrus*	*Clymenum*	*och*	DZA
114435	*Lathyrus ochrus*	*Clymenum*	*och*	ESP
64802	*Lathyrus ochrus*	*Clymenum*	*och*	GRC
114437	*Lathyrus ochrus*	*Clymenum*	*och*	ITA
107844	*Lathyrus ochrus*	*Clymenum*	*och*	MAR
65373	*Lathyrus ochrus*	*Clymenum*	*och*	SYR
66120	*Lathyrus tingitanus*	*Lathyrostylis*	*tin*	DZA
109122	*Lathyrus tingitanus*	*Lathyrostylis*	*tin*	TUN
66144	*Lathyrus tingitanus*	*Lathyrostylis*	*tin*	DZA
65554	*Lathyrus cilicicus*	*Lathyrostylis*	*cilic*	SYR
65571	*Lathyrus cilicicus*	*Lathyrostylis*	*cilic*	SYR
132519	*Lathyrus cyaneus*	*Lathyrostylis*	*cyn*	AZE
135235	*Lathyrus cyaneus*	*Lathyrostylis*	*cyn*	AZE
65551	*Lathyrus digitatus*	*Lathyrostylis*	*dig*	SYR
64744	*Lathyrus pallescens*	*Lathyrostylis*	*pal*	TUR
136784	*Lathyrus amphicarpos*	*Lathyrus*	*amp*	SYR
66060	*Lathyrus amphicarpos*	*Lathyrus*	*amp*	UNK
66151	*Lathyrus annuus*	*Lathyrus*	*ann*	DZA
119696	*Lathyrus annuus*	*Lathyrus*	*ann*	ESP

64757	*Lathyrus annuus*	*Lathyrus*	*ann*	PAL
65306	*Lathyrus annuus*	*Lathyrus*	*ann*	SYR
65372	*Lathyrus annuus*	*Lathyrus*	*ann*	SYR
65270	*Lathyrus annuus*	*Lathyrus*	*ann*	SYR
65533	*Lathyrus annuus*	*Lathyrus*	*ann*	SYR
65884	*Lathyrus annuus*	*Lathyrus*	*ann*	TUR
65267	*Lathyrus basalticus*	*Lathyrus*	*bas*	SYR
66023	*Lathyrus basalticus*	*Lathyrus*	*bas*	SYR
66031	*Lathyrus basalticus*	*Lathyrus*	*bas*	SYR
65828	*Lathyrus belinensis*	*Lathyrus*	*bel*	TUR
66064	*Lathyrus blepharicarpus*	*Lathyrus*	*ble*	JOR
137145	*Lathyrus blepharicarpus*	*Lathyrus*	*ble*	LBN
64986	*Lathyrus blepharicarpus*	*Lathyrus*	*ble*	SYR
65716	*Lathyrus blepharicarpus*	*Lathyrus*	*ble*	TUR
66061	*Lathyrus blepharicarpus*	*Lathyrus*	*ble*	TUR
64782	*Lathyrus cassius*	*Lathyrus*	*cas*	GRC
64978	*Lathyrus cassius*	*Lathyrus*	*cas*	IRQ
65322	*Lathyrus cassius*	*Lathyrus*	*cas*	SYR
65368	*Lathyrus cassius*	*Lathyrus*	*cas*	SYR
135427	*Lathyrus cassius*	*Lathyrus*	*cas*	SYR
65958	*Lathyrus cassius*	*Lathyrus*	*cas*	TUR
65746	*Lathyrus cassius*	*Lathyrus*	*cas*	TUR
140965	*Lathyrus chloranthus*	*Lathyrus*	*chl*	ARM
140966	*Lathyrus chloranthus*	*Lathyrus*	*chl*	ARM
126278	*Lathyrus chloranthus*	*Lathyrus*	*chl*	ARM
64725	*Lathyrus chloranthus*	*Lathyrus*	*chl*	IRN
65586	*Lathyrus chrysanthus*	*Lathyrus*	*chr*	SYR
108322	*Lathyrus chrysanthus*	*Lathyrus*	*chr*	SYR
136797	*Lathyrus chrysanthus*	*Lathyrus*	*chr*	SYR
65603	*Lathyrus chrysanthus*	*Lathyrus*	*chr*	SYR
135420	*Lathyrus chrysanthus*	*Lathyrus*	*chr*	SYR
66131	*Lathyrus cicera*	*Lathyrus*	*cic*	DZA
64868	*Lathyrus cicera*	*Lathyrus*	*cic*	GRC
64865	*Lathyrus cicera*	*Lathyrus*	*cic*	GRC
64833	*Lathyrus cicera*	*Lathyrus*	*cic*	GRC
66049	*Lathyrus cicera*	*Lathyrus*	*cic*	JOR
64987	*Lathyrus cicera*	*Lathyrus*	*cic*	SYR

65690	*Lathyrus cicera*	*Lathyrus*	*cic*	SYR
65691	*Lathyrus cicera*	*Lathyrus*	*cic*	SYR
65255	*Lathyrus cicera*	*Lathyrus*	*cic*	SYR
64990	*Lathyrus cicera*	*Lathyrus*	*cic*	SYR
65873	*Lathyrus cicera*	*Lathyrus*	*cic*	TUR
66056	*Lathyrus cicera*	*Lathyrus*	*cic*	UNK
65080	*Lathyrus ciliolatus*	*Lathyrus*	*cilio*	SYR
135411	*Lathyrus ciliolatus*	*Lathyrus*	*cilio*	SYR
136920	*Lathyrus gorgonii*	*Lathyrus*	*gor*	LBN
64743	*Lathyrus gorgonii*	*Lathyrus*	*gor*	TUR
65902	*Lathyrus gorgonii*	*Lathyrus*	*gor*	TUR
65215	*Lathyrus gorgonii*	*Lathyrus*	*gor*	JOR
63034	*Lathyrus gorgonii*	*Lathyrus*	*gor*	SYR
65695	*Lathyrus gorgonii*	*Lathyrus*	*gor*	TUR
65264	*Lathyrus heirosolymitanus*	*Lathyrus*	*hie*	SYR
65345	*Lathyrus heirosolymitanus*	*Lathyrus*	*hie*	SYR
65763	*Lathyrus heirosolymitanus*	*Lathyrus*	*hie*	TUR
66078	*Lathyrus hierosolymitanus*	*Lathyrus*	*hie*	JOR
136846	*Lathyrus hierosolymitanus*	*Lathyrus*	*hie*	LBN
65285	*Lathyrus hierosolymitanus*	*Lathyrus*	*hie*	SYR
65396	*Lathyrus hierosolymitanus*	*Lathyrus*	*hie*	SYR
65588	*Lathyrus hierosolymitanus*	*Lathyrus*	*hie*	SYR
65742	*Lathyrus hierosolymitanus*	*Lathyrus*	*hie*	TUR
65758	*Lathyrus hierosolymitanus*	*Lathyrus*	*hie*	TUR
66179	*Lathyrus hirsutus*	*Lathyrus*	*hir*	AZE
140446	*Lathyrus hirsutus*	*Lathyrus*	*hir*	AZE
66190	*Lathyrus hirsutus*	*Lathyrus*	*hir*	GEO
64764	*Lathyrus hirsutus*	*Lathyrus*	*hir*	TUN
64766	*Lathyrus hirsutus*	*Lathyrus*	*hir*	TUN
116381	*Lathyrus hirsutus*	*Lathyrus*	*hir*	TUR
66044	*Lathyrus marmoratus*	*Lathyrus*	*mar*	EGY
64982	*Lathyrus marmoratus*	*Lathyrus*	*mar*	IRQ
65526	*Lathyrus marmoratus*	*Lathyrus*	*mar*	SYR
65381	*Lathyrus marmoratus*	*Lathyrus*	*mar*	SYR
65524	*Lathyrus marmoratus*	*Lathyrus*	*mar*	SYR
65957	*Lathyrus marmoratus*	*Lathyrus*	*mar*	TUR
65964	*Lathyrus marmoratus*	*Lathyrus*	*mar*	TUR

65721	*Lathyrus marmoratus*	*Lathyrus*	*mar*	TUR
62145	*Lathyrus odoratus*	*Lathyrus*	*odo*	ITA
66029	*Lathyrus odoratus*	*Lathyrus*	*odo*	UNK
65214	*Lathyrus pseudocicera*	*Lathyrus*	*pseud*	JOR
65276	*Lathyrus pseudocicera*	*Lathyrus*	*pseud*	SYR
65862	*Lathyrus pseudocicera*	*Lathyrus*	*pseud*	TUR
65871	*Lathyrus pseudocicera*	*Lathyrus*	*pseud*	TUR
66174	*Lathyrus rotundifolius*	*Lathyrus*	*rotun*	ARM
141485	*Lathyrus rotundifolius*	*Lathyrus*	*rotun*	ARM
65223	*Lathyrus sativus*	*Lathyrus*	*sat*	CYP
66133	*Lathyrus sativus*	*Lathyrus*	*sat*	DZA
66045	*Lathyrus sativus*	*Lathyrus*	*sat*	EGY
65068	*Lathyrus sativus*	*Lathyrus*	*sat*	FRA
64903	*Lathyrus sativus*	*Lathyrus*	*sat*	GRC
107512	*Lathyrus sativus*	*Lathyrus*	*sat*	MAR
112149	*Lathyrus sativus*	*Lathyrus*	*sat*	MAR
107703	*Lathyrus sativus*	*Lathyrus*	*sat*	MAR
64720	*Lathyrus sativus*	*Lathyrus*	*sat*	TUR
65803	*Lathyrus stenophyllus*	*Lathyrus*	*sten*	TUR
65830	*Lathyrus stenophyllus*	*Lathyrus*	*sten*	TUR
64773	*Lathyrus sylvestris*	*Lathyrus*	*sylv*	DNK
140204	*Lathyrus tuberosus*	*Lathyrus*	*tuber*	TJK
66147	*Lathyrus inconspicuus*	*Linearicarpus*	*inc*	DZA
65420	*Lathyrus inconspicuus*	*Linearicarpus*	*inc*	SYR
65346	*Lathyrus inconspicuus*	*Linearicarpus*	*inc*	SYR
64999	*Lathyrus inconspicuus*	*Linearicarpus*	*inc*	TUR
65739	*Lathyrus inconspicuus*	*Linearicarpus*	*inc*	TUR
65395	*Lathyrus sphaericus*	*Linearicarpus*	*sph*	SYR
65839	*Lathyrus sphaericus*	*Linearicarpus*	*sph*	TUR
65875	*Lathyrus vinealis*	*Linearicarpus*	*vin*	TUR
65881	*Lathyrus vinealis*	*Linearicarpus*	*vin*	TUR
65883	*Lathyrus vinealis*	*Linearicarpus*	*vin*	TUR
65915	*Lathyrus vinealis*	*Linearicarpus*	*vin*	TUR
63389	*Lathyrus nissolia*	*Nissolia*	*niss*	SYR
65529	*Lathyrus nissolia*	*Nissolia*	*niss*	SYR
65443	*Lathyrus setifolius*	*Orobastrum*	*set*	SYR
65445	*Lathyrus setifolius*	*Orobastrum*	*set*	SYR

66016	*Lathyrus setifolius*	*Orobastrum*	*set*	SYR
65800	*Lathyrus setifolius*	*Orobastrum*	*set*	TUR
65434	*Lathyrus occidentalis*	*Orobus*	*occi*	SYR
66167	*Lathyrus laxiflorus subsp. laxiflorus*	*Preatensis*	*lax*	GEO

Appendix 2. Morphological character set

Character	Abbrev.	Description	States
CH1	LF	Life form	1. annual; 2. biennial; 3. perennial
CH2	PLSTAT	Plant Status	1. sturdy; 2. slender to sturdy; 3. slender; 4. rigid
CH3	GH	Growth habit	1.erect; 2. ascending; 3. prostrate; 4. procombent
CH4	Veg. Pub.	Vegetative pubescence	1. glabrous; 2. glabrescent
CH5	HRTY	Type of hair	1. glaucous; 2. pilous; 3. villose; 4. no hairs
CH6	PL.HT	Plant height/cm.	In cm
CH7	STMSH	Stem shape	1. winged; 2. terete; 3. ridgid; 4. angled
CH8	LFTST	Leaflet status	1. present; 2. reduced
CH9	NLFT/LF	Number of Leaflets per leaf	Number
CH10	LFTARR	Leaflet arrangement	1. paripinnate; 2. subdigitate; 3. pinnate; 4. phyllodic; 5. sub-sessile; 6. reduced
CH11	LFRAC	Leaf rachis	1. laminate; 2. not laminate
CH12	RACEND	Rachis ends in	1. murco; 2. tendril; 3. aristate
CH13	LFTSH	Leaflet shape	1. linear; 2. elliptic; 3. oblong; 4. lanceolate; 5. obovate; 6. ovate; 7. sub-orbicular; 8. spatulate; 9. tendrillous
CH14	LFTAPSH	Leaflet apex shape	1. mucronate; 2. acute; 3. emarginate; 4. acuminate; 5. subobtuse; 6. obtuse; 7. undulate-margined; 8. aristate; 9. absent
CH15	LFTLN	Leaflet length/mm.	mm
CH16	LFTWD	Leaflet width/mm.	mm
CH17	LFTVN	Leaflet venation	1. pinnate; 2. parallel; 3. reticulate; 4. not applicable
CH18	LFTHR	Leaflet hairiness	1. glabrous; 2. glabrescent; 3. pubescent; 4. gland dotted on lower face; 5. not applicable
CH19	STPSH	Stipule shape	1. subulate; 2. lanceolate; 3. ovate; 4. oblong; 5. suborbicular; 6. triangular; 7. filiform
CH20	STPBS	Stipule base shape	1. hastate; 2. semi-hastate; 3. sagittate; 4. semi-sagittate; 5. variable
CH21	STPMRG	Stipule margin	1. entire; 2. dentate; 3. incised; 4. variable
CH22	STPPUB	Stipule pubesent	1 glabrous; 2 pubescent
CH23	STPLN	Stipule length mm.	mm
CH24	STPWD	Stipule width mm.	mm

CH25	STPLNPL	Stipule length/petiol length	1. shorter than petiol length; 2. equal to petiol length; 3. longer than petiol length; 4. sub-eqaul to petiol length; 5. not applicable
CH26	PEDLLN	Peduncle length/mm.	mm
CH27	PDNLNLFL	Peduncle length/leaf length	1. shorter than leaf length; 2. equal to leaf length; 3. longer than leaf length; 4. not applicable
CH28	PDCLN	Pedicle length mm.	mm
CH29	FLNO	Flowers number	mm
CH30	PETCLR	Flower petal colour	1. concolorous; 2. not concolorous
CH31	CORCLR	Corolla colour	1. white; 2 .cream; 3. yellow; 4. orange; 5. pink; 6. brick-red; 7. blue; 8. violet; 9. purple
CH32	FLLN	Flower length/mm.	mm
CH33	STDLN	Standard length/mm.	mm
CH34	STDVNN	Standard vein number	1. absent; 2. 3-5 veins; 3. more than 5 veins
CH35	STDAPSH	Standard apex shape	1. strongly emarginated; 2. emarginated; 3. emarginated with mucro; 4. obtuse
CH36	WNGCLR	Wing colour	1. white; 2 .cream; 3. yellow; 4. orange; 5. pink; 6. brick-red; 7. blue; 8. violet; 9. purple
CH37	WNGLN	Wing length/mm.	mm
CH38	WNGLMBLN	Wing limb length/mm.	mm
CH39	WNGLMBWD	Wing limb width/mm.	mm
CH40	WNGCLLN	Wing claw length/mm.	mm
CH41	KEELLN	Keel length/mm.	mm
CH42	CLXLN	Calyx length/mm.	mm
CH43	CLXTHLN	Calyx teeth length/mm.	mm
CH44	CLXBLN	Calyx base length/mm.	mm
CH45	CLXBSH	Calyx base shape	1. gibbous; 2. not gibbous
CH46	CLXTH	Calyx teeth	1. equal; 2. unequal
CH47	CLXTHOR	Calyx teeth orientation	1. straight; 2. reflexed
CH48	CLXHR	Calyx hairs	1. glabrous; 2. glabrescent; 3. pubescent
CH49	CLTHLNTBLN	Calyx teeth length/tube length	1. shorter than tube; 2. equal the tube length; 3. longer than tube
CH50	CLXLWTHTBLN	Calyx lowest teeth length/ tube length	1. lowest tooth shorter than tube; 2. lowest tooth equal to tube; 3. lowest tooth longer than tube
CH51	STYLN	Style length/mm.	mm

CH52	STYCON	Style contortion	1. straight; 2. twisted
CH53	STYSH	Style shape	1. linear; 2. oblong; 3. spathulate; 4. canaliculate; 5. arcuate
CH54	OVRSH	Ovary shape	1. linear; 2. intermediate; 3. oblong
CH55	OVRLN	Ovary length/mm.	mm
CH56	OVRWD	Ovary width/mm.	mm
CH57	LEGOR	Legume orientation	1. straight; 2. beaked; 3. incurved
CH58	LEGSH	Legume shape	1. linear; 2. oblong; 3. canescent
CH59	LEGLN	Legume length/mm.	mm
CH60	LEGWD	Legume width/mm.	mm
CH61	LEGHR	Legume hairiness	1. glabrous; 2. glabrescent; 3. pubescent; 4. tomentose
CH62	LEGDEH	Legume at matuirity	1. dehiscent; 2. indehiscent
CH63	LEGVLV	Legume valve	1. hairy; 2. not hairy
CH64	LEGVLVPTR	Legume valve pattern	1. reticulate-nerved; 2. obscurely-nerved; 3. gland-dotted; 4. tuberculate; 5. longitudinally-nerved; 6. glandular-verrucose; 7. obliquely-nerved; 8. tuberculate-pilose; 9. eglandular; 10. glabrous
CH65	UPLEGSUT	Upper legume suture	1. broadly winged; 2. narrowly-winged; 3. not 2-winged
CH66	SUTTYP	Suture type	1. keeled; 2. canaculate
CH67	SDSURF	Seed surface	1. smooth; 2. tuberculate; 3. reticulate; 4. coarsely-tuberculate; 5. ruminate-rugulose; 6. punctate; 7. verrucose; 8. viscose; 9. pappilose
CH68	SDNOPD	Seed number/pod	number
CH69	HILLN	Hilum length/mm.	mm
CH70	SDDIA	Seed diameter/mm.	
CH71	LNSHIL	Relation of lens to hilum	
CH72	SDCLR	Seed colour	1. white; 2. yellow; 3. grey; 4. brown; 5. purplish-brown; 6. purple; 7. blackish; 8. dark brown; 9. dark- green
CH73	SDSH	Seed shape	1. compressed; 2. round; 3. angular; 4. oval; 5. cubical; 6. globose
CH74	LWPDSUT	Lower Suture of pod	1. ciliate; 2. not ciliate
CH 75	AMPH	Amphicarpic pod	1. yes; 2. no

CHAPTER FOUR

ECOGEOGRAPHIC SURVEY AND GAP ANALYSIS FOR DIFFERENT SECTIONS OF *LATHYRUS* L.

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4.1 Abstract

The genetic diversity of the genus *Lathyrus* is of significant importance, particularly for its role in sustaining the livelihoods of local communities living under very harsh conditions and its potential to adapt to climate change. Grass pea (*L. sativus* L.) is the most widely used species and to a lesser extent *L. cicera* and *L. ochrus*, all used for both feed in many parts of the world and food in poor regions, but the over-consumption of seeds could lead to lathyrism disease caused by neurotoxins. The continuation and the expansion of cultivation of *Lathyrus* species are tightly linked to the ability of breeders to access genetic resources to solve the problem of lathyrism and other biotic and abiotic constraints. This study has added substantial information and accuracy to the existing global *Lathyrus* database by combining diverse multiple datasets and by adding information of major herbaria from Europe. This Global *Lathyrus* database, available at ICARDA, was used to conduct gap analysis to guide future

collecting missions and *in situ* conservation efforts for 37 species originating from the Mediterranean Basin, and Caucasus, Central and West Asia region. The results showed the highest concentration of *Lathyrus* priority species in the countries of the Fertile Crescent, France, Italy and Greece. The region extending from South-Central Turkey, through the western Mediterranean mountains of Syria to the northern Bekaa valley in Lebanon, and precisely the area around the Lebanese / Syrian border near Tel Kalakh region in Homs, was identified as the hotspot and the overall priority location for establishing genetic reserves. The gap analysis for *ex situ* conservation shows that only 6 species (representing 16.6%) of the 37 priority species are adequately sampled. Only *L. cicera*, has already been well sampled among the closely related species to cultivated species *L. sativus*, showing the need for more collecting missions in the areas underrepresented, and for collecting closely related wild species such as *Lathyrus amphicarpos*, *L. belinensis*, *L. chrysanthus*, *L. hirticarpus* *L. hirsutus* and *L. marmoratus*. In addition, six priority *Lathyrus* species have no *ex situ* collections *(L. lentiformis*, *L. lycicus*, *L. phaselitanus*, *L. trachycarpus*, *L. tremolsianus* and *L. undulatus*) requiring also further targeted *ex situ* collecting. Future collecting missions could also be targeting useful adaptive traits.

Keywords: *Lathyrus*, *ex situ* conservation, *in situ* conservation, gap analysis, Mediterranean Basin, Central and West Asia.

4.2 Introduction

Although national, regional and international efforts are ongoing for collecting and conserving *ex situ* the genetic resources, new approaches are needed to fill the gaps in the existing collections (Amri *et al.*, 2008, unpublished report). There has been relatively little effort in conserving *in situ*/on-farm the landraces and wild relatives of major crops as these

were not targeted with most of the existing genetic reserve (Guarino *et al.*, 1995; Hawkes *et al.*, 2000, Amri *et al.*, 2008 unpublished report).

One such novel approach to help prioritise conservation action is genetic gap analysis. As stated in Jenenings, 2000, Burley (1988) proposed four steps to identify the gaps in conservation efforts: (1) identifying and classifying biodiversity; (2) locating areas managed primarily for biodiversity; (3) identifying biodiversity that is under-represented in the managed areas; (4) setting priorities for conservation action (Jennings, 2000). The approach of conservation gap analysis proposed by Maxted *et al.* (2008a) is based on comparing natural diversity with current conservation actions to identify the gaps to revise the conservation strategy. He recommended four steps for gap analysis starting with identification of priority taxa, identification of ecological breadth and complementary hotspots using distributional data, matching the identified ecogeographic breadth and complementary hotspots with the existing conservation actions, and ending with the formulation of a revised *in situ* and *ex situ* conservation strategy. Gap analysis can also be applied to taxonomic and genetic diversity and its distribution in existing wild populations, as illustrated in the gap analysis of cowpea *Vigna unguiculata* and its wild relatives from Africa (Maxted *et al.,* 2004). There is now an extensive literature associated with gap analysis and broader conservation evaluation techniques in ecosystem conservation, which essentially identifies areas in which selected elements of biodiversity are under-represented (Margules, 1989; Margules and Pressey, 2000; Balmford, 2003; Brooks *et al.*, 2004; Dietz and Czech, 2005; Riemann and Ezcurra, 2005). Maxted *et al.* (2009) conducted the gap analysis for six genera *Cicer, Lathyrus, Lens, Medicago, Pisum* and *Vicia* in the Mediterranean region and found that their biodiversity hotspots were identified in the Syrian/Lebanese border which is not covered by any of the existing internationally recognised protected areas.

The *Lathyrus* gene pool is an ideal candidate for this application of a gap analysis due to its adaptation to harsh environments and the agricultural importance of some species, such as grass pea as food and feed for poor people. A review of *ex situ* conservation efforts of *Lathyrus* was done through the *Lathyrus* conservation strategy undertaken in 2007 by the Global Crop Diversity Trust in collaboration with ICARDA (GCDT, 2007), which holds the second largest collection in the world. The model of plant genetic conservation (Maxted *et al.*, 1997c) was applied to develop an efficient strategy for *in situ* and on-farm conservation of *Lathyrus* (Maxted *et al.*, 2003). The ecogeographic distribution of *Lathyrus* species is poorly understood and was provisionally studied by Baggott (1997). GIS tools are extensively used to define biodiversity hotspots, including Diva-GIS (Hijmans *et al,.* 2005) and FloraMap softwares, used extensively by Jones and Gladkov (1998) and Maxted *et al.* (2004).

The objective of this paper is to present a genetic gap analysis for *Lathyrus* species to guide future complementary efforts of *in situ* and *ex situ* conservation at national and international levels.

4.3 Materials and Methods

4.3.1 Collating of existing taxon level data

The study group is the *Lathyrus* species from the Mediterranean Basin and the Caucasus, Central and West Asia regions. Significant digitized ecogeographic datasets from the International Center for Agricultural Research in the Dry Areas (ICARDA) and Global Biodiversity Information Facilities (GBIF) as well as datasets collected by the author were used for this study. As recommended by Maxted *et al.* (2006), the species to concentrate on were those defined using gene pools (GP) and/or taxon groups (TG) concepts, where the closest *Lathyrus* species would be found in GP1B and GP2, or if gene pool distinction were

unavailable, in TG1b and TG2. A total of 37 species with 18,147 accessions were used in this study, excluding all accessions of *Lathyrus sativus* (grass pea) because *L. sativus* is belongs to GP1 (Maxted *et al.*, 2009).

4.3.2 Collecting of existing accession level data

The total data used in this study were derived from 61,081 unique herbarium and germplasm accessions of 97 *Lathyrus* species, and 18,147 unique herbarium and germplasm accessions of 37 priority species. The *Lathyrus* ecogeographic data were obtained from eight datasets, mainly from ICARDA, GBIF, Global *Lathyrus* and from personal ecogeographic surveys in seven major international herbaria (the Royal Botanic Gardens in Kew, UK, the Royal Botanic Gardens in Edinburgh, UK, the Natural History Museum, London, UK, the Natural History Museum, Paris, France, the University of Montpellier, France, the Botanic Gardens in Geneva, Switzerland, and Florence University in Italy). The largest group of data came from the Global Biodiversity Information Facility (http://www.gbif.org/), the Global Database of *Lathyrus* collection (ICARDA, Syria), with additional data from different collections by Nigel Maxted, and from several ecogeographic surveys of food and forage legumes undertaken jointly by the International Center for Agricultural Research in the Dry Areas (ICARDA) and the University of Birmingham from 1998 to 2010. The lead author has visited the seven herbaria and examined all *Lathyrus* specimens available. Datasets are freely available from the author on request, and a summary is provided in Appendix 1.

4.3.3 Data processing

Data were standardized to a single format and duplicate observations identified and removed to avoid bias in the final results. In addition, occurrences identified as being outside of the natural range of the species were considered to be introductions and therefore were not

considered in the final analysis. Where latitude and longitude and location name were missing, these records were also removed. In addition, the distribution of all the geo-referenced data was checked using the ArcView GIS version 3.3 to identify and fix the outliers. Each data field was indexed, and errors and invalid entries were manually corrected. Basic statistics describing the taxonomic, geographic, curatorial and ecological data were derived for the database content. The combined, corrected dataset of *Lathyrus* species accessions was then spatially analyzed. In addition to the entire dataset of accessions of all species in the genera, a second priority set of accessions was produced using gene pool and taxon group concepts for the crops present in the genera to identify the closest crop wild relatives (CWR) species. The final dataset included 37 species with a total of 18,147 accessions.

4.3.4 Spatial Analysis

ArcView GIS 3.3 program was used to produce distribution maps from the *Lathyrus* dataset of 97 species, represented by 61,081 accessions as the main dataset, and the distribution maps of the thirteen sections of genus *Lathyrus* and of the 37 priority species. *In situ* and complementarity analysis of species richness (identifying complementary areas to conserve the maximum number of species) was carried out using DIVA-GIS version 7.1.7 (www.diva-gis.org). Species richness (Hijmans *et al.,* 2005) was used to map the distribution of species and to identify hotspots of species diversity within each section. Secondly, putative reserves were selected using the iterative reserve selection method implemented in DIVA-GIS (Hijmans *et al.* 2005), which identifies the minimum number of 100 x 100 km^2 grid cells that will capture the maximum number of species.

4.3.4.1 *Ex situ* conservation gap analysis

In addition to ArcView version 3.3, DIVA-GIS version 7.1.7 (www.diva-gis.org) and the global climatic data with 2.5 min resolution (diva_worldclim_2-5m.zip) were used in the Bioclim method (Hijmans *et al.*, 2005) to produce predictive distribution maps based on the climatic data. For each *Lathyrus* taxon, a comparison was made between the distribution map based on the actual *ex situ* germplasm accession data, the herbaria information and the predicted distribution maps generated from their climatic envelope data. *Ex situ* conservation gaps were identified as regions where the species was predicted to occur but had not been previously collected, or areas predicted to be under sampled. The level of *ex situ* conservation priority for each of the *Lathyrus* species was ranked (high, medium and low) as follows: High priority: Species with, 200 germplasm accessions conserved *ex situ* and/or species for which *ex situ* collections inadequately represented their geographic range with several predicted under-sampled regions; Medium priority: Species well represented in *ex situ* collections across their geographic range, with only a few predicted under-sampled regions, but with <500 germplasm accessions conserved *ex situ*. Low priority: Species well represented throughout their geographic range with more than 500 accessions conserved *ex situ* and only a few, if any, under-sampled areas predicted.

4.3.4.2 *In situ* species richness and complementarity analysis

The DIVA-GIS software was used to identify the optimal locations for the establishment of future *in situ* reserves required to conserve the maximum species diversity within the *Lathyrus* genus. The method of 'number of different classes (richness)' (Hijmans *et al.,* 2005) was used to map the distribution of species richness in order to identify hotspot regions. The circular neighborhood point-to-grid method was selected with a default cell size of 18 resolutions. The 'number of observations' method (Hijmans *et al.*, 2005) was also used in DIVA-GIS, to map the density of germplasm collections for all *Lathyrus* species to avoid the

bias of not selecting randomly the accessions. DIVA-GIS was used to study species complementarity using the iterative procedure (Rebelo and Siegfried, 1992; Rebelo, 1994) in the 'reserve selection' manner described by Hijmans *et al.* (2005), which identifies the minimum number of 100 x 100km^2 grid cells that are complementary to each other in maximizing conserved *Lathyrus* diversity, assuming that that when recommending a site for the establishment of a genetic reserve for CWR species, it will be preferably within an existing protected area (Maxted *et al.*, 1997; Heywood and Dulloo, 2006; Iriondo *et al.*, 2008). But *Lathyrus*, like many other CWR species, are located both within and outside existing protected areas. It is preferable to select existing natural reserves where the targeted species are already managed over a long period, or areas where it is relatively easy to amend the existing site management to facilitate genetic conservation of CWR species, in order to avoid the large costs and social problems associated with the establishment of new reserves (Iriondo *et al.,* 2008). As such, DIVA-GIS was used to compare the distribution of complementary hotspot sites with the World Database on Protected Areas (WDPA–www.unep-wcmc.org). A spatial comparison is conducted between the complementary hotspots identified, and the WDPA highlights potential national protected areas in optimal locations for the establishment of the active *in situ* conservation of *Lathyrus* priority species.

4.3.5 Production of ecogeographic report and conspectus

The foundation of the ecogeographic survey was primarily literature based with additional data collated from the passport data of herbarium specimens and several databases. The main purpose of the specimen survey was to collect taxonomic information and to fill the gaps in species descriptions and in the selected characters. The survey covered *Lathyrus* species in the Mediterranean Basin and Caucasus, Central and West Asia regions. Each specimen has

been characterized to obtain the morphological and botanical characters, as well the ecological and geographical information.

The herbarium specimen and gene bank accession data were collated directly into a database to facilitate data checking and analysis and also to avoid transcription errors. The basic structure of the database file is shown in Table 4.1 with an explanation of the content of the fields.

Table 4.1. Field structure and content of the ecogeographic database.

Field	Data Type	3 Field Name	Field Description
1	Taxonomic	SECTION	*Lathyrus* section to which species belongs
2		SPECIES	Accepted *Lathyrus* species name
3		SUBSPECIES	Subspecies name, if appropriate
4		VARIETY	Varietal name, if appropriate
5	Curatorial	H_OR_G	Whether herbarium specimen or gene bank accession
6		COLLECTION	Collection where herbarium specimen or gene bank accession was located, herbarium codes following Holmgren *et al.* (1990)
7		COLLECTOR	Name of collector(s)
8		COLL_NOS	Number given by collector to specimen
9		COLL_DATE	Collection date
10	Descriptive	FLOWERS	Flower: present / absent
11		FLOWER_COL	Color of flower
12		FRUIT	Fruit: present / absent
13	Geographic	COUNTRYCOD	Country code
14		PROVINCE	Province
15		TOWN	Name of nearest town
16		LOCALITY	Name of nearest settlement
17		DISTANCE	Distance from nearest town
18		DIRECTION	Direction from nearest town
19		LATITUDE	N = +; S = -

20		LONGITUDE	E = +; W = -
22	Ecological	ELEVATION	Height in meters
23		HABITAT	Ecological habitat where specimen found
24		VEGETATION	Vegetation type at site of collection
25		SOIL_COLOU	Color of soil where specimen found
26		SOIL_TEXTU	Texture of soil where specimen found
27		SITE_STONI	Stoniness / rockiness where specimen found
28		PARENT_ROC	Type of parent rock
29		SLOPE	Slope of ground
30		ASPECT	Aspect of collection site
31		EXPOSURE_T	Degree of openness of site
32		DRAINAGE	E (excessive) / G (Good) / M (Moderate) / P (Poor)
33		LAND_USE	Principle use of land
34		BIOTIC_FAC	Any noted biotic interaction with site where the specimen was found
35		ABIOTIC_FA	Any noted abiotic interaction with site where the specimen was found
36		FREQUENCY	Estimation of population size at site where the specimen was found

The database was indexed (*i.e.* the records were rearranged in alphabetical or numerical order) on each field in turn to highlight typing errors or invalid entries. Exploratory mapping using the latitude and longitude fields also revealed location errors; specimens placed on the sea or from a different geographical unit, were corrected whenever possible.

4.4 Results

4.4.1Data content

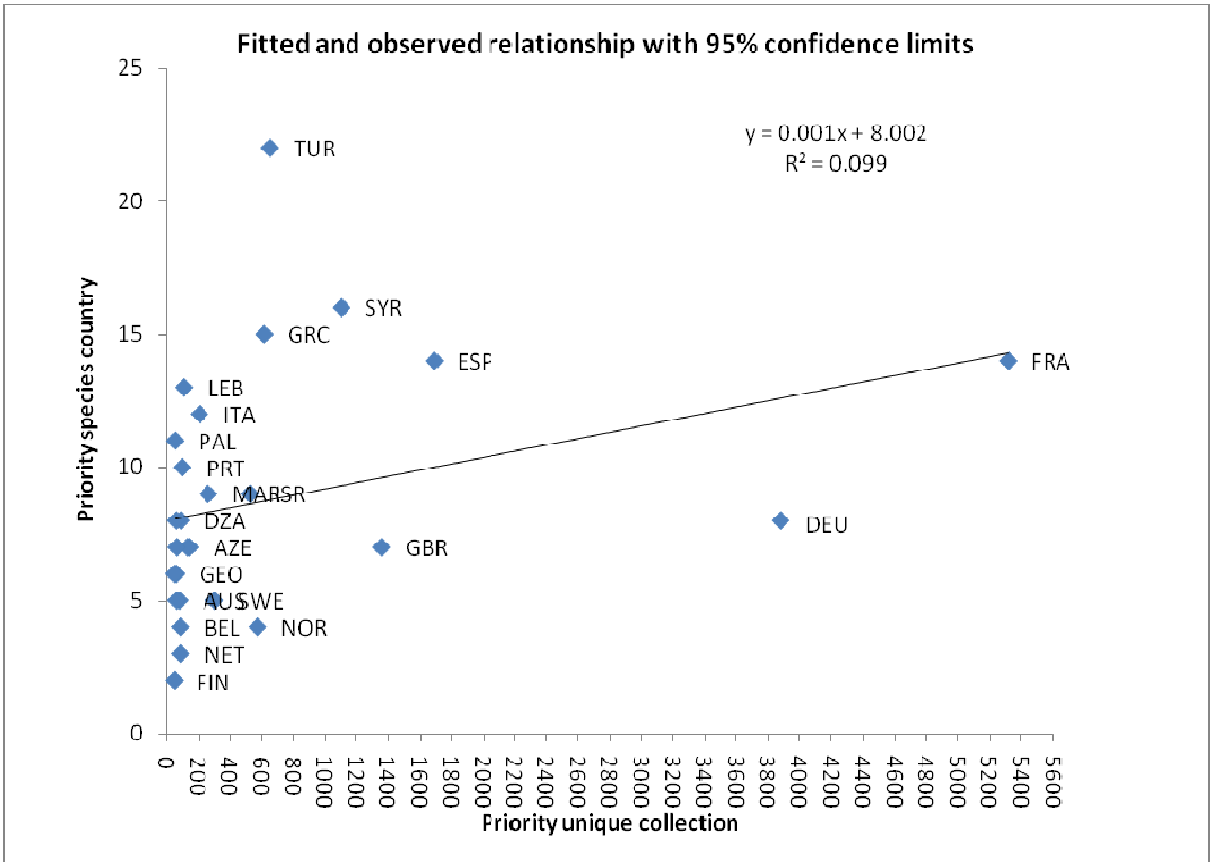
The general dataset revealed that the most frequently recorded species were: *Lathyrus pratensis* (16,567), *L. linifolius* (10,183), *L. sylvestris* (3992), *L. tuberosus* (3563), *L. aphaca* (3538), *L. vernus* (3313), *L. latifolius* (3,176), *L. niger* (2536), *L. nissolia* (1666), *L. hirsutus* (1532) and *L. cicera* (1321), which reflects their frequent and widespread distribution; together they account for 83.8% (total of 51,208 accessions) (see Appendix 1). Among these common species, only *L. sylvestris*, *L. tuberosus*, *L. latifolius*, *L. hirsutus* and *L. cicera* are among the crop wild relatives species of *Lathyrus* L.. There are 31 rare and restricted species with less than ten known records; including: *L gloeospermus* (9), *L. belinensis* (5), *L. lycicus* (4), *L. hirticarpus* (4), *L. undulatus* (4), *L. trachycarpus* (2), *L. phaselitanus* (2), and *L. lentiformis* (1). It is to be noted that the numbers above reflect unique accessions as duplicates were removed.

4.4.2 Geographic distribution

It is well known that *Lathyrus* is one of the genera that has a Mediterranean-Western Asiatic centre of species diversity (Maxted and Bennet, 2001); it also has secondary centers in South America (Kupicha, 1976, 1983). The analysis of all collections of *Lathyrus* showed that 10.3% (6,318 unique accessions) were collected in Central and West Asia and North Africa region (CWANA), 89.5% (54,677 accessions) from Europe, and less than one percent (86 accessions) from southern and East Asia, and Africa. However, this can be attributed to intensive collecting efforts and data availability rather than true species concentration. If the numbers of species in each region are considered, there are 77 species and 31 priority species present in CWANA, 61 species and 25 priority species present in Europe, and 8 species and 1

priority species present in southern and East Asia. So although there are significantly more collections available with geo-referenced data for Europe than CWANA, the highest concentration of all species and priority species is clearly in CWANA and fewer collections and species are found in southern and East Asia, and Africa. The seven countries with the highest number of species were Turkey (57), Spain (43), France (39), Syria (30), Greece (30), Russian Federation (29) and Lebanon (23), while for priority species they were Turkey (22), Syria (16), Greece (15), Spain (14), France (14) and Lebanon (13) (Appendix 2). However, even absolute numbers of species masks concentration; for example, although Syria has a relatively high number of species (including priority species), they are restricted to a relatively small part of the country, mainly in semi-arid and humid regions, compared to Turkey where the species distributions are more evenly spread throughout the whole country.

Under-estimation of species richness in the under-sampled areas can also come from unequal sampling across a species' native range (Maxted *et al.*, 2004). This was tested using regression analysis of the number of priority *Lathyrus* species recorded in each country and the number of accessions collected from that country. The regression line ($y = 8.0027 + 0.0012 \log_{10}X$) with 95% confidence intervals is presented in Figure 4.1. For clarity in understanding the figure, country labels have only been added for outlying countries. Figure 4.1 showed that none of the countries rich in *Lathyrus* species can be considered over-sampled, recommending more collecting missions in Turkey, Syria, Spain, Greece and Lebanon to find additional diversity. France, Germany and the United Kingdom are shown to be well represented and further collection should not be a priority.



AZE: Azerbaijan, AUT: Austria, BEL: Belgium, DEU: Germany, DZA: Algeria, ESP: Spain, FIN: Finland, FRA: France, GBR: Great Britin, GEO: Georgia. GRC: Greece, ITA: Italy, ISR: Israel, NET: Netherlands, NOR: Norway, LEB: Lebanon, MAR: Morocco, PAL: Palestine, PRT: Portugal, SWE: Sweden, SYR: Syria, TUR: Turkey.

Figure 4.1. Regression of *Lathyrus* priority species against the number of accessions collected from each country

4.4.3 Species richness and complementarity analysis

Both species richness and complementarity were analyzed for all *Lathyrus* species, as well as for the priority species. Also, the species richness and the complementarity were analyzed for all sections of *Lathyrus* (Figures 2-5). Figure 4.2 shows that the *Lathyrus* species are distributed from North Western Europe/North Africa to Central Asia, Afghanistan and India, however, the highest species concentration is found in both the Iberian Peninsula and the Fertile Crescent regions. Complementarity analysis for all *Lathyrus* showed that the major

diversity hotspots are found in North East Spain, with 17-21 species, and around Tel Kalakh in Homs Province in Syria, with 14-17 priority *Lathyrus* species, followed by the locations in eastern Central Turkey and in Palestine (Figure 4.3).

Figure 4.4 highlights species richness for priority *Lathyrus* species in Western Europe through to Central Asia and Afghanistan, with the highest priority species concentration found mainly in the Fertile Crescent region. Based on complementarity analysis of priority species, their major diversity hotspot is found around Tel Kalakh in Homs Province in Syria, with 10-12 priority species, and in eastern Central Turkey in the regions of Elazig and Diyarbakir and in Palestine with 3-5 species (Figure 4.5).

Both species richness and complementarity analysis were conducted for all species within the sections *Aphaca*, *Clymenum, Linearicarpus, Lathyrostylis, Lathyrus, Orobus, Orobon, Orobastrum, Nissolia, Neurolobus, Notolathyrus, Pratensis* and *Viciopsis* of genus *Lathyrus.*

Figure 4.6 shows that species in section *Aphaca* are distributed over Western Europe through Central Asia and Afghanistan, with the remarkable species concentration found in the Fertile Crescent, and the location from complementarity analysis was found in Kasab, northern Syria by the Turkish border (Figure 4.7). Figure 4.8 highlights the species richness for all species of the section *Clymenum* spreading over Western Europe and North Africa through Greece, the Aegean Sea to the Fertile Crescent regions, with the northeast of Spain as the location identified by complementarity analysis, with 1-3 species present (Figure 4.9). Figure 4.10 shows the species richness for all section *Linearicarpus* species in Western Europe through the Aegean Sea region to Afghanistan, with a high concentration in the Caucasus region and in the north of Spain as the locations identified by the complementary

analysis, with 2-3 species (Figure 4.11). The results of the species richness and complementary analysis for all the species belonging to the section *Lathyrostylis* show the species distribution in western Europe through the Aegean Sea region to the Caucasus region, with the highest species concentration found in the Fertile Crescent region and the priority hotspot in Osmaniye Province in Turkey, with 2-3 priority species (Figures 12 and 13). Figure 4.14 shows the species richness for section *Lathyrus* species with a broad distribution ranging from the UK, Spain and Morocco in the west, through the Aegean Sea, Fertile Crescent and Central Asia regions, to Afghanistan and Pakistan in the East, with a scattering of collections throughout Europe and a clear species concentration in the Fertile Crescent. Based on complementarity analysis, the hotspot for the section *Lathyrus* is located in the vicinity of Tel Kalakh in Homs Province in Syria, with 10-12 species (Figure 4.15). For the section *Orobus*, the species are found in Western and Northern Europe with scattered collections in Central Europe and the Caucasus region (Figure 4.16), with the hotspot located in North Spain, where 6-7 species are present (Figure 4.17). Figure 4.18 highlights the species richness for all species of *Pratensis* section in Western and Northern Europe through the Aegean and Central Asian regions and with scattered collections in the Caucasus region and highest concentration in North Turkey. The hotspot location for this section identified using complementary analysis is in North Turkey in Kastambuli province with 2-3 species present (Figure 4.19). The sections *Nissolia, Neurolobus, Notolathyrus, Orobon, Orobastrum and Viciopsis*, contain relatively small numbers of species, and none of them are priority species. The distributions of these sections are presented in Figure 4.20.

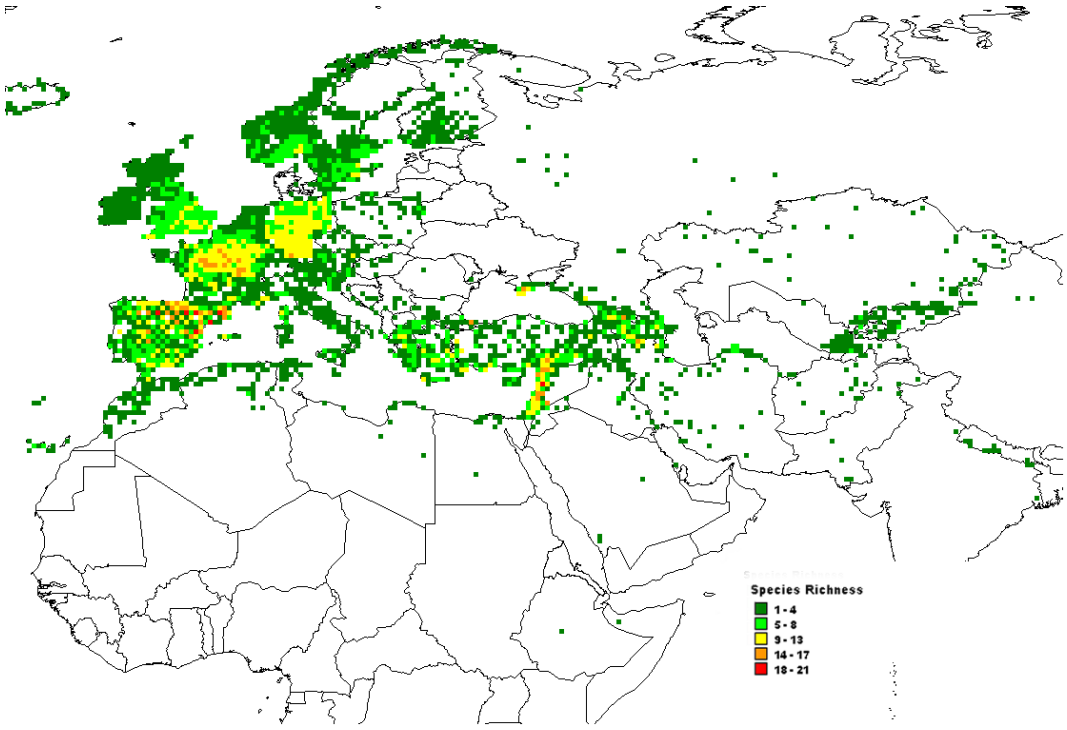


Figure 4.2. Species richness for all unique accessions of *Lathyrus* species in 100 x 100 km grid cells

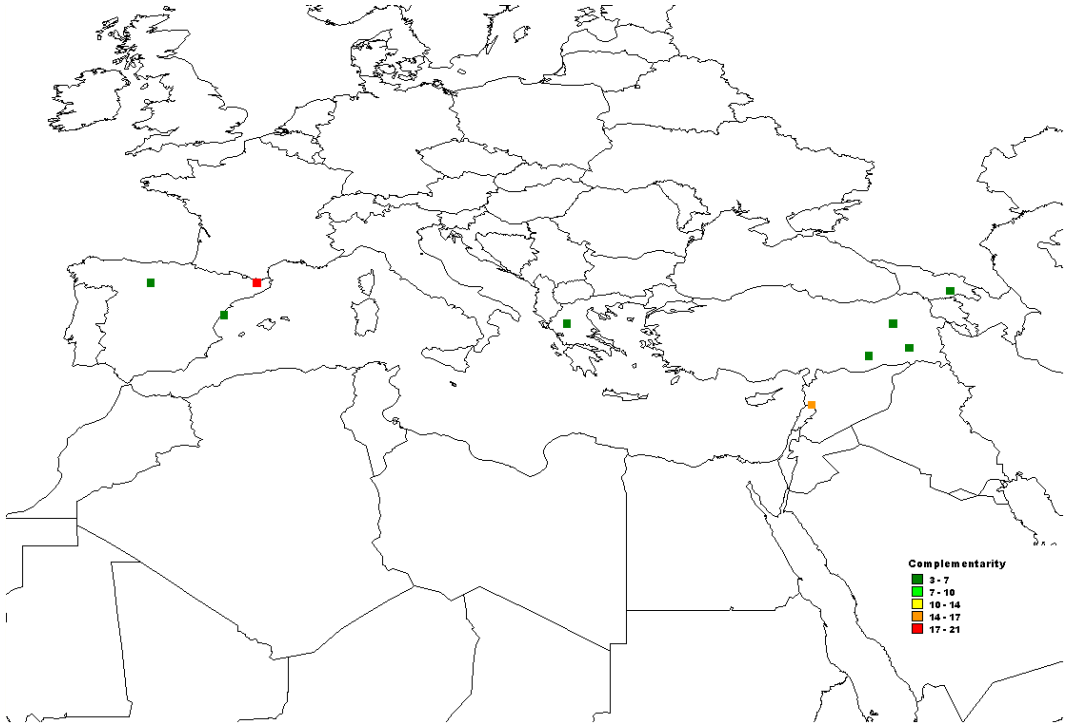


Figure 4.3. Location of *Lathyrus* species diversity hotspots identified using complementarity analysis

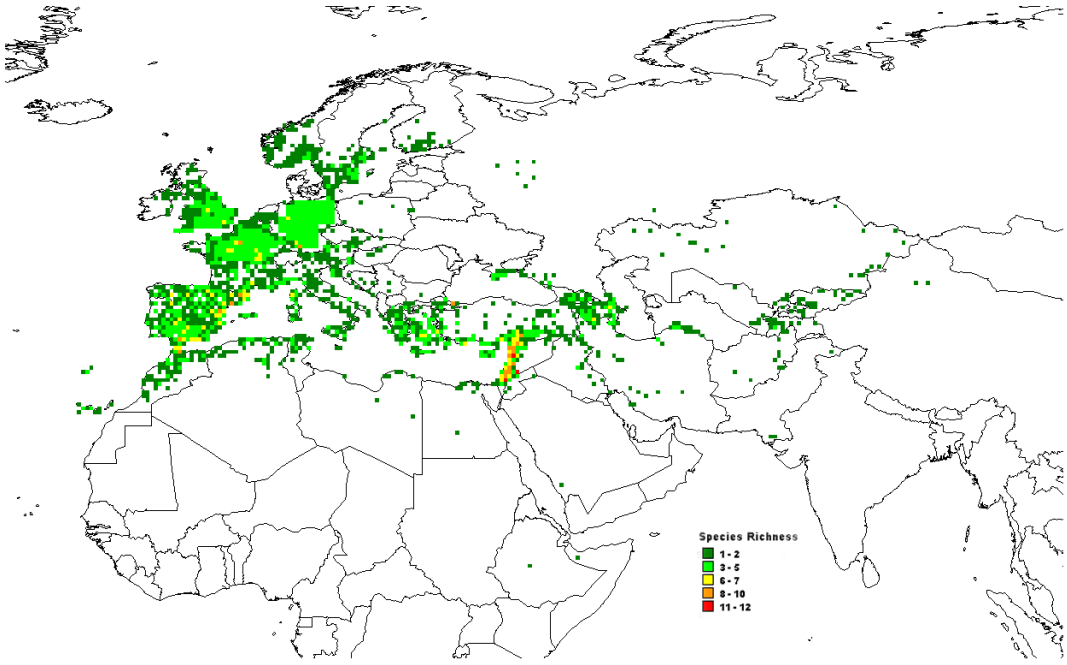


Figure 4.4. Species richness for priority *Lathyrus* species in 100 x 100 km grid cells

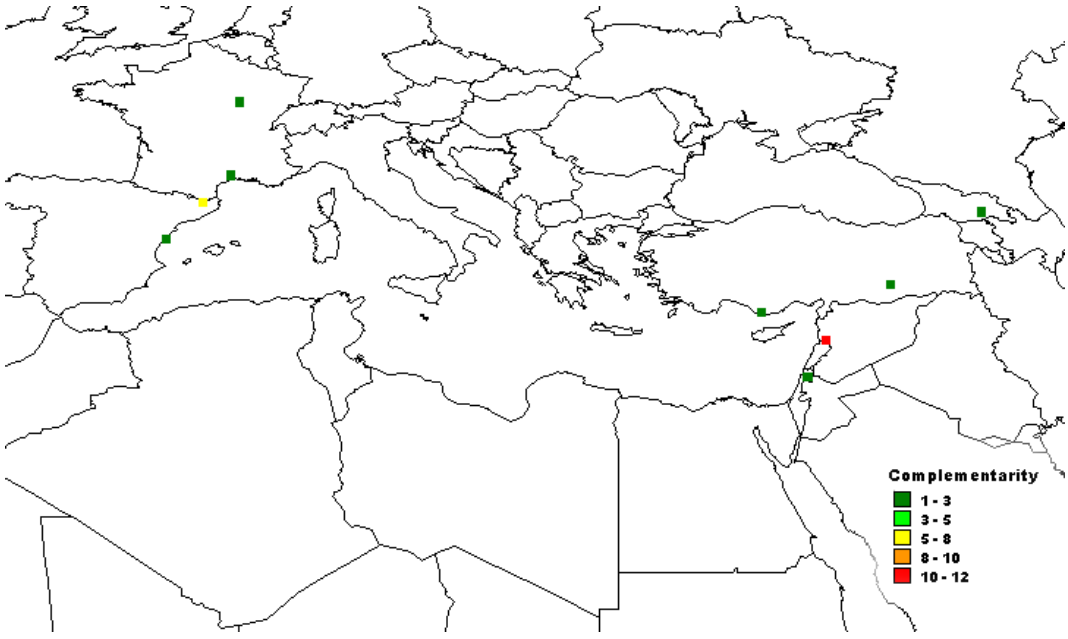


Figure 4.5. Location of hotspots of priority *Lathyrus* species diversity identified using complementarity analysis

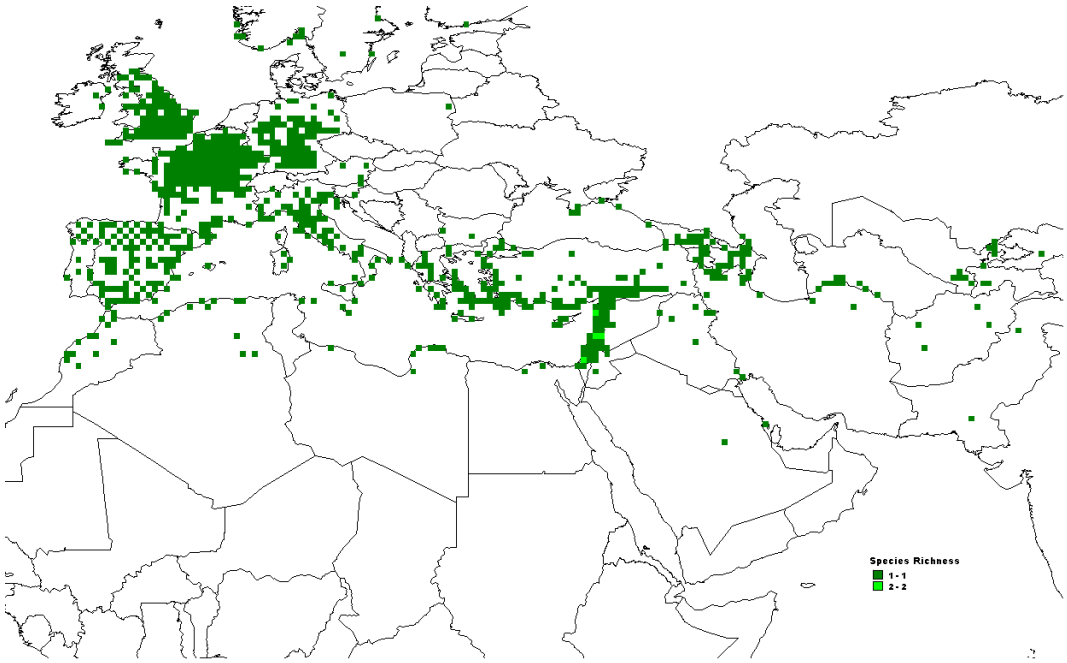


Figure 4.6. Species richness for priority species of the section *Aphaca* in 100 x 100 km grid cells

Figure 4.7. Location of hotspot of species of *Aphaca* section using complementarity analysis

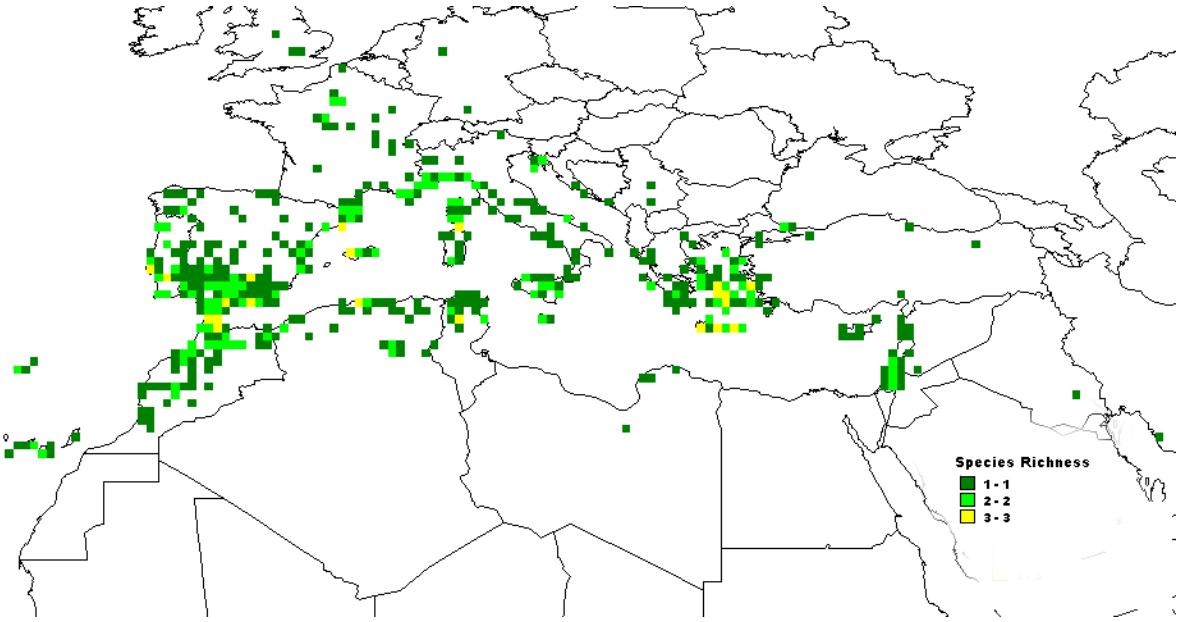


Figure 4.8. Species richness for priority species of section *Clymenum* in 100 x 100 km grid cells

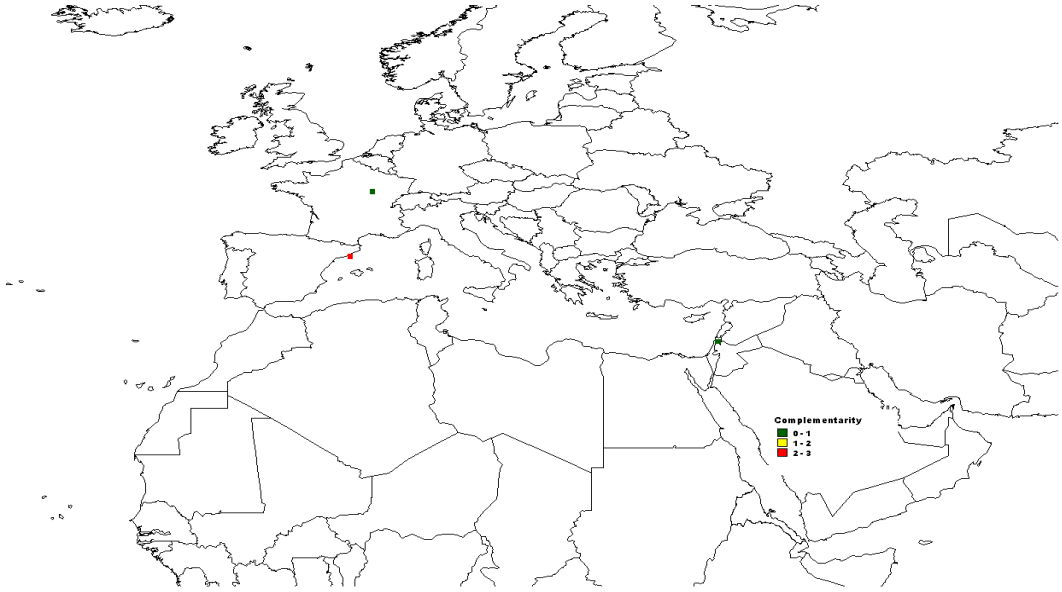


Figure 4.9. Location of hotspots of priority section *Clymenum* species using complementarity analysis

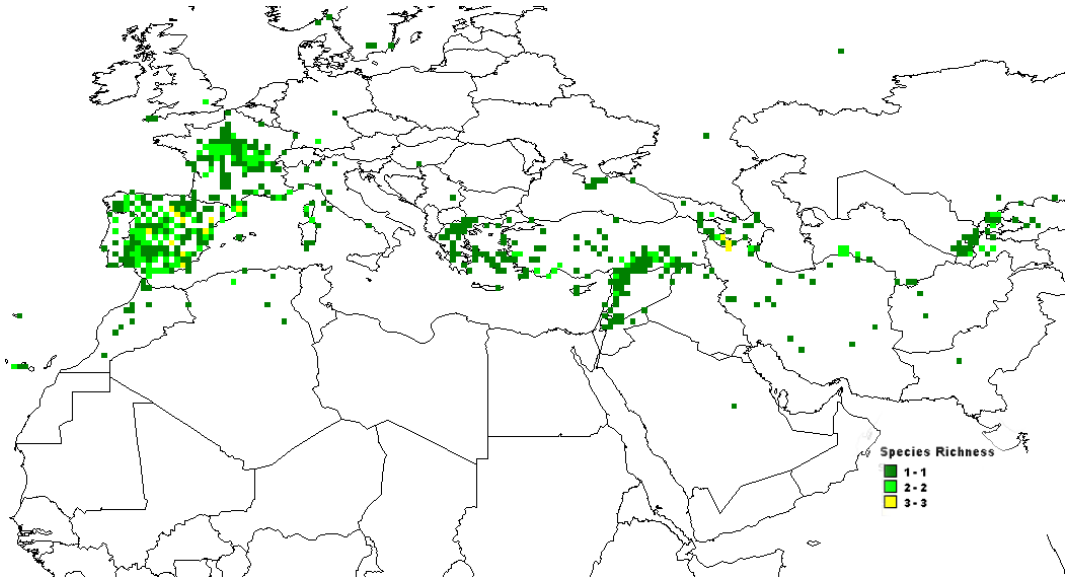


Figure 4.10. Species richness for priority species of section *Linearicarpus* in 100 x 100 km grid cells

Figure 4.11. Location of hotspot of priority section *Linearicarpus* species using complementarity analysis

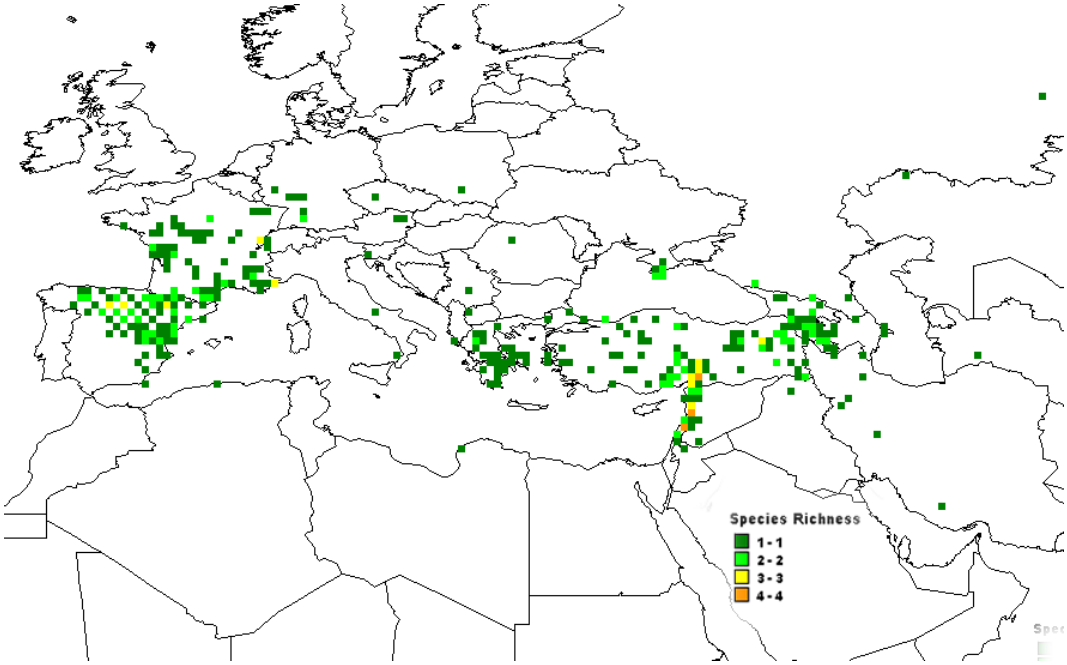


Figure 4.12. Species richness for priority species of section *Lathyrostylis* in 100 x 100 km grid cells

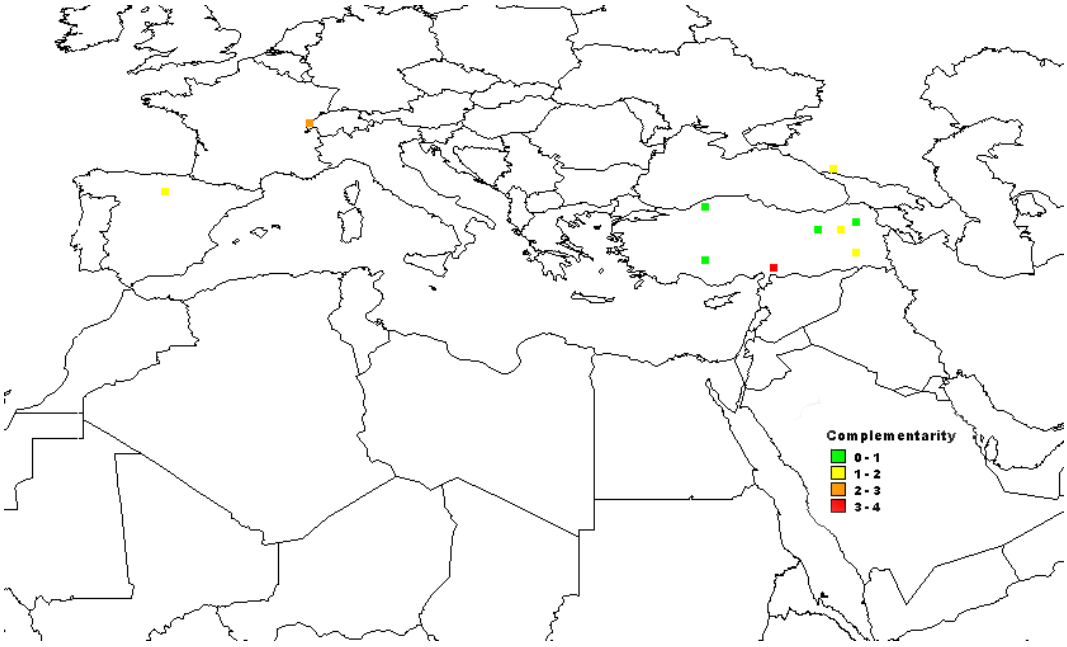


Figure 4.13. Location of hotspots using complementarity analysis for priority species of the section *Lathyrostylis*

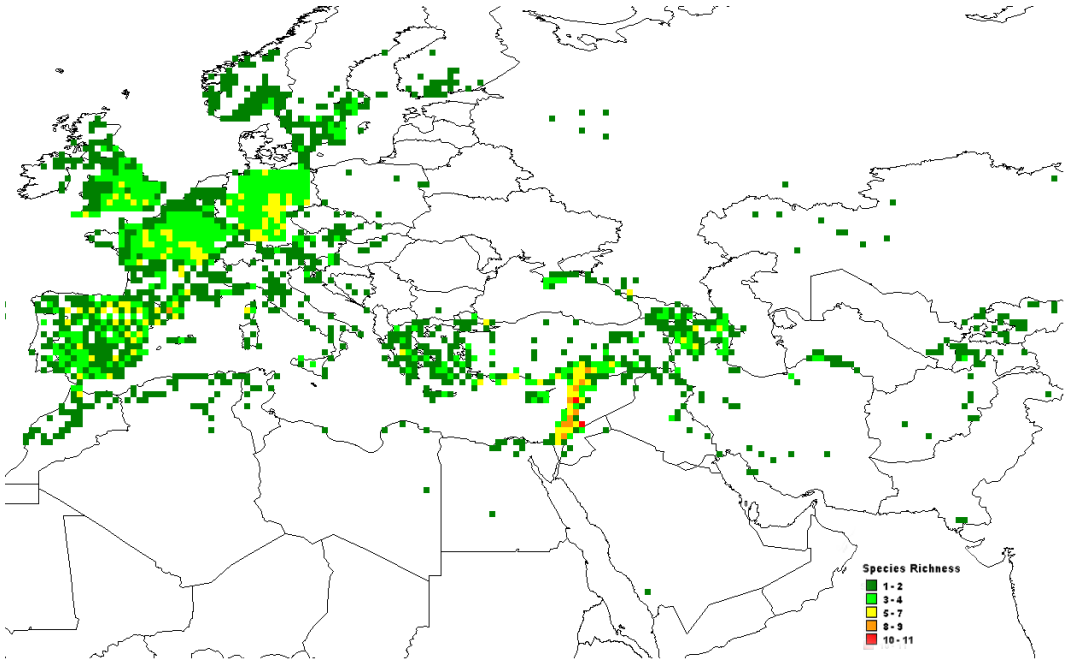


Figure 4.14. Species richness for priority species of section *Lathyrus* in 100 x 100 km grid cells

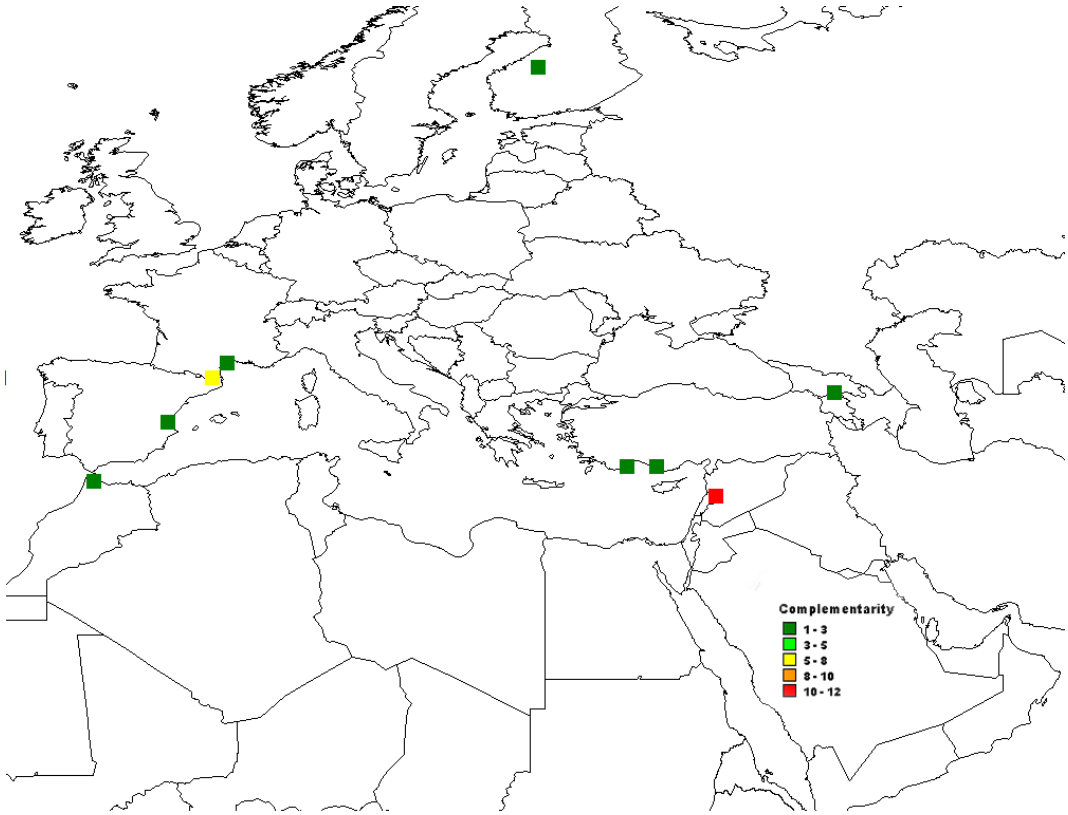


Figure 4.15. Location of hotspots of priority section *Lathyrus* species diversity using complementarity analysis

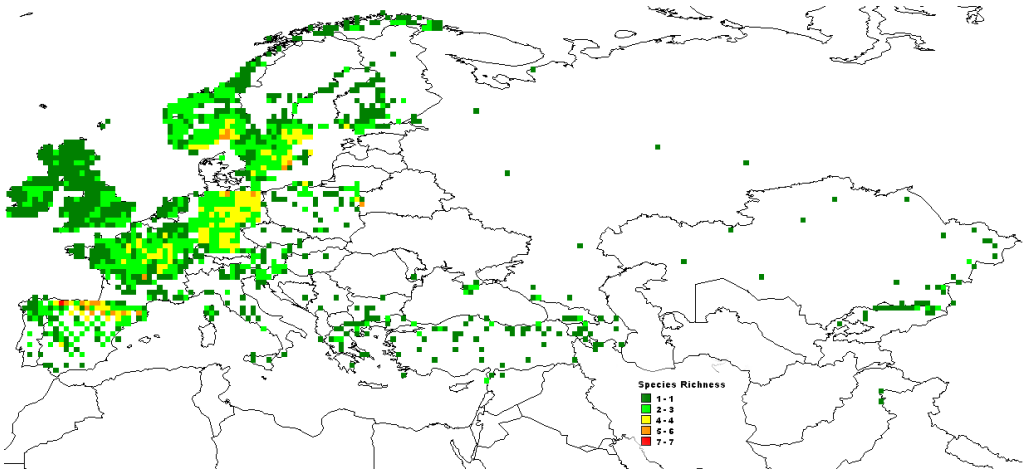


Figure 4.16. Species richness for priority species of section *Orobus* in 100 x 100 km grid cells

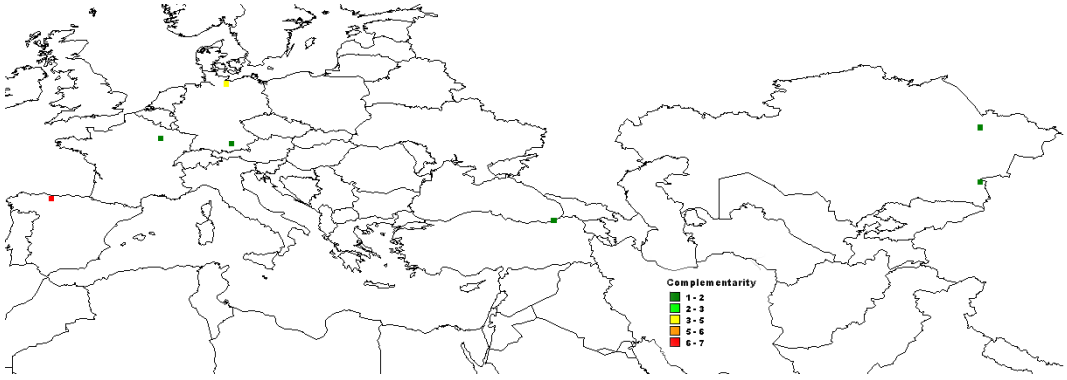


Figure 4.17. Location of hotspot of priority section *Orobus* species using complementarity analysis

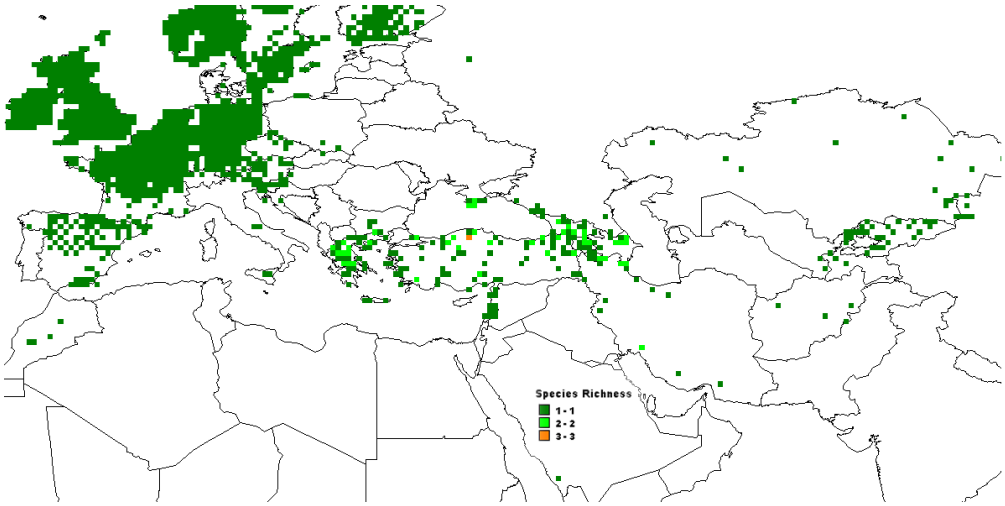


Figure 4.18. Species richness for priority species of section *Pratensis* in 100 x 100 km grid cells

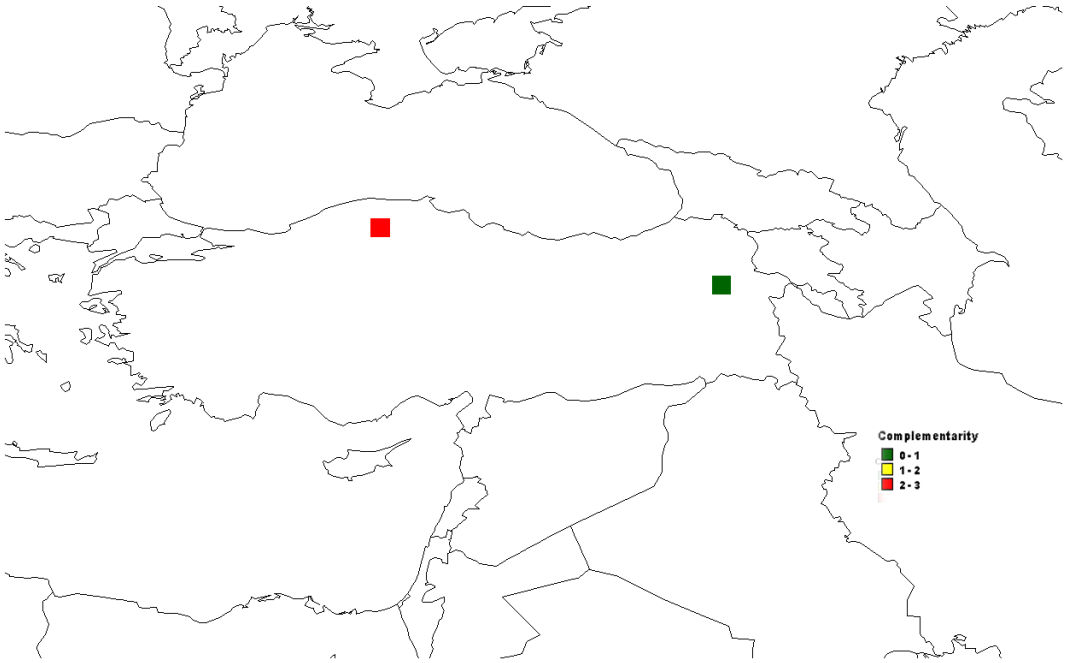


Figure 4.19. Location of hotspot for priority section *Pratensis* species diversity using complementarity analysis

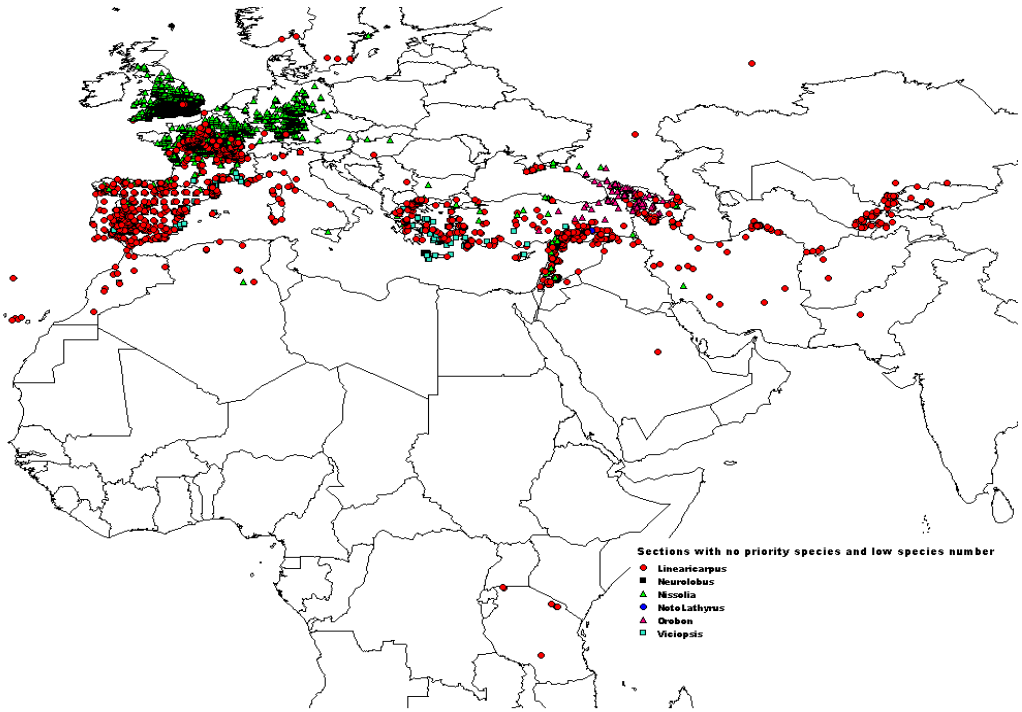


Figure 4.20. Geographical distribution of sections *Nissolia, Neurolobus, Notolathyrus, Orobon, Orobastrum and Viciopsis*, contain relatively small numbers of species, none of which are priority species.

re4.4.4 *Ex situ* conservation gap analysis

A summary of the gene bank holdings for the three most comprehensive online databases for *Lathyrus* priority species, together with numbers of georeferenced herbaria and gene bank accessions included in the study is presented in Table 4.2. The largest collection, composed of 1,256 accessions, is recorded in the EURISCO web-based catalogue, which provides information about *ex situ* plant collections maintained in Europe and being held in European national collections, while 882 accessions of *Lathyrus* are held by the Consultative Group on International Agricultural Research (CGIAR) centers, indicated by the Systemwide Information Network of Genetic Resources (SINGER) holdings, the bulk of which are held at the genebank of the ICARDA. The smallest national collection is composed of 273 accessions held by United Stated Department of Agriculture (USDA). For the analysis only georeferenced germplasm and herbaria accessions were included, but by comparing the total gene bank holdings with the numbers of georeferenced individuals, it can be seen that for most species the numbers of herbaria specimens is significantly larger than the numbers of gene bank accessions, particularly as not all *ex situ* collections have been georeferenced.

It is generally accepted that without knowledge of a taxon's pattern of genetic diversity distribution, a random sample of 50 sites per species per region would provide an adequate minimum sample of genetic diversity (Brown and Marshall, 1995); so assuming the conservationist would wish some additional safety collections in excess of the minimum, and allowing for a certain percentage of duplication of conserved germplasm samples between SINGER, EURISCO and USDA collections, a figure of 100 germplasm collections would be an adequate sample of natural diversity of a priority species (Hawkes *et al.*, 2000). Table 4.2 shows that only 6 species (16%) out of the 37 priority species are adequately sampled (indicated green in the Table) and 18 priority species (indicated red in the Table) have less

than 10 samples conserved *ex situ* including some close wild relatives of crops such as *Lathyrus basalticus, L. ciliolatus, L. amphicarpos, L. cirrhosus, L. stenopyhyllus, L. gloeospermus, L. heterophyllus, L. hirticarpus, L. belinensis, L. grandiflorus* and *L. mulkak.* Six priority species are not completely conserved *ex situ,* but have specimens in the herbaria.

Table 4.2. Ecogeographic data set of the priority species included in the analysis

Species	Accessions in SINGER	Accessions in EURISCO	Accessions in USDA	Total germplasm accessions	Georeferenced accession & herbaria samples
cicera	214	558	42	814	1321
ochrus	160	185	25	370	486
hirsutus	29	129	21	179	1532
hierosolymitanus	129	7	4	140	444
clymenum	18	84	25	127	947
tingitanus	18	81	4	103	111
odoratus	4	33	52	89	12
pseudocicera	74	2	1	77	178
annuus	33	30	7	70	665
gorgoni	61	8	1	70	306
tuberosus	7	38	20	65	3563
latifolius	4	36	12	52	3176
blepharicarpus	48	0	1	49	403
rotundifolius	5	29	11	45	174
marmoratus	36	4	1	41	264
sylvestris	4	1	32	37	3992
chloranthus	4	19	2	25	34
cassius	8	4	2	14	61
basalticus	6	0	1	7	28
ciliolatus	3	1	3	7	28
amphicarpos	4	2	0	6	15
chrysanthus	4	1	1	6	24
cirrhosus	1	1	2	4	28
stenophyllus	2	0	2	4	27

gloeospermus	2	1	0	3	9
heterophyllus	0	2	0	2	115
hirticarpus	2	0	0	2	4
belinensis	1	0	0	1	5
grandiflorus	0	0	1	1	37
mulkak	1	0	0	1	27
lentiformis	0	0	0	0	1
lycicus	0	0	0	0	4
phaselitanus	0	0	0	0	2
trachycarpus	0	0	0	0	2
tremolsianus	0	0	0	0	118
Undulates	0	0	0	0	4
Total	**882**	**1,256**	**273**	**2,411**	**18,147**

4.4.5 *In situ* conservation gap analysis for *Lathyrus* priority species

Mapping species richness distribution and complementarity site analysis showed the diversity hotspots for all priority species and for major *Lathyrus* sections having priority species. The analysis for priority species in the genus *Lathyrus* clearly identifies the western Fertile Crescent, South-Central Turkey, western Syria and northeast Lebanon, and North Spain as the areas in which to focus *in situ* conservation efforts. The highest concentration of all priority species, and therefore the most species rich hotspot, is in the north of the Bekaa valley in Lebanon and adjoining Tel Kalakh region in Homs Province, Syria (Figure 4.21).

It has been argued if the existing protected areas are contributing to *in situ* conservation of CWR species. This is done by placing the identified hotspots in relation to the existing International Union for Conservation of Nature (IUCN) recognized protected areas. There are ten existing IUCN-recognized protected areas that are within a 100 km radius of the hotspots, but only one of these has official IUCN designation (Figure 4.21, Table 4.3). For *Lathyrus*, none of these protected areas are comparable in terms of habitat to the *Lathyrus*

identified hotspots in the Fertile Crescent, but more surveys focusing on priority *Lathyrus* are needed to identify the potential sites for establishing genetic reserves.

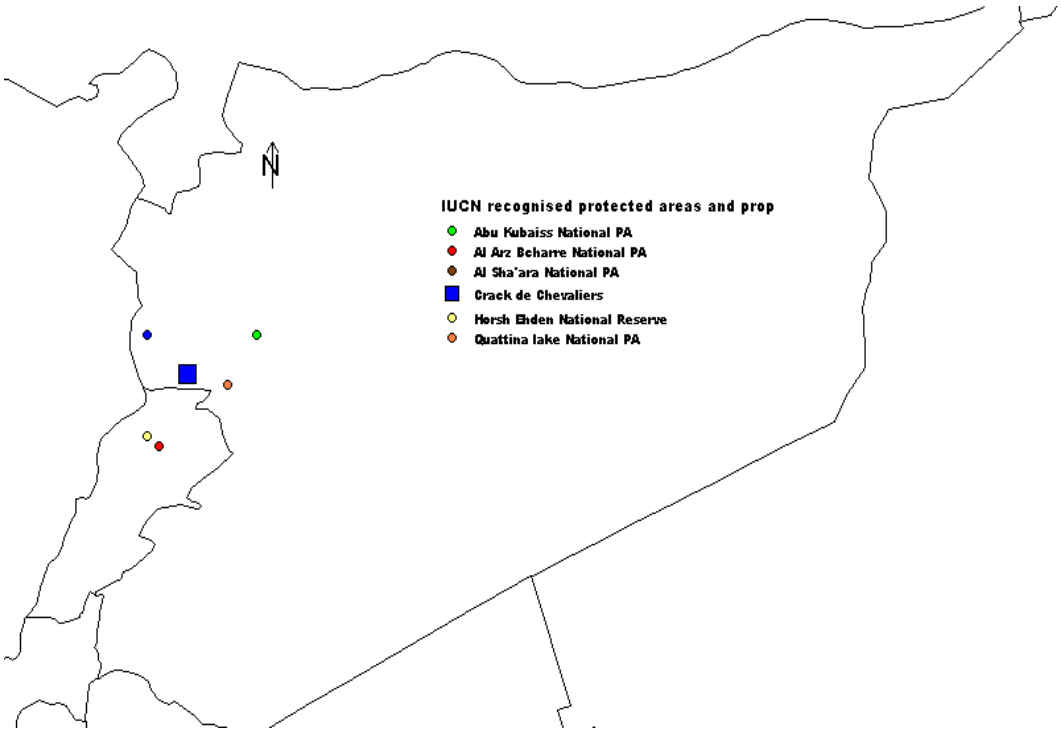


Figure 4.21. Location of *Lathyrus* genera priority species complementary species diversity hotspots with associated IUCN recognized protected areas

Table 4.3. List of IUCN recognized protected areas within 100 km radius of the complementary species diversity hotspots

Country	**Protected area name**	**Type of protected area**	**Location**	**Area** (ha)
Syria	Al Sha'ara National PA	Protected Area	36.00 N 35.00 E	1,000
	Abu Kubeiss National PA	Protected Area	36.80 N 35.00 E	11,000
	Quttina lake National PA	Protected Area	36.58 N 34.67 E	6,000
Lebanon	Horsh Ehden National Reserve	National Reserve	36.00 N 34.32 E	-
	Arz Bcharreh National Protected Zone	Protected Zone	36.08 N 34.25 E	-

The Global Environment Facility (GEF)-funded, ICARDA-coordinated regional project on 'Conservation and Sustainable Use of Dryland Agrobiodiversity in the Fertile Crescent' has undertaken ecogeographic surveys in 65 monitoring sites in Jordan, Lebanon, the Palestinian Authority, and Syria during the period of 2000-2005, and 2009 in Syria only and 2010 in Jordan, Lebanon and Syria to assess the status and trends of species diversity and its threats. The results of the surveys are shown in Table 4.4, where a total of 15 identified priority species with some non-identified species were recorded. Most of the sites have 1 to 2 priority species and only the sites in Irbid, north of Jordan, and in Sweida, South of Syria, have 4-5 priority species. Protected areas are adjacent to sites in Irbid and Sweida and should be assessed for the presence of the targeted species. Based on these surveys, eleven sites were recommended for *in situ* conservation of wild relatives of cereals, legumes and fruit trees, including the previous mentioned sites in addition to sites in northeast Lebanon at Aarsal and Baalbek, close to the identified hotspots. These two reserves contain significant *Lathyrus* priority species diversity. Therefore, it is recommended that the *in situ* genetic conservation of this diversity is made a priority at these two sites. It should be noted that although the same project established two genetic reserves in Syria, these were not in the priority locations identified.

Table 4.4. Number of priority *Lathyrus* species and number of observations in different sites surveyed in Jordan, Lebanon, Palestinian Authority and Syria.

Country	Target area	Project site	Priority species number	No of Observations
JOR	Irbid	Al Wahadneh	4	19
JOR	Irbid	Baoun	4	56
JOR	Irbid	Samta	4	39
JOR	Irbid	Wadi Rayyan	3	46
LEB	Baalbek	Ham	2	6
LEB	Baalbek	Nabha	1	13
PAL	Hebron	Dahriyyeh	3	3
PAL	Hebron	Sair-Wadi Sair	1	15
PAL	Jenin	Deir Abu Deif	4	6
PAL	Jenin	Tayasir	1	1
SYR	Lattakia	Birin	3	7
SYR	Lattakia	Haffeh	1	1
SYR	Lattakia	Sharifa	1	2
SYR	Lattakia	Teshreen	1	1
SYR	Lattakia	Wadi Kars	2	4
SYR	Sweida	Kanawat	4	8
SYR	Sweida	Mushannaf	4	27
SYR	Sweida	Rashida	5	30
SYR	Sweida	Sahwet Al Khodr	4	7
SYR	Sweida	Sahwet Al-Balata	3	7

4.5 Discussion

This study has added substantial information and accuracy to the existing *Lathyrus* database by combining diverse multiple datasets, upgrading it and by examining and collating the information from the herbaria visited. For most priority *Lathyrus* species, there are larger numbers of herbaria specimen records than the seed accessions held in genebanks, and this will be used to guide future collecting missions to sample the species and populations which are not sampled yet as seeds. This difference could also be due to the objectives of herbaria focusing on describing the flora of a given country compared to the collecting missions which focus on sampling only the populations of the species found in the sites visited.

The results of the species richness analysis for all *Lathyrus* species considered has shown that their distribution extends from the Canarias Islands to Bangladesh and extends north to Iceland, the Scandinavian countries and Siberia, covering different climatic zones from arid-hot to cold. The highest concentration of *Lathyrus* priority species is found in the countries of the Fertile Crescent, France, Italy and Greece. These results confirm the conclusions by Kupicha (1981; 1983) that the Mediterranean, Fertile Crescent and the Irano-Turanian regions are the major centers of diversity for *Lathyrus* priority species; and the importance of the Fertile Crescent as reported previously by Vavilov (1926) and Harlan (1992). This study has identified the hotspots for each section having priority species and should guide future efforts of *in situ* and *ex situ* conservation. The region extending from South-Central Turkey, through the western Mediterranean mountains of Syria to the northern Bekaa valley in Lebanon, and precisely the area around the Lebanese / Syrian border near Tel Kalakh region in Homs, was identified as the hotspot and the overall priority location. Establishing a genetic reserve in this area should have the highest priority, as the site would facilitate complementary *in situ* conservation for the priority species of the main sections *Lathyrus* and *Clymenum*. This area

is also indicated as the individual generic hotspot as suggested by Maxted *et al.* (2009) to have the highest temperate legume species concentration including *Lathyrus*, *Medicago* and *Vicia*. At present there are no protected areas within the locations identified but there are protected areas in adjacent regions, and these should be surveyed to assess the feasibility of these hosting genetic reserves. The results of this gap analysis reinforce the field survey results, conducted in this region over many years by the author and others from ICARDA and the University of Birmingham, identifying the highest concentration of target taxa in the valley below Crack de Chevalier (Qal'at Al Hosn). The unique concentration of diversity in this valley was first highlighted by Maxted (1990) who identified it as a priority site for the *in situ* conservation of *Vicia faba* wild relatives. This valley also contains extensive cereal diversity: *Triticum baeoticum* Boiss., *T. urartu* Tumanian ex Gandilyan, *T. turgidum* L. subsp. *dicoccoides* (Körn. Ex Asch. & Graebn.) Thell., several *Aegilops* species (Valkoun *et al.*, 1998; Maxted *et al.*, 2008b); *Hordeum vulgare* L. subsp. *spontaneum* (C.Koch.) Thell.; *H. bulbosum* L. and *H. maritimum* L. subsp. *gussoneanum* (Parl.) Asch. & Graeb. (Vincent *et al.*, 2009); and *Avena clauda* Durieu, *A. damascena* Rajhathy et B. R. Baum, *A. sativa* L. and *A. sterilis* L. (Patsiou *et al.*, 2009); as well as wild vegetable (flax *Linum usitatissimum* L.) and fruit tree (e.g. *Pistacia* spp., *Malus* spp., *Pyrus* spp.) crop wild relatives. As such, this valley has national, regional and global importance for *in situ* conservation of temperate food and agricultural crop wild relative's diversity. However, this site is highly threatened by over-grazing and the destruction of natural habitats for agriculture and urbanization purposes. Keisa *et al.* (2007) showed this area is being developed rapidly for tourism, which could affect negatively the rich biodiversity; however, much of the development is concentrated in a restricted ribbon around the most fertile soil of the valley bottom, and they concluded that suitable sites for *in situ* conservation could still be found above this development in the

traditionally farmed or abandoned terraces. More systematic surveys in the Qal'at Al Hosn valley are required, and the designation and establishment of the genetic reserve is an urgent global priority which requires national and international support for better management of the selected site. This management could include technological options using water-harvesting, combined with community managed grazing, to investigation of alternative sources of income to support the livelihood of local communities to continue their efforts to conserve the remaining agrobiodiversity. Promoting eco-tourism, targeting awareness increase and effective contribution to conservation activities could be developed as an alternative source of income for the custodians of local biodiversity. In addition, enabling policies to empower local communities and general public awareness actions should be developed.

Having stressed the need for surveying of adjacent existing protected areas to ground truth the hotspot predictions and the designation of novel sites in which to establish genetic reserves, it should be stressed that CWR are often located in pre-climax communities (Jain, 1975; Maxted *et al.*, 1997b; Stolton *et al.*, 2006); therefore, the likely site management to maintain pre-climax conditions in the genetic reserve may need to be intensive. Although protected areas do not have to be established in climax vegetation and they can contain agricultural lands, the option of conserving *in situ* crop wild relatives diversity outside of traditional protected area should also be considered, especially where crop wild relatives population maintenance can be associated with traditional farming practices (see Maxted *et al.*, 2008d). The *in situ* conservation of crop wild relative's diversity outside of protected areas, although discussed, has yet to be enacted; therefore, it should clearly not be seen as an alternative to protected area conservation but as a means of complementary conservation.

The gap analysis for *ex situ* conservation shows that none of the priority species of *Lathyrus* have over 100 germplasm collections, and only *Lathyrus cicera* with 814 accessions could be

likely well sampled, but it would be necessary to confirm by better understanding of the full distribution of the genetic diversity within the species. The species is common and this could result in large number of accessions collected without representing the extent of its large distribution. Only 6 species (representing 16.6%) of the 37 priority species are adequately sampled with more than 100 germplasm accessions each, 12 priority species have more than 10 samples and the rest (19 species) having less than 10 accessions conserved *ex situ*. Only *L. cicera*, has already been well sampled among the closely related species to cultivated species *L. sativus*, showing substantial collecting efforts are needed to collect the other closely related wild species such as *Lathyrus amphicarpos*, *L. belinensis*, *L. chrysanthus*, *L. hirticarpus* *L. hirsutus* and *L. marmoratus*, which are under-represented in gene bank collections. In addition, six priority *Lathyrus* species have no *ex situ* collections *(L. lentiformis*, *L. lycicus*, *L. phaselitanus*, *L. trachycarpus*, *L. tremolsianus* and *L. undulatus*) requiring also further targeted *ex situ* collecting. A more focused analysis of the *ex situ* conserved accessions would be required to confirm whether the species were sampled from throughout their distribution range, population samples adequately reflect the total variation present per site and to indicate for those species under-represented where within their range further sampling should be targeted. It is evident that wild *Lathyrus* species provide an invaluable gene source for the improvement of food and forage legume cultivars (Maxted and Bennett, 2001). The efficient conservation of these species is essential in order to assist plant breeders in fulfilling the high production demands thought to be required in the future if food security is to be maintained and adaptation to the adverse effects of climate change is to be achieved. Future collecting missions and *in situ* conservation efforts could also be guided, in addition to herbaria, by the traits sought by various users including breeders. Accessions with adaptation to heat, drought, salinity and other abiotic and biotic stresses can be targeted

special efforts by mapping the distribution of *Lathyrus* species to environmental gradients with typical stresses. The conserved germplasm can not only serve the breeding purpose, but could be use for the rehabilitation of degraded ecosystems. Similar analysis should be done specifically for grass pea and its closest wild relatives to find out where to conduct collection.

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4.7 Appendices

Appendix 1. List of species included in the dataset. Records in bold are those included in the priority species level analysis as close relatives of crops.

Genus	**Species**	**Number of records**
Lathyrus	*alpestris*	2
Lathyrus	***amphicarpos***	**15**
Lathyrus	*angulatus*	539
Lathyrus	***annuus***	**665**
Lathyrus	*aphaca*	3538
Lathyrus	*armenus*	6
Lathyrus	*aureus*	73
Lathyrus	***basalticus***	**28**
Lathyrus	*bauhinii*	37
Lathyrus	***belinensis***	**5**
Lathyrus	*bijugas*	1
Lathyrus	***blepharicarpus***	**403**
Lathyrus	*boissieri*	16
Lathyrus	*brachypterus*	12
Lathyrus	***cassius***	**61**
Lathyrus	***chloranthus***	**34**
Lathyrus	***chrysanthus***	**24**
Lathyrus	***cicera***	**1321**
Lathyrus	*cilicicus*	20
Lathyrus	***ciliolatus***	**28**
Lathyrus	***cirrhosus***	**28**
Lathyrus	***clymenum***	**947**
Lathyrus	*cyaneus*	83
Lathyrus	*czeczottianus*	18
Lathyrus	*davidii*	8
Lathyrus	*digitatus*	119
Lathyrus	*elongatus*	10

Lathyrus	*filiformis*	115
Lathyrus	***gloeospermus***	**9**
Lathyrus	*gmelinii*	22
Lathyrus	***gorgoni***	**306**
Lathyrus	***grandiflorus***	**37**
Lathyrus	***heterophyllus***	**115**
Lathyrus	***hierosolymitanus***	**444**
Lathyrus	***hirsutus***	**1532**
Lathyrus	***hirticarpus***	**4**
Lathyrus	*humilis*	8
Lathyrus	*hygrophilus*	7
Lathyrus	*inconspicuus*	511
Lathyrus	*incurvus*	36
Lathyrus	*japonicus*	474
Lathyrus	*karsianus*	4
Lathyrus	*komarovii*	3
Lathyrus	*krylovii*	1
Lathyrus	*laevigatus*	56
Lathyrus	***latifolius***	**3176**
Lathyrus	*laxiflorus*	276
Lathyrus	*layardii*	3
Lathyrus	***lentiformis***	**1**
Lathyrus	*libani*	4
Lathyrus	*linifolius*	10183
Lathyrus	***lycicus***	**4**
Lathyrus	***marmoratus***	**264**
Lathyrus	***mulkak***	**27**
Lathyrus	*neurolobus*	8
Lathyrus	*niger*	2357
Lathyrus	*nissolia*	1666
Lathyrus	*nivalis*	9
Lathyrus	*occidentalis*	61
Lathyrus	***ochrus***	**486**

Lathyrus	***odoratus***	**12**
Lathyrus	*pallescens*	45
Lathyrus	*palustris*	1206
Lathyrus	*pannonicus*	206
Lathyrus	*pannonicus*	2
Lathyrus	***phaselitanus***	**2**
Lathyrus	*pisiformis*	22
Lathyrus	*pratensis*	16567
Lathyrus	***pseudocicera***	**178**
Lathyrus	*pyrenaicus*	6
Lathyrus	*quadrimarginatus*	1
Lathyrus	*quinquenervius*	1
Lathyrus	*roseus*	107
Lathyrus	***rotundifolius***	**174**
Lathyrus	*satdaghensis*	2
Lathyrus	*saxatilis*	107
Lathyrus	*setifolius*	215
Lathyrus	*spathulatus*	44
Lathyrus	*sphaericus*	678
Lathyrus	*stenolobus*	7
Lathyrus	***stenophyllus***	**27**
Lathyrus	*sylvestris*	1
Lathyrus	***sylvestris***	**3992**
Lathyrus	*tauricola*	2
Lathyrus	***tingitanus***	**111**
Lathyrus	***trachycarpus***	**2**
Lathyrus	***tremolsianus***	**118**
Lathyrus	***tuberosus***	**3563**
Lathyrus	*tukhtensis*	8
Lathyrus	***undulatus***	**4**
Lathyrus	*variabilis*	14
Lathyrus	*venetus*	57
Lathyrus	*vernus*	3313

Lathyrus	*vinealis*	30
Lathyrus	*vivantii*	7
Total		**61081**

Appendix 2. Geographical distribution of *Lathryus* collections.

Country	All dataset unique collections	Priority species unique collections	% of unique collections	Total species / country	Priority species / country
Afghanistan	16	8	0.03	7	4
Albania	1	0	0.00	1	0
Algeria	113	90	0.19	13	8
Andora	24	6	0.04	11	3
Armenia	357	132	0.58	21	7
Austria	273	82	0.45	16	5
Azerbaijan	283	144	0.46	17	7
Belgium	1111	87	1.82	10	4
Bosnia	3	2	0.00	2	1
Bulgaria	32	1	0.05	8	1
China	15	2	0.02	3	1
Croatia	27	20	0.04	8	5
Cyprus	75	64	0.12	10	7
Czech Republic	20	11	0.03	8	3
Denmark	17	0	0.03	3	0
Egypt	16	14	0.03	5	4
Ethiopia	1	1	0.00	1	1
Finland	509	51	0.83	9	2
France	15,820	5314	25.90	39	14
Georgia	201	60	0.33	23	8
Germany	9504	3879	15.56	23	8
Gibraltar	1	1	0.00	1	1
Greece	1194	617	1.95	30	15
Greenland	10	0	0.02	1	0
Herzegovina	1	1	0.00	1	1
Hungary	9	4	0.01	7	2
Iceland	166	0	0.27	3	0
India	2	0	0.00	1	0
Iran	72	27	0.12	16	7

Iraq	46	31	0.08	14	9
Ireland	2139	16	3.50	9	4
Israel	715	528	1.17	14	9
Italy	399	209	0.65	26	12
Japan	43	0	0.07	2	0
Jordan	77	40	0.13	9	6
Kazakhstan	84	22	0.14	11	2
Korea, South	2	0	0.00	1	0
Kyrgyzstan	63	24	0.10	8	3
Lebanon	174	109	0.28	23	13
Libya	29	9	0.05	5	3
Luxembourg	2	0	0.00	2	0
Malta	17	13	0.03	3	2
Mongolia	3	0	0.00	2	0
Montenegro	3	1	0.00	2	1
Morocco	312	263	0.51	15	9
Nepal	14	0	0.02	1	0
Netherlands	1073	87	1.76	10	3
Norway	9400	575	15.39	12	4
Pakistan	11	5	0.02	5	2
Palestine	68	54	0.11	14	11
Poland	313	37	0.51	12	2
Portugal	129	98	0.21	17	10
Romania	1	0	0.00	1	0
Russian Federation	85	28	0.14	29	9
Saudi Arabia	5	1	0.01	4	1
Serbia	3	0	0.00	3	0
Slovakia	5	2	0.01	5	2
Slovenia	96	24	0.16	14	5
Soviet Union	147	50	0.24	17	6
Spain	3263	1690	5.34	43	14
Sweden	2341	303	3.83	15	5
Switzerland	8	2	0.01	7	2
Syria	1784	1105	2.92	30	16
Tajikistan	57	31	0.09	8	4

Tanzania	7	0	0.01	1	0
Tunisia	64	63	0.10	6	5
Turkey	1662	652	2.72	57	22
Turkmenistan	19	10	0.03	5	2
Ukraine	178	60	0.29	17	6
United Kingdom	6276	1358	10.27	16	7
Uzbekistan	89	27	0.15	10	5
Yugoslavia	2	2	0.00	1	1

CHAPTER FIVE

CORE COLLECTION AND ALTERNATIVE "COLLECTION" SUBSETS FOR *LATHYRUS* L.

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5.1 Abstract

The genetic diversity of *Lathyrus* genus is of significant importance. This is because several of its species are well placed to help meet the increasing global demand for animal feed and to provide food for the poor and crops for a diversity of farming systems, particularly those to be affected by climate change. Increasing breeding efforts are devoted to human consumed species *L. sativus*, *L. cicera* and *L. ochrus*, mainly targeting lowering the β-ODAP (beta-N-oxalyl-diamino-propionic acid) neurotoxin content, adaptation to heat, drought and salinity, and resistance to major diseases and pests. Several methods were used to develop manageable subsets which capture most of the variation from the original dataset and with high probability of finding sought traits. MaxEnt, PowerCore programs and R language platform facilitated subsets were derived from 2674 accessions belonging to 31 *Lathyrus* species originating from the Mediterranean Basin and the Caucasus, Central and West Asia regions. Focused Identification of Germplasm Strategy (FIGS) was also used to derive a heat and drought tolerance subset based on maximum temperature and aridity index. The results

showed that, PowerCore had the highest Shannon diversity index based on species, but does not capture enough accessions within species, which could be due to low number and nature of variables considered. MaxEnt subset and random subsets selected on the basis of taxon and geographic representativity, appear to capture most the variability in the original population. The diversity index could be improved by adding accessions of species not included in the selected random samples using any of the methods. FIGS has allowed for the selection of more accessions of species well known for their adaptation to drought and heat. The availability of information phenotypic and genotypic, along with the environmental layers could improve further the selection of appropriate subsets. These subsets, with manageable size and higher probability of finding the sought traits, will allow to link conservation with utilization of genetic resources and will reduce the pressure of regeneration of species with cross-pollination, as is the case of some species of *Lathyrus*.

Keywords: *Lathyrus*, genetic resources, core collections, FIGS approach, heat and drought tolerance

5.2 Introduction

Lathyrus is a large genus containing around 160 species (Lewis *et al.*, 2005; ILDIS, 2010), located mainly in Europe, Asia and North America, and extending to temperate South America and tropical East Africa, but with its centre of diversity primarily in the Mediterranean and Irano-Turanian regions (Kupicha, 1983). Several *Lathyrus* species are cultivated for human consumption, animal feed, and fodder, as well as for ornamental purposes, in addition to their benefits as soil nitrifiers and as dune stabilizers (Davis, 1970; Lal *et al.,* 1986; Campbell *et al.,* 1994; Sarker *et al.*, 2001; Tadesse and Bekele, 2003; Agrawal *et al.*, 2011). Three main *Lathyrus* species are grown and used for human consumption: *Lathyrus sativus, L. cicera*, *L. ochrus* and to a lesser extent *L. clymenum.*

The genetic diversity of *Lathyrus* genus is of significant importance, particularly for its potential use within the rainfed cropping systems of many countries, and as a genepool for the improvement of grass pea (*Lathyrus sativus*), which is used as feed in many parts of the world and as food by poor communities living under harsh conditions in Ethiopia and South Asia (Smartt, 1990; Campbell *et al.,* 1994; Siddique *et al.,* 1996; Getahun *et al.,* 2005; Milczak *et al.,* 2001; Crino *et al.,* 2004; Vaz-Patto and Rubiales, 2009). Grass pea constitutes the only food crop producing green and forage where other crops are decimated by droughts or floods. Its seeds are rich in crude protein (24-31%) and complement cereals in amino acid composition for a balanced diet for poor people in its major production zones (Aletor *et al.,* 1994; Akalu *et al.*, 1998; Hanbury *et al.,* 2000a). However, in drier years, excessive human consumption of its grains could cause a neurological disorder, lathyrism, caused by the presence of a neurotoxin in the seed known as either beta-N-oxalyl-diamino-propionic acid (β-ODAP) or beta-(N)-oxalylamino-L-alanine acid (BOAA). The toxicity results in irreversible paralysis, characterized by lack of strength in, or inability to move the lower limbs. It is particularly prevalent in some areas of Bangladesh, Ethiopia, India and Nepal, and affects more men than women. The total acreage of grass pea, estimated at 1.50 million ha, is decreasing in India and Nepal following the ban of its cultivation by governments (ICAR, 2009; MOAC, 2009). Because of its inherent adaptation to harsh conditions, its importance as a survival food for some of the poorest people in the world and its potential to adapt to climate change, and yet recognizing the dangers that its excessive consumption can cause, grass pea is considered as a crop of global importance and was included as such in the Annex 1 list of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, 2004). The Global Crop Diversity Trust (GCDT) in collaboration with the International Center for Agricultural Research in the Dry Areas (ICARDA) developed in

2008 the long-term conservation strategy for food legumes, including *Lathyrus* (GCDT, 2009).

Relatively little research efforts have been directed in the past to improvement of grass pea, but interest is renewed in grass pea with the growing concerns with climate changes (Yang and Zhang, 2005; Polignano *et al.,* 2009; Grela *et al.*, 2010; Agrawal *et al.*, 2011). The limited breeding efforts around the world are focusing on three main pulse species *L. sativus, L. cicera*, *L. ochrus* and to a lesser extent *L. clymenum.* Their aim is to improve yield, resistance to biotic and abiotic stresses and, most importantly, to reduce the percentage of, or ideally eliminate, the neurotoxin from the seed (Malek *et al.*, 1996; Tadesse *et al.,* 1997; Sarker *et al.*, 2001; Agrawal *et al.*, 2011). Species in the primary, secondary and tertiary gene pools may play an important role for the genetic improvement of cultivated *Lathyrus* species, including for lowering beta-ODAP content (Sarker *et al.,* 2001).

The *Lathyrus* database, produced as a result of the *Lathyrus* global conservation strategy, contains around 23,000 accessions with main collections held by University of Pau in France (4477 accessions), ICARDA (3239 accessions), National Board of Plant Genetic Resources in India (2619 accessions), and Genetic Resources Center in Bangladesh (1841 accessions). The ICARDA collection is unique because 45% and 54% of the accessions are respectively wild relatives and landraces, mainly of *L. sativus*, followed by *L. cicera* and *L. ochrus* (GCDT, 2009; ICARDA-database, 2010). However, to date, an extensive and systematic approach to global collection, conservation and evaluation of *Lathyrus* has not been adopted. Furthermore, it is necessary to study the genetic diversity of the available collections in order to understand their full utilization potential (Maxted *et al.*, 2003). ICARDA has characterized more than 60% of the accessions for main descriptors (ICARDA-GRS database), with more than 1,082 accessions belonging to 30 species evaluated for 21

descriptors and agronomic traits at the ICARDA station at Tel Hadya (Robertson and Abd-El-Moneim, 1997, ICARDA-GRS database, 2010). A small proportion (10%) of this collection was evaluated for other traits, and around 1200 accessions were evaluated for β-ODAP content (Agrawal *et al*, 2010, unpublished data).

The large size of the collections poses a problem on the capacity to evaluate all the accessions for sought traits. To avoid sending random samples, core collection concept was defined and introduced by Frankel in 1984 as "a limited set of accessions representing, with a minimum of repetitiveness, the genetic diversity of a crop species and its wild relatives". The core would maintain "useful variability" while keeping the number of accessions to a manageable size. The size of the core to represent 5-10% of the original collection was based on the sampling theory put forward by Ewens in 1972. Based on this theory, Brown (1989a) found that the fraction of alleles retained in a sample increases only slowly or disproportionally when the sampling goes above 10% (Yonezawa *et al.* 1995). He found that most of the original allelic variation could be retained in 10% randomly drawn accessions, either from a population or from its subgroups after its stratification (Brown 1995). Practically, the core collection is developed from the data associated with the accessions, and there are basically two types of methods based on the data: the branching and the clustering methods (van Hintum, 1995). The branching methods use passport data in combination with a priori information/knowledge while clustering methods use characterization that can go as close as to molecular characterization (van Hintum, 1995). To capture the above spectrum of methods, several strategies have been adopted ranging from sampling of a constant number of accessions per region (C strategy), to sampling in proportion to the logarithm of the number of accessions available per region (L strategy), to marker-assisted strategy (M strategy) where the sampling is based on marker allele richness. The strategies above combine both statistical

procedures, such as principal component analysis (Hamon *et al.*, 1995), and clustering (Hu *et al.*, 2000) with random selection to setup the core. As mentioned above passport data of accessions are used to develop groups based on the geographical origin of the accessions, and the sampling is carried out randomly within these groups. The clustering methods include molecular and morphological to guide the sampling (Schoen and Brown, 1993).

A number of core collections have been developed for a large number of crops since the concept has been established, the barley core collection (Hintum *et al.,* 1990), which is designed to guide the genebank customer to a relevant smaller subset of barley accessions from all partner genebanks sharing information on their barley accessions with the central database hosted by IPK Gatersleben in Germany (Endresen, 2011). As the core collections develop, the core paradigm expands to include the issue of evaluation, since the problem of genetic resources is not only the size but also the lack of evaluation that hinder their effective use. The core was proposed to overcome the problem of limited use of genetic resources in addition to their large size. Modifications were added to the core to accommodate these concerns, such as specific collections (Macky, 1990, 1995; Brown, 1995; van Hintum, 1999; Brown and Spillane, 1999). Although the shift has helped in the stimulation of use of genetic resources (Holbrook *et al.*, 2000), there is more emphasis to add modifications towards more specific and thematic collections because of the challenge to retain all the variation that users might need (Polignano *et al.*, 2001). Among modification there is also development of mini core collections to address the concern above, in particular the use in relation to traits (Upadhyaya *et al.* 2002; Holbrook and Dong, 2005). Upadhyaya and Ortiz (2001) developed a two-stage strategy: first, to develop a core collection using characterization data from the entire collection and then, to evaluate core collection accessions for various traits to develop a mini core collection. In both stages, the intention is to ensure that over 80% of the variability

from the entire collection (for developing core) or from the core collection (for developing mini core) is sampled. Several mini core collections were developed for finger millet, pearl millet, sorghum, chickpea, peanut and cowpea (Rao and Rao, 1995; Upadhyaya *et al.*, 2006a; Bhattacharjee *et al.*, 2007; Upadhyaya *et al.*, 2009), and rice (Yan *et al.*, 2007). The differences between means of the core and mini core collections were found to be nonsignificant for all the traits, while variances differed only for few traits. The previous studies also reported similar or slightly lower H` in mini core than core collections (Upadhyaya *et al.*, 2002, 2006b, 2009b). In other crops, when mini core collections were evaluated, researchers were able to identify new sources of variation, for example, drought tolerance in chickpea and groundnut; salinity tolerance in chickpea, groundnut, and pigeon pea; low temperature tolerance (at germination) in groundnut; resistance to pest (pod borer) and diseases (*Ascochyta* blight, *Botrytis* gray mold, dry root rot, and *Fusarium* wilt) in chickpea; early maturity and/or large-seed size in chickpea and groundnut; and large-seed size and high grain yield in chickpea (reviewed in Upadhyaya *et al.*, 2009a). This mini core collection can also be used for molecular characterization to select genetically diverse germplasm to maximize diversity and broaden the genetic base of finger millet cultivars.

In terms of use, there are concerns that the core may not capture the desired trait variation, and other alternatives need to be developed. This was recognized in the early years of the development of core collections (Macky, 1990; Johnson R.C. and T. Hodgkin, 1999). For adaptive traits however, core collections may not capture the needed diversity (Brown & Spillane, 1999, Polignano *et al.* 2001, Gepts 2006; Dwivedi *et al.* 2007, Pessoa-Filho *et al.* 2010, Xu 2010). The need to rationalize the search for rare and adaptive traits has led to the use of alternative approaches, including the development of specific or thematic genetic resource collections (Gollin *et al.* 2000, Gepts 2006; Dwivedi *et al.* 2007; Pessoa-Filho *et al.*,

2010; Xu, 2010). As an alternative to the core, the Focused Identification of Germplasm Strategy (FIGS) has been developed. The FIGS approach is a trait-based approach within the crop improvement perspective to develop a subset of accessions with high probability of identification of desired genetic material (Macky, 1990, 1995; Macky and Street, 2004). It is based on the assumption that the distribution patterns of adaptive traits might be, similar to taxonomic taxa distributions, the result of ecological and evolutionary factors, including, but not limited to, environmental factors, natural selection and local selection pressures that are hard to quantify, such as interactions with humans. FIGS approach to selecting germplasm from genetic resource collections has shown that they are more prone to provide useful and novel genes (Mackay and Street 2004, El-Bouhsini *et al.* 2009, Bhullar *et al.* 2010, El-Bouhsini *et al.* 2010). Relationships between adaptive traits and collection site attributes have also been revealed by recent studies using multi-linear and multi-way models such as *N*-PLS (non-orthogonal PLS) (Endresen 2010, Endresen *et al.* in press). Modeling of stem rust resistance using geographical information systems (GIS) has also led to the detection of a relationship between geographical areas and incidence of resistance to stem rust (Bonman *et al.* 2007).

Following the increased interest for core collections, a range of software was developed and adopted to assist in the core subset selection, using different strategies (Brown, 2005). MSTRAT software uses M-strategy to maximize the number of observed alleles at the marker loci (Gouesnard *et al.*, 2001). PowerCore uses advanced M-strategy using heuristic search to establish core sets (Kim *et al.*, 2007). MaxEnt is a program for maximum entropy modeling of species geographic distributions, and its use could help in selecting core subsets based on whatever information is available, including only climatic variables.

The present study aims at developing core subsets for *Lathyrus* genetic resources using different approaches and at introducing new and alternative approaches for selecting best bet sets for adaptive traits.

5.3 Materials and Methods

5.3.1 Collection of existing accession level data

A total of 2674 accessions originating from major *Lathyrus* collections were used to derive core subsets using different methods. Only the accessions with georeferenced and climatic data were selected from the *Lathyrus* global database maintained by ICARDA (Table 5.1). Half of the data is from the ICARDA genebank. The number of accessions per species is included in Table 5.2.

Table 5.1. Number of *Lathyrus* germplasm selected from different genebank collections for developing core subsets

Genebank	Number of accessions
ICARDA	1332
ESP004	347
DEU146	289
IBCR	155
UKR008	134
HUN003	83
NGLRP	75
FRA243	46
GRC005	40
SVK001	31

CYP004	30
ATFCC	28
PRT005	24
CZE122	18
PRT001	11
BGR001	10
PRT083	8
RUS001	8
BARI	2
AUT001	1
PRT084	1
ROM007	1
Total	2674

ATFCC Australian Temperate Field Crops Collection, Horsham, Australia; **AUT001:** Agrobiology Linz - Austrian Agency for Health and Food safety / Seed Collection, Linz, Austria; **BARI:** Bangladesh Agricultural Research Institute, Bangladesh; **BGR001:** Institute for Plant Genetic Resources 'K.Malkov', Plovdiv, Bulgaria; **CYP004:** National (CYPARI) Genebank, Cyprus; **CZE122:** Genebank Department, Division of Genetics and Plant Breeding, Research Institute of Crop Production, Prague, Czech Republic; **DEU146:** Genebank, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany; **ESP004:** Instituto Nacional de Investigacin y Tecnologیa Agraria y Alimentaria. Centro de Recursos Fitogenéticos, Spain; **FRA243:** Reseau Plantes Fourrageres et à Gazon, Geves, France; **GRC005:** Greek Genebank, .National Agricultural Research Foundation, Greece; **HUN003:** Institute for Agrobotany, Hungary; **IBCR:** Institute of Biodiversity Conservation and Research (former PGRC/E), Ethiopia; **ICARDA:** International Centre for Agricultural Research in the Dry , Aleppo, Syria; **NGLRP:** National grain Legume Research Program, Nepal; **PRT001:** Banco Português de Germoplasma Vegetal, Braga, Portugal; **PRT005:** Banco de Germoplasma - Departamento de Recursos Genéticos e Melhoramento, Portugal; **PRT083:** Sector de Proteaginosas, Departmento de Pastagens, Forragens e Proteaginosas, Elvas, Portugal; **PRT084:** Sector de Pastagens e Forragens, Departamento de Pastagens, Forragens e Proteaginosas, Elvas Codex, Portugal; **ROM007:** Suceava Genebank; Romania. **RUS001:** N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry, St. Petersburg, Russia; **SVK001:** Research Institute of Plant Production Piestany, Slovakia; **UKR008:** Ustymivka Experimental Station of Plant Production, Ukraine.

Table 5.2. Number of accessions per *Lathyrus* species considered in deriving core subsets

Species	No. of accessions
L. amphicarpos	4
L. annuus	82
L. articulatus	93
L. basalticus	5
L. belinensis	1
L. blepharicarpus	47
L. cassius	11
L. chloranthus	9
L. chrysanthus	7
L. cicera	440
L. ciliolatus	2
L. cirrhosus	1
L. clymenum	45
L. gloeospermus	3
L. gorgoni	*67*
L. heterophyllus	1
L. hierosolymitanus	112
L. hirsutus	70
L. hirticarpus	1
L. latifolius	12
L. marmoratus	27
L. mulkak	1
L. ochrus	214
L. odoratus	4
L. pseudocicera	76
L. rotundifolius	6
L. sativus	1261
L. stenophyllus	2
L. sylvestris	31
L. tingitanus	19
L. tuberosus	20
Total	**2674**

Passport information was taken from the global *Lathyrus* database maintained at ICARDA and the GIS layers were obtained from the GIS-Unit at ICARDA (De-Pauw *et al.*, 2011).

5.3.2 Data processing

Different methods were used to extract core collection subsets from the original data of *Lathyrus* collection. A 10% sample was used as recommended by previous studies and also to allow for comparison among different selection methods. In case of *Lathyrus* collection, 10% of the existing accessions is a manageable size for both the distribution by genebanks and for evaluation by users. The first core subsets were derived for a process involving a selection of a 10% sample at random (Subset random) or after the stratification of the original data by taxon (Subset taxon) and by origin (Subset geographic) of accessions. The sampling process was carried out using R language platform (R Development Core Team, 2011).

- ***Use of MaxEnt***

MaxEnt is a general-purpose method for making predictions or inferences from incomplete information. Its use can be extended to form core subsets based on modeling of species distributions, and is applicable for cases where evaluation and molecular data are limited or non existent. The idea of MaxEnt is to estimate a target probability distribution by finding the probability distribution of maximum entropy (i.e., that is most spread out, or closest to uniform), subject to a subset of constraints that represent our incomplete information about the target distribution. In this study, MaxEnt was chosen because it can use climatic variables available for *Lathyrus* collection.

Two subsets were derived from MaxEnt, the proposed subset and the other constructed by adding accessions from species not included in the proposed subset.

- ***Use of PowerCore***

This program applies advanced M-strategy using heuristic search for establishing core or allele mining sets (Kim *et al*, 2007). The software can be downloaded for free as a Windows system compatible binary from: http://www.genebank.go.kr/eng/PowerCore/powercore.jsp.

Some modifications were included in the input data, which considered eight variables: species, geographic origin, mean annual temperature, annual, average, minimum and maximum temperatures, FAO classes of aridity and altitude. Classes were defined for each variable and accessions were randomly selected using the criteria of proportional number of each cluster. The core subset was then derived to represent maximum diversity of species richness, climatic conditions and eco-geographic zones. Two subsets were constructed using this approach, one selecting at random and the other with minimum number os accessions representing all clusters

- ***Use of FIGS approach***

The Focused Identification of Germplasm Strategy (FIGS) was introduced to provide modification to core collections towards more specific and thematic collections because of the challenge to retain all the variation that users might need. The FIGS approach is a trait-based and user-driven approach to select potentially useful germplasm for crop improvement. It was conceived to provide indirect evaluation of germplasm for specific traits using, as a surrogate, the environment based on the hypothesis that the germplasm is likely to reflect the selection pressures of the environment in which it was originally sampled (Mackay 1990, Mackay 1995, Mackay and Street 2004). FIGS, as a focused approach, combines both the development of a priori information (dataset template or specialized knowledge as per Gollin

et al. (2000)) based on the quantification of the trait-environment relationship and the use of this information to define a subset of accessions with a higher probability of containing the sought after traits. This study developed a subset for heat and drought tolerance in *Lathyrus* collection by developing clusters based on the aridity index and maximum temperature. Kelley-Gardner-Sutcliffe penalty function was used to define five clusters.

All the methods above used the same dataset and considered only the climatic variables. The comparison of the outcome of the different approaches to select these core collections was based on number of species represented in the subsets and the Shannon diversity index (H') calculated at the level of species richness:

$$H' = \sum p_i \log (p_i)$$

where p*i* is the frequency of the i*th* entity (taxon).

5.4 Results

Out of 2674 accessions belonging to 31 *Lathyrus* priority species, the sample size for different methods used to derive core and FIGS subsets ranged from 272 (representing 10% selection criteria used for some method) down to 102 and 82, when using respectively PowerCore random subset and PowerCore selecting the minimum number of accessions representing all taxa (Table 5.3). MaxEnt method selected 181 accessions when using random selection at the species level, but was increased to 219 when adding random accessions from minor species not present in former MaxEnt subset. The number of species selected when using random selection were 16 and 21 taxa in case of MaxEnt subsets, 19 in case of random sampling using R-language platform program and 31 when using PowerCore. All the 31 species were included in the random PowerCore subset when using stratified selection based on geographical distribution. FIGS approach allowed for the inclusion of 18 species. The core

subsets constructed to represent the taxon diversity, included as expected, all the 31 species. The larger the sample size, the bigger the number of species included in the random core subsets and the bigger the number of accessions included in the subsets, except in the case of FIGS approach. All the species with large numbers of accessions were included in the random core subsets as well as in the FIGS subset, as is the case for *L. annuus, L. articulatus, L. cicera, L. gorgoni, L. hierosolymitanus, L. ochrus, L. pseudocicera, L. sativus, L. sylvestris, L. tingitanus, L. tuberosus* and *L. cassius*, with the exception of *L. clymenum, L. hirsutus, L. latifolius*, and *L. marmoratus*. These latter exception species, along with those with very few accessions were not selected by MaxEnt random approaches. The FIGS subset included more accessions of *L. annuus, L. blepharicarpus, L. articulatus, L. gorgoni, L. ochrus* and *L. sativus* compared to other constructed subsets.

The efficiency of selection was assessed using Shannon diversity index for species richness (Table 5.3). This index ranged from 0.76 in case of FIGS subset to 1.16 in case of PowerCore random subset, compared to a value of 0.84 for the original dataset. The subsets including all species have given high values of the Shannon diversity index. Core subsets from PowerCore random and MaxEnt representing all the taxa, gave respective values of 1.08 and 1.02. Values ranging from 0.83 to 0.9 were exhibited by the remaining subsets, selected either at random or through stratified selection based on taxon representation or geographic distribution.

Table 5.3. Number of accessions per *Lathyrus* species included in different core subsets and FIGS subset selected using different methods

Species	Original subset	Random subset (10%)	Taxon subset (10%)	Geographic subset (10%)	MaxEnt subset all taxa	MaxEnt random subset	PowerCore random subset	PowerCore maximum subset	FIGS Subset (10%)
L. amphicarpos	4		1		3		1	1	1
L. annuus	82	10	8	9	7	7	1	2	10
L. articulatus	93	10	9	5	7	7	1	1	25

L. basalticus	5		1		3		1	1	1
L. belinensis	1		1		1		1	1	
L. blepharicarpus	47	5	4	4	4	4	1	1	8
L. cassius	11	2	1	1	1	1	1	1	3
L. chloranthus	9	1	1		6		1	2	1
L. chrysanthus	7	1	1	1	3		1	1	
L. cicera	440	47	44	46	32	32	6	5	16
L. ciliolatus	2	1	1		2		1	1	
L. cirrhosus	1		1		1		1	1	
L. clymenum	45	8	4	6	3	3	2	1	3
L. gloeospermus	3		1		2		1	1	
L. gorgoni	67	4	6	7	5	5	2	1	9
L. heterophyllus	1		1		1		1	1	
L. hierosolymitanus	112	10	11	11	10	10	3	1	6
L. hirsutus	70	6	7	7	4	4	3	4	3
L. hirticarpus	1		1		1		1	1	
L. latifolius	12		1		7		1	1	
L. marmoratus	27	3	2	5	2	2	1	2	1
L. mulkak	1		1	1	1		1	1	
L. ochrus	214	23	21	28	12	12	3	3	40
L. odoratus	4		1		3		2	2	
L. pseudocicera	76	9	7	6	7	7	3	1	1
L. rotundifolius	6	1	1		3		1	1	1
L. sativus	1261	121	126	121	82	82	49	30	136
L. stenophyllus	2		1		1		1	1	
L. sylvestris	31	3	3	7	2	2	6	6	
L. tingitanus	19		2	2	1	1	2	2	2
L. tuberosus	20	2	2	1	2	2	2	4	
Total accessions	**2674**	**267**	**272**	**268**	**219**	**181**	**102**	**82**	**267**
Number of species	**31**	**19**	**31**	**18**	**31**	**16**	**31**	**31**	**18**
Shannon index H'	**0.85**	**0.84**	**0.90**	**0.83**	**1.08**	**0.84**	**1.02**	**1.16**	**0.76**

Common accessions were used to roughly compare among different methods used to develop core subsets and FIGS subset (Table 5.4). FIGS subset has more common accessions with the core subsets except for the subset derived using PowerCore method. The PowerCore

subset has 25 common accessions with MaxEnt subset. This latter has the lowest number of common accessions with the random, geographic and taxon subsets.

Table 5.4. Common accessions among various *Lathyrus* core and FIGS subsets

	FIGS Subset	PowerCore Maximum Subset	MaxEnt Subset all Taxon
FIGS Subset	-		
PowerCore Maximum Subset	9	-	
MaxEnt Subset Taxon	24	25	-
Random Subset	23	7	2
Geographic Subset	28	14	4
Taxon Subset	29	13	1

The comparison between different subsets was also assessed by the superposition of maps of the distribution of the accessions included in different subsets, using DIVA-GIS program. In all maps, the highest concentration of accessions selected by different subsets fit to the areas with large accession numbers in the original dataset, as in the case of Morocco and Spain, the Fertile Crescent region, India and Bangladesh, and Ethiopia. MaxEnt subset extended well over the areas of distribution of *Lathyrus* species and followed well the geographical distribution of the random sample, except for the Russian regions and in Afghanistan (Figure 5.1). When the comparison of MaxEnt subset was done with PowerCore subset, this latter did not appear to cover as much geographic diversity as done with MaxEnt (Figure 5.2). FIGS subset had major areas of concentration of selected accessions in South-East Pakistan, the eastern region between Jordan to South-Turkey, the region including Cyprus, South Greece and South Italy, and along the coastal areas of Morocco and Tunisia (Figure 5.3). When comparing the three subsets, MaxEnt showed more geographic expansion, followed by PowerCore, while the FIGS subset confirmed its concentration in limited regions

(Figure 5.4). FIGS subset, however, had more concentration in terms of accessions for some specific areas. These taxa are ranked based on their occurrence in FIGS subset (Table 5.5).

Table 5.5. Percent of accessions of major species included in in different subsets

Species	Random subset	Taxon subset	Geographic subset	MaxEnt subset all taxa	MaxEnt ran-core subset	PowerCore maximum subset.	FIGS subset
cassius	**0.18**	**0.09**	**0.09**	**0.09**	**0.00**	**0.09**	**0.27**
articulatus	**0.11**	**0.10**	**0.05**	**0.08**	**0.05**	**0.01**	**0.27**
amphicarpos	0.00	0.25	0.00	0.75	0.00	0.25	**0.25**
basalticus	0.00	0.20	0.00	0.60	0.40	0.20	**0.20**
ochrus	0.11	0.10	0.13	0.06	0.07	0.01	**0.19**
blepharicarpus	0.11	0.09	0.09	0.09	0.17	0.02	**0.17**
rotundifolius	0.17	0.17	0.00	0.50	0.33	0.17	0.17
gorgoni	0.06	0.09	0.10	0.07	0.01	0.01	**0.13**
annuus	0.12	0.10	0.11	0.09	0.07	0.02	0.12
chloranthus	0.11	0.11	0.00	0.67	0.22	0.22	0.11
sativus	0.10	0.10	0.10	0.07	0.06	0.02	**0.11**
tingitanus	0.00	0.11	0.11	0.05	0.11	0.11	**0.11**

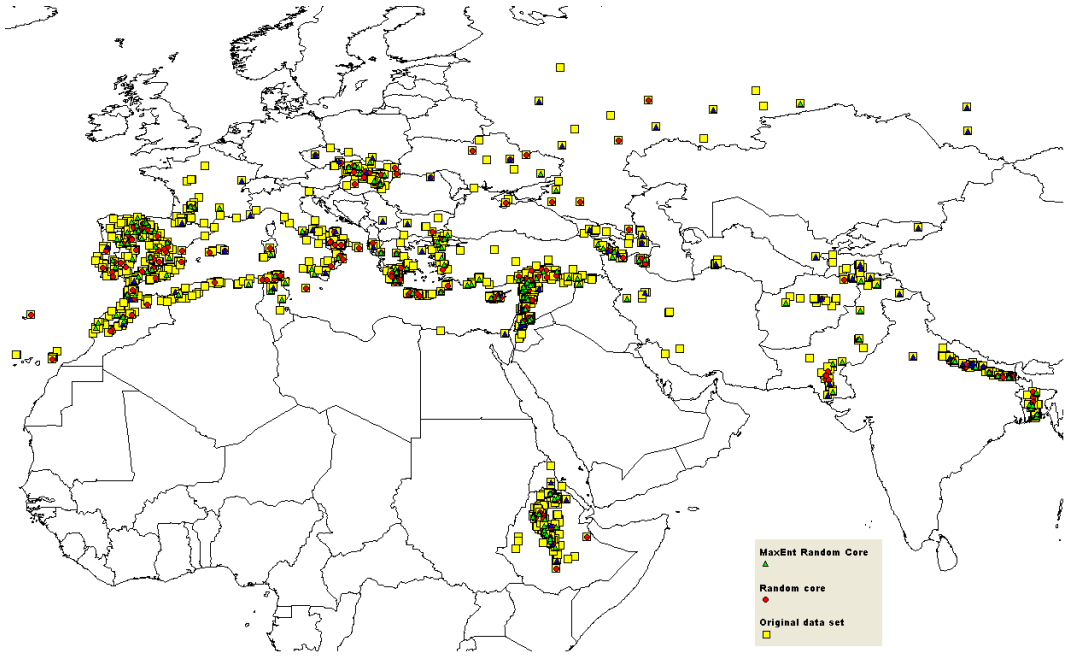

Figure 5.1. Distribution map of *Lathyrus* accessions selected using MaxEnt and R-random approaches

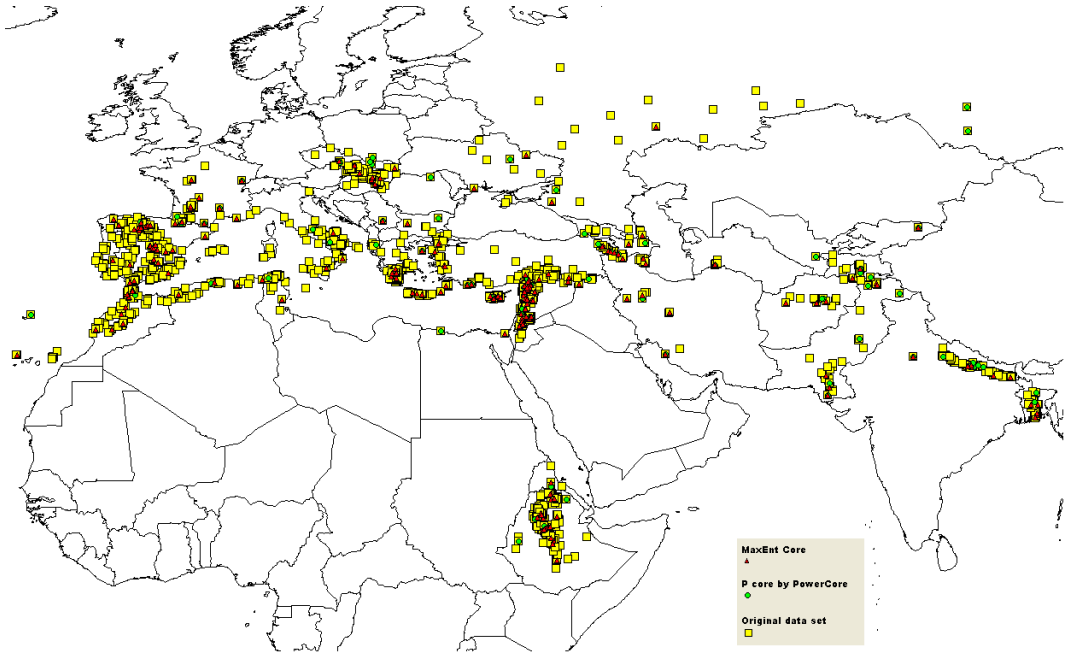

Figure 5.2. Distribution map of *Lathyrus* accessions selected using MaxEnt and PowerCore approaches

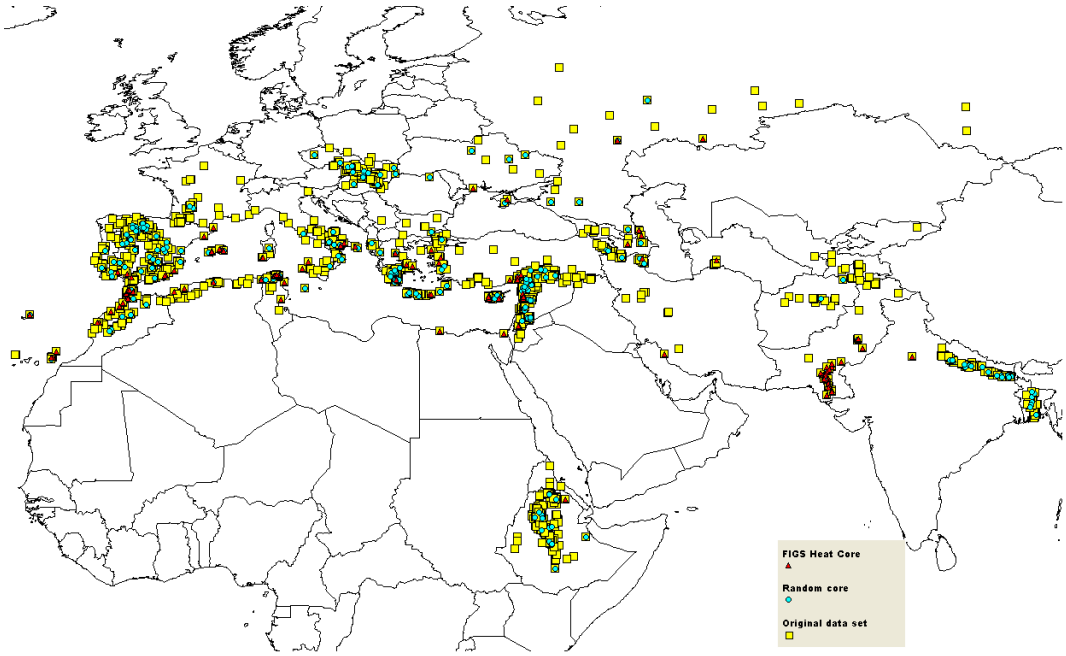

Figure 5.3. Distribution map of *Lathyrus* accessions selected using FIGS and random approaches

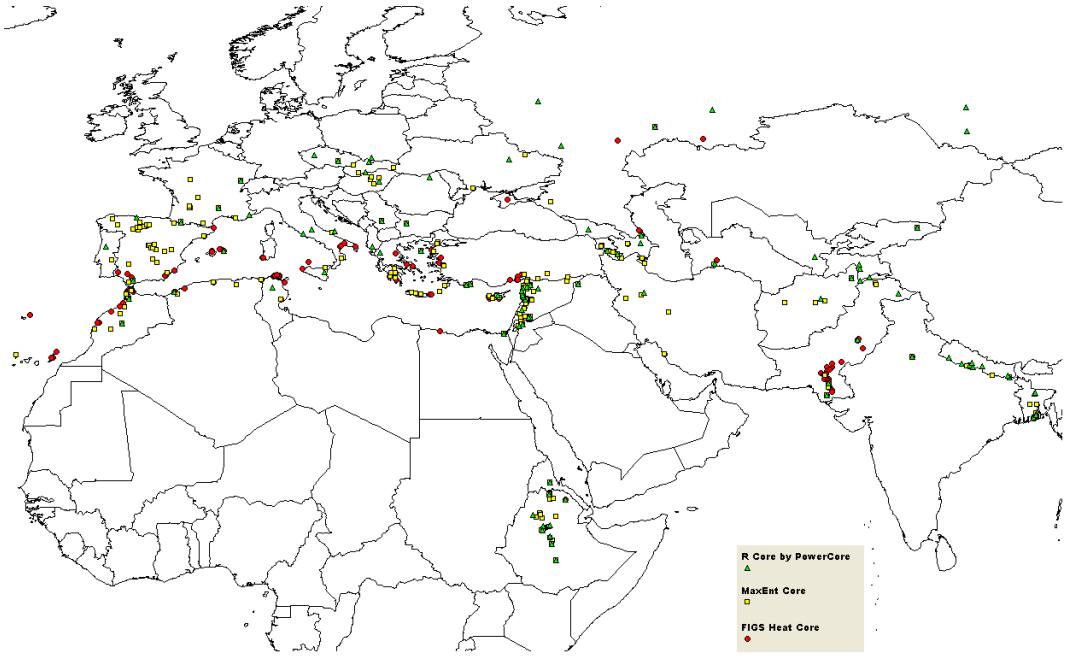

Figure 5.4. Distribution maps of *Lathyrus* accessions selected using MaxEnt, PowerCore and FIGS approaches

5.5 Discussion

Most of the accessions held by many genebanks are not georeferenced as we were able to include only 49% of accessions of *Lathyrus* priority species included in the global dataset, indicating the need for more efforts to assign georeferenced information for the remaining accessions, mainly from some national genebank in Ethiopia and Central, West Asia and North Africa. We were constrained to use only climatic and georeferenced information to conduct the selection of different core sets and FIGS set because of the limited information available on characterization and evaluation of genetic resources.

All core subsets selection approaches showed similar or higher values of Shannon diversity index than the original data and all succeeded in including many accessions from the species with high sample size, when applying 10% selection rate. PowerCore approach, mainly used for development of allele mining sets (Kim *et al*, 2007), was adapted to serve the purpose of selecting core sets, but has selected only 1-3 accessions for species with low sample size and even for species with larger number of accessions like *L. annuus, L. articulatus, L. blepharicarpus, L. clymenum, L. gorgoni, L. marmoratus,* and *L. pseudocicera*. Although this approach gives the highest Shannon diversity index and includes all species in the sets constructed, it might not be able to reflect the needed within species variation for various adaptive traits. This limitation can be overcome to some extent by increasing the rate of representative sample size above 10% applied in this study or by increasing the number of variables and clusters considered in the analysis.

MaxEnt in the absence of phenotypic and genotypic information can serve to form a better representative set which can capture more variation at the species level. This approach has been advocated by many researchers to study the distribution of species as reported by

Elith *et al*., (2011) and Phillips and Dudik (2008). Its use can be extended to select core sets, to understand correlations between environmental layers and species traits, and to predict species richness or diversity, towards better conservation planning of *Lathyrus* and also to target accessions with needed traits. MaxEnt approach, which can use eco-geographic information allows to grasp the diversity of the original population and accounts for a good geographic distribution and representation. It allowed to select more accessions per selected species compared to PowerCore approach.

The use of stratified random selection based on taxonomic representativity improved the Shannon diversity index as shown for Max-Ent and R-facilitated subsets. The Shannon diversity index was highly improved when the accessions from minor species were added to the random MaxEnt set. This also will be the case if the accessions of minor species were added to the random subsets. This recommends that all approaches can be adapted to extract core subsets using environmental characteristics provided that a process is developed to allow the inclusion of accessions from minor species (not selected by random approaches because of their few accessions).

FIGS approach was used to extract best bet set for heat and drought tolerance based on maximum temperature and aridity index. Although it has its accessions concentrated in some regions, as expected, FIGS has shown more common accessions with the other subsets. The commonality among different methods can be improved if neighborhood analysis can be done to relate the accessions of the same species and found within the same environment. Most of the selected accessions using FIGS belong to the species *L. annuus, L. blepharicarpus, L. articulatus, L. gorgoni, L. ochrus* and *L. sativus*, which are known to be found more in areas prone to drought and heat (Table 5). It has permitted the selection of an acceptable size set of accessions to be further tested for tolerance to heat and drought under

field or controlled conditions using either direct evaluation of performances or physiological or metabolic traits related to tolerance to these abiotic stresses.

The efficiency of all the approaches used in forming subsets out of the original collection could be improved substantially if characterization and evaluation of phenotypic or genotypic traits are available. This will allow for selection using a two stage approach: the development of a priori information (dataset template or specialized knowledge) as per suggested by Gollin *et al*. (2000) to determine the trait-environment relationship, followed by the use of this information to define a subset of accessions with a higher probability of containing the sought after traits.

To ensure the continuum between conservation and utilization, proper selection of the accessions from large collections will help to better focus the evaluation efforts on a subset with higher probability of finding the sought traits. While core collections have contributed to some extent the selection of subsets, FIGS approach and other similar initiatives could combine both the objective of manageable size with the selection of accessions with higher probability of finding the needed traits. ICARDA is leading further development of the FIGS approach, adding more algorithms. Actually most of distributed material form ICARDA genebank is done using FIGS approach.

5.6 References

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CHAPTER SIX

FIELD GUIDE PRODUCTION

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6.1 Abstract

A Field Guide for the "Grass pea and Chicklings (*Lathyrus* L.)" of the Mediterranean Basin and the Caucasus, Central and West Asia regions is produced including 76 taxa with line drawings for 54 taxa, to assist local plant genetic resources (PGR) workers in species identification. This will be an easy to use identification aid, which will avoid the jargonistic pit-falls of conventional taxonomic keys. This aid is using different illustrations (line drawings, photographs, paintings, etc.) of the key features of the species, hence avoiding recourse to complex botanic terminology. In addition, it includes well detailed texts containing scientific and vernacular names, diagnostic descriptions, iconography, and alliances to other species, distribution maps, phenology, ecological preferences, geographic distribution and conservation status. DELTA software is used to produce Lucid outputs in accordance to the characterization and observations of the morphological characters for *Lathyrus* species studied. These outputs aimed to be user-friendly and therefore more widely accessible. Both DELTA and Lucid have flexibility for iincorporating characters within the keys.

Keywords: *Lathyrus* field guide, taxonomy, DELTA, LUCID, ecogeographic survey, geographical distribution, identification and keys.

6.2 Introduction

6.2.1Necessity for conservation field guide to Chicklings (*Lathyrus* L.)

The genetic diversity of the genus *Lathyrus is* of significant importance, particularly for potential use in rain-fed cropping systems of many countries and as a genetic resource for the improvement of *L. sativus* L. Several other species are also cultivated for human consumption, animal feed, and fodder, as well as for ornamental purposes but there is a potential for further exploitation of the *Lathyrus gene* pool. Therefore, the collection, conservation, characterization, study of genetic diversity and utilization of the genus *Lathyrus* deserves immediate attention as a priority research area. There is an urgent need to conserve the genetic diversity of the genus using both *ex situ* (gene banks) and *in situ* (natural habitats) conservation methods. This will permit a critical assessment of the genetic diversity and the genetic erosion of the genus, along with enhancing its exploitation.

The inability of many conservationists and field biologists to use traditional identification aids has led to confusion of specimen identity and has undoubtedly resulted in the application of poor conservation and as a result poor utilisation of much valuable material. Plant species that are not accurately identified, either remains uncollected, because their value is not recognised when encountered, or is collected, but remains unidentified and is incorporated into gene banks as "unknown legume species" or "*Lathyrus* sp.", or is collected, but misidentified, leading to spurious results when utilised. The growing out and re-identification of several large germplasm collections (Maxted, 1989, 1992; Maxted and Bisby, 1986, 1987) have estimated that a significant proportion, between 10 and 35%, of *ex situ* conserved material currently held in

national and international gene banks is either wrongly identified or is not identified at all. In this state, the material is of limited conservation value and is unlikely to ever be utilisation. If the justification for active conservation is potential use, then the onus must be on the conservationists to promote effective conservation and link that conservation to use by supplying the conserved diversity to the user community in the most appropriate manner. Species recognition based on experience is also of limited value for field biologists, particularly in the centres of diversity, because as Morse (1971) concluded initial recognition depends on either being self-taught or learned from an expert, which implies the requirement for taxonomic expertise which in West Asia and North Africa region is in short supply. In contrast, comparison covers a broad array of approaches, including searching through museum specimens for matching specimen, reading descriptions, reviewing illustrations and flicking through named photographs. This approach works but is impractically time-consuming and requires access to an extensive range of named comparative materials. By far, the most practical and efficient means of identification is the use of user friendly keys or related identification aids. Keys offer a step-by-step approach to identify a species commonly employing a dichotomous hierarchical tree in which the user follows a sequential path to the end of the branch, at which time the species of interest is identified. Despite their widespread use by the taxonomic community itself (Fortuner, 1989 and Thompson, 1999), traditional dichotomous keys have serious limitations due to the amount of technical botanical terminology used to describe plant parts and their stylized format. Also writing keys is highly skilled and badly written keys abound. The use of these traditional keys remains a seriously limiting problem for those who lack formal biological training and is an unnecessary limitation to plant conservation and exploitation in many regions of the world.

To surmount these ‘problems’ of plant identification and aid conservation and use of genetic diversity, it is necessary for a paradigm shift in biodiversity identification. The

conservation and other non-taxonomic communities need to benefit from recent but well-established developments in computer science to apply innovative methods of plant identification and computer-aided-learning programs, which can be used by professional and amateur communities alike. This "Conservation Field Guide" is the second, after that of *Medicago* L. of a proposed series that will employ these contemporary techniques to aid non-experts field botanists identify the plant diversity that requires conservation (Al-Atawneh, *et al.*, 2009).

6.2.2 Content of conservation field guide

There is no universal format for what constitutes a field guide. It might focus on a particular habitat, region or taxon, and it commonly presents taxonomic background, morphological descriptions, habitats, behaviour, ecology, distribution maps, uses, conservation notes and simple dichotomous keys suitable for field use, possibly annotated with line drawings, photographs or paintings. This "Conservation Field Guide" to Grass peas and Chicklings of the Mediterranean Basin and the Caucasus, Central and West Asia regions include all of these components, plus notes on the current conservation status, threats assessment and usage which will be include within each taxon statement, as shown in Figure 6.1. This guide, in an attempt to specifically address the problem of non-expert identification, the printed version will be accompanied by a CD with an interactive identification system. Lucid was developed specifically as a means of helping taxonomists communicate their data sets to non-specialists who wish to identify animals, plants and other organisms. Once constructed, the keys can be made freely available via the Lucid website (www.lucidcentral.com). Lucid based keys are accessed via the Lucid Player which provides the interface for users to load and interact with the Lucid keys, using text, images, videos and sounds to help select those taxonomic, diagnostic or other features that best describe the particular case being investigated.

The key works by elimination so that as the user selects character states, the program eliminates the taxa that do not possess that state, and this is repeated until a single taxon remains and the identification is achieved. Once a specimen has been identified to a particular taxon, the user is provided with a full range of multimedia fact sheets, sub-keys for infra-specific taxa or links to websites for further information or recommendations.

Page A	Page B
Accepted name	Line drawing
Synonyms	
Description	
Habitat	
Distribution map	
Geographical distribution	Photographs
Conservation assessment	
Threat assessment	
Actual and potential usage	

Figure 6.1. Conservation Field Guide Individual Taxon Information Layout.

Lucid keys can be built in various languages and use terminology familiar to the user, allowing the package to be used internationally and across a wide range of capabilities. Potential users range from biologists, geologists, agriculturists, veterinary and medical scientists to university and high-school students and the public at large. Details on operation of the Lucid key are provided in Appendix 6.1.

6.3 Materials and Methods

6.3.1 Conservation field guide construction

Although the production of this field guide is directly associated with the problems encountered during the field survey activities of the Global Environment Facility (GEF) and the United Nations Development Programme (UNDP) funded and ICARDA coordinated project on "Conservation and Sustainable Use of Dryland Agrobiodiversity in Jordan, Lebanon, the Palestinian Authority and Syria", the realisation of the need for "Conservation Field Guides", and therefore the gestation of the project, has been much longer. In fact at least since the explosion in crop and wild species conservation in the 1960s, the problem of identification of wild species has been appreciated by professional conservationists. As such the data sets used to construct this volume have been accruing for the same time period.

The methodology employed for Conservation Field Guide construction is an adaptation of Maxted (1996) and involves the following stages:

2. The taxon group is selected and delimited.
3. Diagnostic characters were selected which distinguish each taxon from related taxa, All taxa are scored for the character set producing a data matrix.
4. The dataset was then:
   i. Entered into DELTA (Dallwitz *et al.*, 2000) format and descriptions and dichotomous keys generated using the DELTA associated programs TONAT and KEY.
   ii. The DELTA format descriptive data was imported to LUCID via Lucid Translator, manipulated in Lucid Builder which generated the interactive key that may be

viewed with Lucid Player.

5. To aid visual identification various illustrations (line drawings and photographs) were prepared.
6. An ecogeographic survey was undertaken to collate the necessary geographic, ecological, taxonomic and genetic data to complete the conservation element of the field guide.

The process that was followed for the 'Field Guide to the genus *Lathyrus* L.' is described in the following sections.

6.3.2 Selection and delimitation of the study group

The study group is the *Lathyrus* species in the Mediterranean Basin and the Caucasus, Central and West Asia. *Lathyrus* species are economically important grain and forage legumes, they are globally used as a quality fodder for the livestock and as ornamental species. *Lathyrus* is extremely useful in agricultural systems as green manure and as an environmental plant for the sustainable conservation and land improvement especially in the dry areas, because of its drought tolerance and the fast growing as a green forages. *Lathyrus* is composed of about 170 species (ILDIS, 2010), 74 species and 2 subspecies of these are present in the Mediterranean Basin and the Caucasus, Central and West Asia regions (target regions) and these are covered by the guide. The foundation of the ecogeographic survey was both literature and specimen based information for *Lathyrus* species from the target regions. Specimens were used to study the geographical and taxonomic distribution of *Lathyrus* species in the target regions. This survey is carried out in different international herbaria which have been visited to study the specimens kept.

It is vital when undertaking an ecogeographic study that the target taxon be studied throughout its range to obtain an accurate and unbiased overall picture of the genepool (Maxted

et al., 1995). However, genus *Lathyrus* has been found in Europe, the Mediterranean, and Central, West and Southern Asia and these areas are the most important centres of diversity for *Lathyrus* (Sarker *et al.*, 2001). Secondary centers of diversity exist in South America, North America and Ethiopia, extending into East Africa (Kupicha, 1983). Figure 6.2 shows the natural distribution of *Lathyrus* species in the Mediterranean basin, Central and West Asia and North Africa regions, based on herbarium specimen and gene bank accessions passport data, and the species richness over the same area. The figure highlighting the distribution of various species is produced using DIVA-GIS software (http://www.diva-gis.org); a detailed discussion of the capabilities of DIVA-GIS is provided by Hijmans *et al.* (2005). The highest number of species is found in Turkey, followed by the countries of the Caucasus region and Syria (ILDIS, 2010) (Table. 6.1). It has been observed that *L. ochrus* and *L. sativus* are mostly distributed in coastal, lowland sites, while *L. cicera* is the most common species in highlands and cold temperate sites (Sarker *et al.*, 2001). The natural distribution of *L. sativus* has been completely obscured by cultivation, even in Southwest and Central Asia (Townsend and Guest, 1974). It is quite difficult to clarify the centre of origin of the genus. It can be argued that relative species concentrations can be used to indicate the centre of origin, which would therefore suggest a South Eastern Europe and North Western Asian origin. More than half of the *Lathyrus* taxa are endemic to this area (Sarker *et al.*, 2001). However, due to floristic migration caused by the recent ice ages, Kupicha (1974) concluded that *Lathyrus* evolved in the early tertiary and the centre of origin is likely to have been much further north than is indicated by contemporary concentrations of taxa, (Sarker *et al.*, 2001).

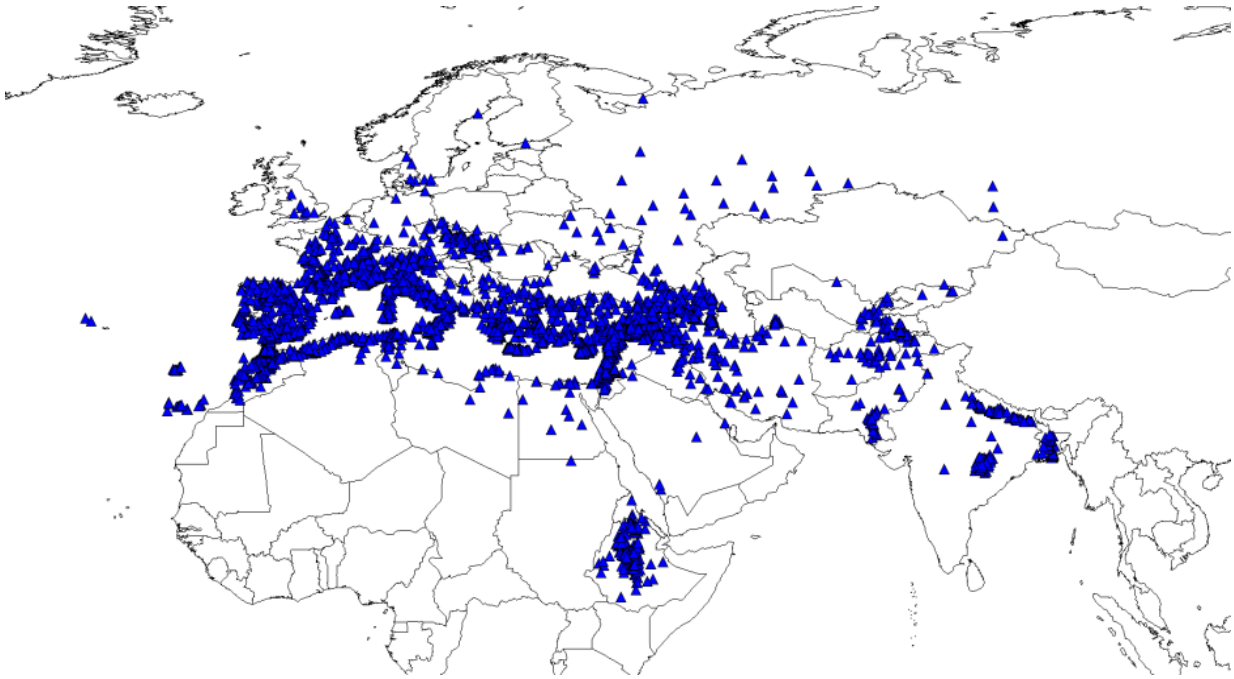

Figure 6.2. Distribution of *Lathyrus* accessions in Europe, the Mediterranean Basin and Caucasus, Central, South and West Asia regions

Table 6.1. Regional distribution of *Lathyrus species* in the Mediterranean Basin and Caucasus, Central and West Asia region

Species	North Africa	West Asia	Caucasus & Central Asia	Eastern Europe	Western Europe
L. amphicarpos L.	√	√			√
L. angulatus L.	√			√	√
L. annuus L.	√	√	√	√	√
L. aphaca L.	√	√	√	√	√
L. armenus (Boiss. & A. Huet) Čelak.		√			
L. aureus (Stev.) Brandza *(Steven)* Bornm.		√	√	√	
L. basalticus Rech.		√			
L. bauhinii P.A. Genty					√
L. belinensis Maxted & Goyder		√			
L. blepharicarpus Boiss		√			

L. boissieri Sirj.		√			
L. brachypterus Čelak.		√			
L. cassius Boiss.		√			
L. chloranthus Boiss.		√	√		
L. chrysantus Boiss.		√			
L. cicera L.	√	√	√	√	√
L. cilicicus Hayek & Siehe		√			
L. ciliolatus Sam.		√			
L. clymenum L.	√	√		√	√
L. cyaneus (Stev.) Koch		√	√		
L. czeczottianus Bässler		√			
L. digitatus (Bieb.) Fiori & Poal.		√	√	√	
L. elongatus (Bornm.) Sirj.		√			
L. gleospermus Warb. & Eig.		√			
L. gorgoni Parl.	√	√			√
L. hierosolymitanus Boiss.	√	√		√	
L. hirsutus L.	√	√	√	√	√
L. hirticarpus Mattatia & Heyn		√			
L. inconspicuus L.	√	√	√	√	√
L. incurvus (Roth.) Willd.		√	√	√	
L. japonicus Willd.				√	√
L. karsianus P.H. Davis		√			
L. laxiflorus (Desf.) Kuntze		√	√	√	
L. layardii J. Ball ex Boiss.		√			
L. libani Fritsch		√			
L. linifolius (Reichard) Bässler	√				
L. lycicus Boiss.		√			
L. marmoratus Boiss. & Bl.	√	√			
L. neurolobus Boiss. & Heldr.				√	
L. niger (L.) Bernh.	√	√		√	√

L. nissolia L.	√	√	√	√	√
L. nivalis Hand.-Mazz.		√			
L. ochrus (L.) DC	√	√		√	√
L. odoratus L.	√	√	√	√	√
L. pallescens (Bieb.) Koch			√	√	
L. palustris L.			√	√	√
L. pisiformis L.			√	√	
L. phaselitanus Hub.-Mor. & Davis		√			
L. pisiformis L.		√	√	√	√
L. pratensis L.	√	√	√	√	√
L. pseudocicera Pamp.	√	√			
L. pygmaeus Gomblaut		√			
L. roseus Stev.		√	√		
L. rotundifolius Willd.		√	√	√	
L. satdaghensis P.H. Davis		√			
L. sativus L.	√	√	√	√	√
L. saxatilis (Vent.) Vis.	√	√	√	√	√
L. setifolius L.	√	√	√	√	√
L. spathulatus Čelak.		√			
L. sphaericus Retz.	√	√	√	√	√
L. stenolobus Boiss.		√			
L. stenophyllus Boiss. & Heldr.		√			
L. sylvestris L.	√	√	√	√	√
L. tauricola P. H. Davis		√			
L. tingitanus L.		√		√	√
L. trachycarpus (Boiss.) Boiss.		√			
L. tuberosus L.		√	√	√	√
L. tukhtensis Czecz.		√			
L. undulatus Boiss.		√		√	

L. variabilis (Boiss. & Ky.) Maly		√			
L. venetus (Miller) Wohlf.		√		√	
L. vernus (L.) Bernh.		√	√	√	√
L. vinealis Boiss. & Noe		√			
L. woronowii Bornm.		√			

Having established the target area, taxonomic and ecogeographic information on local geographical distribution and ecological preferences for *Lathyrus* was obtained from local Floras, ICARDA genebank and ILDIS database.

- **Taxon data collation**

The collection of ecogeographic information followed the model proposed by Maxted *et al.* (1995). Ecogeographic data was collated for *Lathyrus* taxa from existing published and unpublished literature sources, as well as from the passport data associated with gene bank accessions and herbarium specimens.

Most of the ecogeographical distribution information was compield from International Legume Database Information System (ILDIS), and from

GRIN). Additional information was gathered from books, journal articles, CD-ROMs and the internet. An example of the kinds of data that were obtained from from different sources is given in Table 6.2 for *Lathyrus laxiflorus Desf.* subsp. *laxiflorus*. The collation of much of this information was done while visiting major herbaria, which are usually associated with good botanical libraries.

Table 6.2. Example of ecogeographic data obtained from a herbarium specimen.

Specimens or Accessions Data	Example Data
3.1.1.1.1.1.1 Sample identification	*Lathyrus laxiflorus Desf.* subsp. *laxiflorus*
Herbarium or gene bank where deposited	Botanical Gardens, Kew
Collector's name and number	Maxted, Ehrman & Khattab, 2100
Collection date (to derive flower and fruiting time)	01/03/1986
Phenological data (does specimen have flowers or fruit?)	Flower - yes Fruit – yes
Particular area of provenance including Latitude and Longitude information.	Country – Syria Province – Lattakia Location – 5km N Kastal Al-Maaf towards Kassab Latitude, 35 56E Longitude, 35 50N
Altitude	580 m
Habitat	Oak woodland on steep slopes
Soil colour	Black
Soil type	Highly organic
Associated species	3.2 Pinus brutia, Quercus calliprinos
Vernacular names	-
Plant uses	-

- **Gene bank accession and herbarium specimen data collation**

As noted by Davis and Heywood (1963) and Maxted *et al.* (1995), the broader the sampling of ecogeographic data associated with germplasm accessions and herbarium specimens, the more likely the data will prove ecologically and geographically predictive. Therefore, passport data was obtained from international herbaria as well as from gene banks. In all, passport data was obtained.

A full citation of specimens in the text was not possible but a Specimen Citation database is included, with the full *Lathyrus* database included in the the accompanying *Lathyrus* Data CD. Specimens or accessions are listed in Specimen Citation database and the

following data are available: taxon identification, county, locality, town, habitat, whether flowering or fruiting, day / month / year of collection, latitude / longitude, elevation (m), collector and collection number, herbarium or gene bank where deposited. The quality of the passport data associated with gene bank accessions is significantly better than herbarium specimens. Where possible, the following data were collected from herbarium labels and gene bank documentation systems: collector's name, collector's number, date of collection, presence/absence of flowers, presence/absence of seeds, country of collection, nearest town, exact locality, habitat, description of collecting site including soil colour, soil texture, associated species, biotic and abiotic factors observed at the collecting site, and any ethnobotanical data recorded for the specimen. An example of the kinds of information it was possible to collate for *Lathyrus* from passport data associated with herbarium specimens and germplasm accessions is given in Table 6.2. This listing of categories of ecogeographic data is extensive and it was not possible to record this amount of information from every specimen. As with any ecogeographic survey there are, however, certain data that must be recorded for the study to yield predictive results, and these are the first seven fields in Table 6.2 (in bold). The other data types listed would normally be recorded at the collection site by the germplasm conservationist these days, but many of the herbarium specimens seen were collected by botanists several decades ago, when the need for such detailed passport data was not fully appreciated. Databases of ICARDA genebank as well of international gene banks were searched over the web to determine the number and origin of germplasm accession of each species of *Lathyrus* held. Where latitude and longitude were not given, or if errors were suspected, gazetteers were used to determine correct coordinates.

The total data used in this study were derived from 3,360 unique herbarium specimens of 76 *Lathyrus* species, and 3219 unique germplasm accessions of 37 priority species. The

Lathyrus ecogeographic data were obtained from eight datasets, mainly from International Centre for Agricultural Research in the Dry Areas (ICARDA), Global Biodiversity Information Facility (GBIF), Global *Lathyrus* database and from personal ecogeographic surveys in seven major international herbaria (the Royal Botanic Gardens in Kew, UK, the Royal Botanic Gardens in Edinburgh, UK, the Natural History Museum, London, UK, the Natural History Museum, Paris, France, the University of Montpellier, France, the Botanic Gardens in Geneva, Switzerland, and Florence University in Italy). The largest group of data came from GBIF (http://www.gbif.org/), the Global Database of *Lathyrus* collection (ICARDA-GRS, Syria), with additional data from different collections by Nigel Maxted, and from several ecogeographic surveys of food and forage legumes undertaken jointly by ICARDA and the University of Birmingham from 1998 to 2010. The lead author has visited the seven herbaria and examined all *Lathyrus* specimens available. A special effort was undertaken to find out the exact location of the collection sites observed through the survey. It is noticed that the majority of these historical specimens observed, did not have coordinates information. Therefore, the coordinates have been picked up by using different sources and references. Microsoft Encarta software was used to find out the geographical coordinates for the site where the specimens have been collected. Information from each database was standardized and duplicate observations identified and removed. In addition, occurrences identified as being outside of the natural range of the species were considered to be introductions and therefore were taken out of the final dataset. Where latitude and longitude were missing, these records were also removed before the final spatial analysis. The combined, corrected datasets of 2695 *Lathyrus* specimens, and 1988 *Lathryus* accessions were then spatially analysed and used for discussions of the results. Both datasets are freely available from the senior author on request and a summary is provided in Appendix 6.2.

- **Ecogeographic database**

The herbarium specimen and gene bank accession data were collated directly into a database to facilitate data checking and analysis and also to avoid transcription errors. The basic structure of the database file is shown in Table 6.3 with an explanation of the content of the fields.

Table 6.3. Field structure and content of the ecogeographic database.

Field	Data Type	4 Field Name	Field Description
1	Taxonomic	SUBGENUS	*Lathyrus* subgenus to which species belongs
2		SECTION	*Lathyrus* section to which species belongs
3		SPECIES	Accepted *Lathyrus* species name
4		SUBSPECIES	Subspecies name, if appropriate
5		VARIETY	Varietal name, if appropriate
6	Curatorial	H_OR_G	Whether herbarium specimen or gene bank accession
7		COLLECTION	Collection where herbarium specimen or gene bank accession was located, herbarium codes follow Holmgren *et al.* (1990)
8		COLLECTOR	Name of collector(s)
9		COLL_NOS	Number given by collector to specimen
10		DAY_OF_C	Day of collection
11		MONTH_OF_C	Month of collection
12		YEAR_OF_CO	Year of collection
13	Descriptive	FLOWERS	Flower present / absent
14		FLOWER_COL	Colour of flower
15		FRUIT	Fruit present / absent
16	Geographic	FLORCODE	Kew Floristic Region Code
17		COUNTRYCOD	BRU country code
18		PROVINCE	BRU province code
19		TOWN	Name of nearest town
20		LOCALITY	Name of nearest settlement
21		DISTANCE	Distance from nearest town
22		DIRECTION	Direction from nearest town
23		LATITUDE	N = +; S = -

24		LONGITUDE	E = +; W = -
25	Ecological	ELEVATION	Height in metres
26		HABITAT	Ecological habitat where specimen found
27		VEGETATION	Vegetation type at site of collection
28		SOIL_COLOU	Colour of soil where specimen found
29		SOIL_TEXTU	Texture of soil where specimen found
30		SITE_STONI	Stoniness / rockiness where specimen found
31		PARENT_ROC	Type of parent rock
32		SLOPE	Slope of ground
33		ASPECT	Aspect of collection site
34		EXPOSURE_T	Degree of openness of site
35		DRAINAGE	E (excessive) / G (Good) / M (Moderate) / P (Poor)
36		LAND_USE	Principle use of land
37		BIOTIC_FAC	Any noted biotic interaction with site where the specimen was found
38		ABIOTIC_FA	Any noted abiotic interaction with site where the specimen was found
39		FREQUENCY	Estimation of population size at site where the specimen was found

The database was indexed (*i.e.* the records were rearranged in alphabetical or numerical order) on each field in turn to highlight typing errors or invalid entries. Exploratory mapping using the latitude and longitude fields also revealed location errors, specimens collected from the sea or from a different geographical unit, and these errors were corrected whenever possible. A copy of the database is provided on the accompanying *Lathyrus* field guide's CD.

6.3.3 Selection of characters

Selection of characters is an important step for producing a good key. Characters need to have a high content of information and be easy to distinguish in the field or the laboratory. Many literatures used to select the characters for this study include Floras, monographs, published papers, and previous identification keys. For *Lathyrus*, these included classifications of Kupicha,

1983; Davis, 1970; Townsend and Guest, 1974; Meikle, 1977; Maxted *et al.*, 1988; and Maxted, 1993, as well as personal observations by the field guide authors. The characters are of morphological nature most of which are easily determined in the field though some require a hand lens. Seventy-five characters were chosen with maximum possible number of state of fifteen. Most of the characters were given adecquate number of states to avoid the loss of information. The character set is included in Appendix 6.3.

6.3.4 Scoring of taxa for characters

Seventy-six species and sub-species were scored for the selected characters using live specimens and descriptions in the Floras, Monographs, and other literatures listed above. Some species have a comprehensive published description while others are much briefer. For those, which have a brief description, the missing character states were collated from observations of herbarium specimens from major herbaria (the Royal Botanic Gardens in Kew, UK, the Royal Botanic Gardens in Edinburgh, UK, the Natural History Museum, London, UK, the Natural History Museum, Paris, France, the University of Montpellier, France, the Botanic Gardens in Geneva, Switzerland, and Florence University in Italy). However, it proved difficult to score some floral or fruit characters from the older dried specimens and this resulted in a limited quantity of missing data. Character scores were only included if they were recorded by the project team, mentioned in descriptions or seen in the herbarium specimens.

6.3.5 Computer aided description

The DELTA system has three main files, which are constructed from the character list (file - CHARS), the character scores for individual taxa (file - ITMS) and the directive information (file - SPECS). Creating these files is explained in details in the "User's Guide to the DELTA System" Edition 4.12 by Dallwitz *et al.*, (2000). All data were checked for errors using the

standard DELTA error checking directive file (file – CHECK) and any errors encountered corrected. Once the three data files were completed, the description generating directive file (file – TONAT) was used to generate the natural language description and the key generating directive file (file – TOKEY) were used to create natural language descriptions and keys via the DELTA associated programs CONFOR and KEY respectively.

6.3.6 Illustrations

One of the unfortunate problems associated with non-specialists learning to use traditional keys is the amount of technical botanical terminology involved. However, the problem can be circumvented somewhat by providing illustrations (line drawings, photographs, paintings etc) of taxa or the key features necessary for identification, hence avoiding recourse to complex terminology. The use of illustrations, when combined with technical terminology, can not only aid identification, but can also help the user learn and understand the meaning of the botanical terminology used. The relative efficacy of using line drawings, paintings or photographs to aid identification is a matter of subjective assessment and individual preference. However, one problem associated with using photographs for identification is that they can only show what is observed at that time in a two dimensional image, whereas with a drawing or painting, the illustrator can enhance the observed two dimensional image to include features that may be less obvious on an individual specimen or in that particular plane of view. For this reason line drawing illustrations are provided for the majority of taxa, as well as complex character states along with the photographs.

6.3.7 Ecogeographic background

An ecogeographic survey is defined by Maxted *et al.* (1995) as: "an ecological, geographical and taxonomic information gathering and synthesis process. The results are

predictive and can be used to assist in the formulation of collection and conservation priorities". Ecogeographic studies involve the collation and analysis of large and complex data sets obtained from the literature and from the passport data associated with herbarium specimens and germplasm accessions. The data compiled are of four basic kinds: ecological, geographic, taxonomic and genetic. These data are synthesised to produce three basic products: the database - which contains the raw data for each taxon; the conspectus - which summarises the data for each taxon; and the report - which discusses the contents of the database and conspectus, as well as proposing future conservation priorities and a coherent strategy. As such, undertaking an ecogeographic survey is essential for efficient and effective conservation, for example it helps identify centres of plant diversity, taxonomic and genetic diversity, areas that require *ex situ* collection and where *in situ* genetic reserves may best be sited. The methodology used for the ecogeographic survey is that proposed by Maxted *et al.* (1995).

6.4 Results

6.4.1 Ecogeographic and taxonomic analysis

6.4.1.1Ecogeographic analysis

- **Geographical distribution**

Lathyrus is a large genus containing around 170 species (ILDIS, 2010), mainly located in Europe, Asia and North America, and extending to temperate South America and tropical East Africa, but the genus has its centre of diversity primarily in the Mediterranean and Irano-Turanian regions (Kupicha, 1981). It is adapted to temperate regions but can also be found at high altitudes in tropical Africa. Endemic species are present in all continents, except Australia and Antarctica (Kupicha, 1981).

L. sativus L., *L. cicera* L. and *L. ochrus* (L.) DC. provide important human food, animal feed and fodder sources. *L. sativus* is widely cultivated for human consumption, as well as for fodder and green manure. Its primary centres of cultivation are in Southern Asia, particularly in Bangladesh, China, India, Nepal, and Pakistan, and in Ethiopia (Asthana, 1996), with more limited production in southern Europe and West Asia. *L. cicera* is cultivated in Greece, Cyprus, Iran, Iraq, Jordan, Spain and Syria and *L. ochrus* in Cyprus, Greece, Syria and Turkey (Saxena *et al.*, 1993). Some other species are used as minor forage or fodder crops: *L. hirsutus* L. is cultivated in southern United States as a fodder species and *L. clymenum* L. is cultivated on Kos, Greece (Sarker *et al.*, 2001). It is an important low risk aversion crop because it has relatively good tolerance to water-logging (in the case of flooding), good ability to grow on residual moisture after the end of the rains or in case of drought, and because it requires low production costs (Tadesse, 1997).

The geographical distribution of *Lathyrus* species in the Mediterranean Basin, Caucasus,

.Central and West Asia region that have been sampled as either herbarium specimen or germplasm collections and the individual species distribution covered by the guide are summarised in Table 6.4.

The genus contains many restricted endemics, for which only very few sites have been documented or which are bound by specific soil types and climatic regimes (Maxted and Goyder, 1988; Ehrman and Maxted, 1990; Maxted, 1993; Maxted *et al.*, 1990; Bennett *et al.*, 1998, 1999; Shackle *et al.*, 2001). The ecogeographic distribution of all but a few *Lathyrus* species is poorly understood, particularly those in sect. *Notolathyrus* that are endemic to South America. There is a need for a detailed ecogeographic study of the whole genus if it is to be effectively and efficiently conserved and utilized (Sarker *et al.*, 2001).

Turkey has the richest diversity of *Lathyrus* species genetic diversity. Davis (1970) reported the presence of 58 species in Turkey, some of them endemic at local or regional level.

Table 6.4. Geographical and ecological distribution of *Lathyrus* species in the Mediterranean Basin and Caucasus, Central and West Asia regions.

Section (no. species in region/total number of species)	Species / subspecies	Natural distribution range	Natural habitat
Orobus (8/54)	*L. aureus* (Stev.) Brandza (Steven) Bornm.	Asia, CWANA, S.E Europe	Forest and scrub, 15-2000 m.
	L. incurvus (Roth.) Willd.	Asia minor, CWANA, E. Europe	Meadows, among shrubberies, at lakesides, quite often on saline soils and scrub near rivers, 600-2000 m.
	L. venetus (Miller) Wohlf	Asia, Europe, Russia, Ukraine, West Asia.	Forests (*Abies-Fagus*), grazed land, 600-950 m.
	L. libani Fritsch	Palestine, Turkey	*Cedrus-Fagus* forest, rocky limestone pasture, 750-1650 m.
	L. niger (L.) Bernh.	N. Africa, Europe, Asia, Oceania	Forests and *Quercus* scrub, shady places, nr. S.l.-1000 m.
	L. japonicus Willd.	Asia, Europe, N. America,	Sandy seacoast

		S. America	
	L. palustris L.	Asia, Caucasus, Europe, N. America	Marshy ground, Among bushes, and in meadow -1000 m.
	L. vernus (L.) Bernh.	West Asia, Caucasus, Europe	In forest fringes and glades,, scrub, rock ledges, meadows, among shrubberies. 60-1400 m.
Lathyrostylis (14/20)	*L. nivalis* Hand.-Mazz.	Iran, Iraq, Turkey	Rocky slopes and screes, usually on limestone, 2400-3200 m.
	L. tukhtensis Czecz.	Turkey	*Pinus* forests, *Quercus* scrub, mountain pastures, rocky limestone slopes, 700-2000 m.
	L. variabilis (Boiss. & Ky.) Maly	Lebanon, Turkey	Forests, *Quercus* scrub, rocky slopes, 1000-1700 m.
	L. satdaghensis P.H. Davis	Turkey	Stony slopes, 1900-2150 m.
	L. karsianus P.H. Davis	Turkey	Banks, rocky places and meadows at the edge of *Pinus sylvestris* forest, 2000-2300 m.
	L. cyaneus (Stev.) Koch	Caucasus, Iran, Turkey	Moist meadows, rarely on rock

		faces, 1670-2800 m.
L. digitatus (Bieb.) Fiori & Poal.	Libya, Caucasus, Europe, Middle East	Quercus woods, macchie, rocky slopes, scree, 200-1550 m.
L. armenus (Boiss. & A. Huet) Čelak.	Lebanon, Libya, Turkey	Marshy meadows, water channels, fallow fields, 1270-2800 m.
L. pallescens (Bieb.) Koch	Caucasus, Europe, Kazakhstan, Turkey	Mountain steppe, meadows, rocky slopes, 1800-2200 m.
L. brachypterus Čelak.	Turkey	Pastures, rocky slopes, 1500-2500 m.
L. spathulatus Čelak.	W. Asia	*Pinus brutia* forest, deciduous woodlands, *Quercus coccifera* macchie, 400-1520 m.
L. elongatus (Bornm.) Sirj.	Turkey	Woodlands, macchie, phrygana, 350-1520 m.
L. cilicicus Hayek & Siehe	Syria, Turkey	Macchie, cornfields, cliffs, 600-1300 m.
L. boissieri Sirj.	Iran, Iraq, Turkey	Steppe, corn and fallow fields, damp slopes, 1000-2000 m.

Lathyrus (25/35)	*L. rotundifolius* Willd.	Asia minor, Caucasus, Crimea, Iran	Scrub (especially on N. slopes), hedges, lush meadows, corn and fallow fields, Broad-leaved forests and their fringes, among shrubberies. 1000-2200 m.
	L. tuberosus L.	Asia, Caucasus, Europe, N. America	Water meadows, grassy banls, fallow fields, 1000-2150 m.
	L. pygmaeus Gomblaut	Lebanon, Syria, Turkey	
	L. sativus L.	Asia, C. & S. Europe	Filed crop and weed, s.l.-1520 m.
	L. cicera L.	Africa, Caucasus, Europe, M.E.	*Quercus* scrub, *Pinus brutia* forest, rocky slopes. Vineyards, corn and fallow fileds, 5-2000 m.
	L. stenophyllus Boiss. & Heldr.	Syria, Turkey	Rocky limeston slopes, Pinus brutia forest, macchie, in herbage,nr. The coast.
	L. marmoratus Boiss. & Bl.	N. Africa, W. Asia	Rocky hillsides, *Ostrya* forest, 75-1700 m.

L. blepharicarpus Boiss	West Asia	Hills, phrygana, grassy places, 100-600 m.
L. ciliolatus Sam.	Lebanon, Syria	
L. hirticarpus Mattatia & Heyn	Palestine, Syria	Rocky hillsides
L. basalticus Rech.	Lebanon, Syria	Hills, basaltic origin soil
L. gorgoni Parl.	Libya, W. Asia, Italy	Corn and fallow fields, water-meadows, ditches, s.l.-1070 m.
L. pseudocicera Pamp.	N. Africa, W. Asia	
L. chrysantus Boiss.	Lebanon, Syria, Turkey	Fallow fileds, c. 750 m.
L. trachycarpus (Boiss.) Boiss.	Turkey	Plains, c. 700 m.
L. lycicus Boiss.	Turkey	Macchie, s.l.-420 m.
L. phaselitanus Hub.-Mor. & Davis	Turkey	Macchie, c. 70 m.
L. undulatus Boiss.	Crimea , Turkey	Deciduous forest, hedges, roadsides, stony slopes nr. S.l.-600 m.

L. sylvestris L.	Asia, Caucasus, Europe, N. America, Oceania	In forest fringes and glades, meadows, among shrubberies
L. annuus L.	Europe, M.E., N. Africa, C. & W Asia, Oceania.	Scrub, hedges, water-meadows, among rocks, fields, s.l.-1000 m.
L. hierosolymitanus Boiss.	Egypt, Greece, Libya, W. Asia	Cornfields, 500 m.
L. cassius Boiss.	W. Asia	*Pinus brutia* forest, scrub, volcanic out-crops, fallow fileds, s.l.-1650 m.
L. odoratus L.	Africa, Asia, Europe, N. & S. America, Pacific Ocean.	Edge of woods, grassy places, roadsides, rarely cultivated, s.l.-50 m.
L. belinensis Maxted & Goyder	Turkey	
L. hirsutus L.	Asia, Caucasus, Europe, N. Africa, W. Asia,	Bushy and grassy places, cultivated land, s.l.-1000 m.
L. chloranthus Boiss.	Armenia, Iran, Iraq, Turkey	Banks and scrub by streams, igneous slopes, wheatfields, 600-1800 m.

Orobon (1/1)	*L. roseus* Stev.	Caucasus, Iran, Turkey	Forests (*Picea, Pinus*), scrub (*Quercus, Corylus*), 30-1800 m.
Pratensis (4/6)	*L. czeczottianus* Bässler	Turkey	Open forests, rocky slopes, eroded banks, 1150-2200 m.
	L. laxiflorus (Desf.) Kuntze	Caucasus, E. & S. Europe, W. Asia	Forest, scrub, shady banks, etc., sl.-1900 m.
	L. layardii J. Ball ex Boiss.	Iran, Turkey	Water meadows and Salix scrub, 1575-1800 m.
	L. pratensis L.	Africa, Asia, Caucasus, Europe, N. America, Oceania	Water meadows, stream sides, bushy places, nr. s.l.-2300 m.
Aphaca (2/2)	*L. aphaca* L.	Africa, Asia, Caucasus, Europe, M.E.	Rocky limestone slopes in oak scrub, 600-700 m.
	L. stenolobus Boiss.	Lebanon, Palestine, Syria	Woods
Clymenum (3/3)	*L. clymenum* L.	Europe, N. Africa, Turkey	*Phrygana*, fields (as a weed), nr. Water, s.l.-100 m.
	L. gloeospermus Warb. & Eig.	Palestine, Syria	Crop fields
	L. ochrus (L.) DC	N. Africa, S.E. Europe, S.W.	Edge of woods, grassy places,

		Europe, W. Asia	roadsides, rarely cultivated, s.l.-50 m.
Orobastrum (1/1)	*L. setifolius* L.	Caucasus, N. Africa, S.E. Europe, S. W. Europe, W. Asia	Rocky slopes, deciduous *Quercus* forests, macchie, phrygana, 20-800 m.
Viciopsis (1/1)	*L. saxatilis* (Vent.) Vis.	Azerbaijan, Crimea, N. Africa, Europe, W. Asia	Rocky slopes, roadsides, 30-600 m.
Linearicarpus (5/7)	*L. inconspicuus* L.	Algeria, C. Asia, Caucasus, Europe	In corn and fallow fields, nr. S. 1-1500 m.
	L. sphaericus Retz.	Asia, N. Africa, Europe, M.E., N. America	Pine forests, hill-sides, 10-2000 m.
	L. tauricola P. H. Davis	Turkey	Open *Pinus* forest, etc., 800-1300 m.
	L. vinealis Boiss. & Noe	W. Asia	Disturbed steppe, vineyards, fallow fields, 900-1300 m.
	L. woronowii Bornm.	Turkey	Disturbed steppe, vineyards, fallow fields, 900-1300 m.
Nissolia (1/1)	*L. nissolia* L.	Caucasus, Europe, N. Africa,	*Pinus nigra* forest, *Quercus* scrub, grassy places, marshes,

		W. Asia	nr. S.l.-1900 m.
Neurolobus (1/1)	*L. neurolobus* Boiss. & Heldr.	Crete	

➢ Centre of diversity

Europe, the Mediterranean, and West, Centrarl and Southern Asia are the most important centres of diversity for *Lathyrus* although secondary centres of diversity exist in South America, North America and Ethiopia, extending into East Africa (Kupicha, 1983). Figure 6.3 shows the natural distribution of *Lathyrus* species in the Mediterranean basin and Caucasus, Central and West Asia region based on herbarium specimen and gene bank accession passport data, while Figure 6.4 shows the species richness over the same area calculated using DIVA-GIS software (http://www.cipotato.org/diva/); a detailed discussion of the capabilities of DIVA-GIS is provided by Hijmans *et al.* (2001). The hotter colours indicate richness and as can be seen *Lathyrus* species richness is South-west Turkey and the Fertile Crescent.

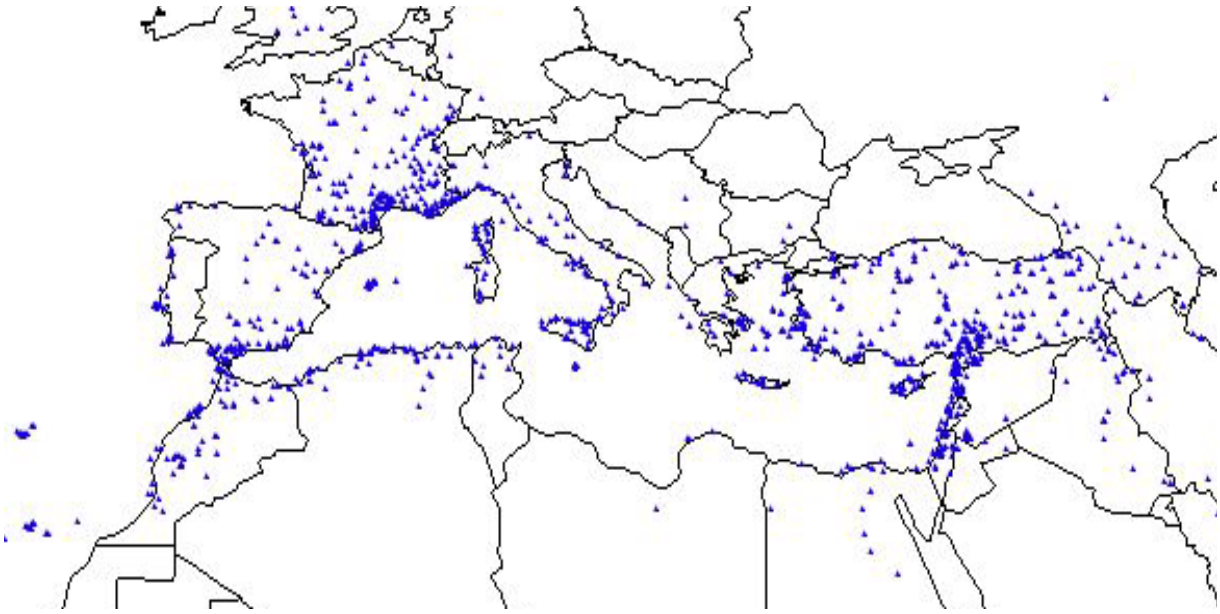

Figure 6.3. General distribution of sampled *Lathyrus* taxa in the Mediterranean Basin, and Caucasus, Central and West Asia region

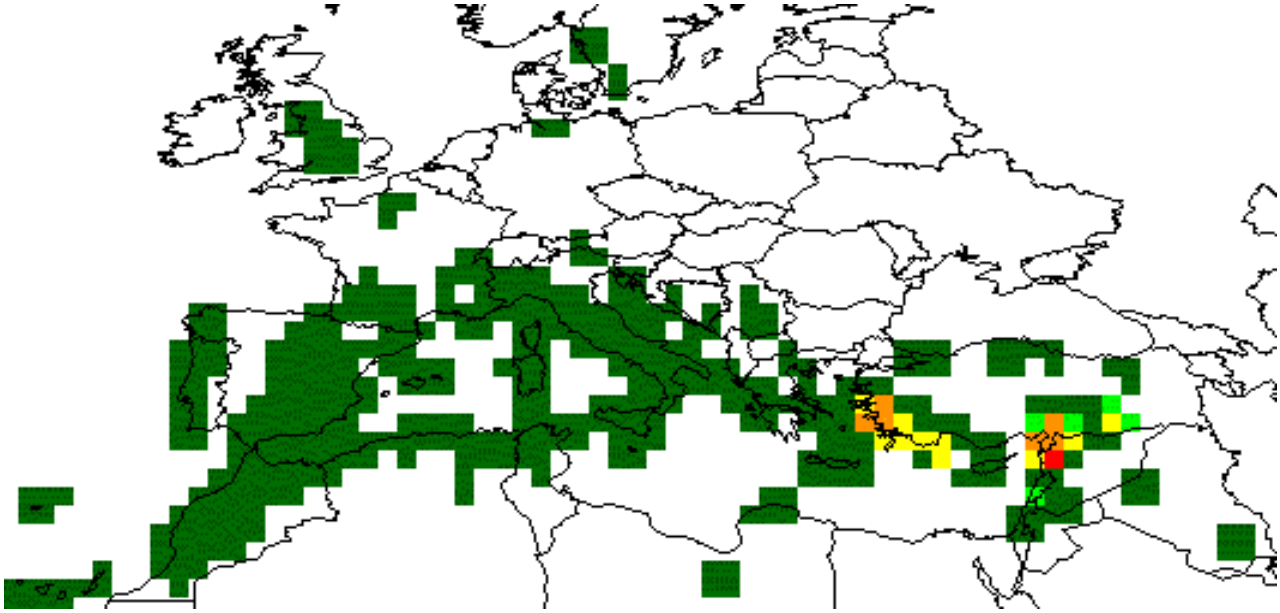

Figure 6.4. *Lathyrus* species richness in Europe, the Mediterranean Basin, and Caucasus, Central, West and South Asia regions

The earliest archaeological remains of *Lathyrus* appear in the Neolithic age in the Balkans and Near East of Bulgaria, Cyprus, Iraq, Iran, and Turkey (Erskine *et al.*, 1994). A single *Lathyrus* seed, presumed to be a field weed, was found in Cayono in Turkey and dated at around 7200 B.C., where bitter vetch was the prevalent pulse (van Zeist, 1972). Compared with the early-domesticated species, lentil, pea and bitter vetch, *Lathyrus* is only found in small quantities in Turkey, Cyprus, Iraq, Iran and Bulgaria, dating from 6750 to 4770 B.C. However, a different picture appears from late Neolithic finds at Dimini in Greece (c. 4000-3500 B. C.), where grass pea is as frequent as pea and lentil (Kroll, 1979). This increased frequency of grass pea is suggestive of domestication. *Lathyrus* was the chief crop component mixed with lentil (c. 2100-1800 B.C.), providing stronger evidence of domestication by the Middle Bronze Age (Helback, 1965). It was also found mixed in substantial quantities with other leguminous crops in later finds. *L. clymenum* was cultivated in the Bronze Age in Thera,

Crete and Melos in Greece (Sarpaki and Jones, 1990) and *L. ochrus* was possibly cultivated on Knossos, Greece at the same time (Jones, 1992). *L. cicera* is believed to have been domesticated in South-west Europe by 4000 - 3000 B.C. (Kislev, 1989).

Written records provide very little knowledge about the origin of grass pea. *Lathyrus* is an ancient Greek plant name probably used for a pulse and possibly for *L. sativus* (Westphal, 1974). The Romans also do not mention *Lathyrus*, which reflects little importance or lack of knowledge of the crop. Thus, the archaeological evidence suggests that domestication of *Lathyrus* possibly occurred during the late Neolithic and surely by the Bronze Age. Prior to that time, it was probably a tolerated weed of other pulses (Erskine *et al.*, 1994).

- **Ecological distribution**

Lathyrus species have been found in many different habitats: open, disturbed-open habitats such as field margins and roadsides; and in closed habitats such as woodlands and steeps (Sarker *et al.*, 2001). The species considered more advanced are generally those found in the more disturbed, open communities. The cultivated species have mostly evolved from disturbed habitats; they were originally the wild and weedy floras of agricultural fields (Vavilov, 1926). Farming systems have therefore had a great influence on the recent evolution of the genus. Their weedy nature would explain the widespread distribution of many species. Although there have been several investigations of genetic diversity within and between related genera in *Vicia*, *Lens* and *Pisum,* there have been no comprehensive studies of genetic diversity in *Lathyrus*.

- **Genetic diversity**

The genetic diversity of the genus *Lathyrus* (Grass pea and Chicklings) is off great importance, particularly for potential use in rain-fed cropping systems of many countries (Campbell *et al.*, 1994) and as a source of genes for the crop improvement of *L. sativus* L.

Several species are cultivated for human consumption, animal feed, and fodder, as well as for ornamental purposes (Sarker *et al.*, 1997), but there is potential for further exploitation of the *Lathyrus* gene pool. Therefore, the collection, conservation, characterization, study of genetic diversity and utilization of the genus *Lathyrus* deserves ample attention as a priority research area. There is an urgent need to conserve the genetic diversity of the genus using both *ex situ* (e.g. gene banks) and *in situ* (e.g. within natural habitats) conservation techniques. This will permit a critical assessment and monitoring of the genetic diversity, evolution and genetic erosion of the genus, as well as enhancing its exploitation (Sabanci, 1996).

Genetic diversity studies of the genus have been carried out by few, Yunus (1990) and Kearney (1993); their attempts were focused on the agricultural importance of grass pea and its close relatives in the sect. *Lathyrus*. These have been found to be predominantly self-pollinating, with anther dehiscence usually occurring before the flower has fully opened. Inter-specific hybridization has been successful between *L. sativus* and two other *Lathyrus* species, though the production of successful hybrids remains low. The first successful inter-specific cross was with *L. cicera* (Saw Lwin, 1956; Davies, 1957; 1958). Yunus (1990) crossed 11 species in sect. *Lathyrus* with *L. sativus*, and found that *L. cicera* and *L. amphicarpos* gave viable seeds. Other species formed pods but these did not form fully developed viable seeds. *L. cicera* is thought morphologically to be the closest relative of *L. sativus* (Yunus & Jackson, 1984). Plitmann *et al.* (1986) arrived at the same conclusion, based on studies of pollen morphology, karyotype and flavonoid aglycones.

It is possible to apply Harlan and De Wet's gene pool concept to this crossability information for *L. sativus* to elucidate its gene pools. The cultivated and wild races of *L. sativus* are included in the primary gene pool. Townsend and Guest (1974) suggested that the primary gene pool is poorly differentiated in terms of morphological characters, as there are

no clear-cut discontinuities between the cultivated and wild forms. Although Smartt (1984) concluded that the white flowered, white seeded varieties are the most highly selected and Jackson and Yunus (1984) suggested that the blue flowered; small speckled seeded forms are primitive. Therefore, it could tentatively place the white flowered, white seeded varieties in GP1A and the blue flowered, small speckled seeded forms in GP1B. The secondary gene pool includes the other biological species that will cross with some difficulty with the crop species. Therefore, GP2 includes: *L. chrysanthus*, *L. gorgoni*, *L. marmoratus* and *L. pseudocicera*, with which *L. sativus* can cross and produce ovules, and possibly more remotely *L. amphicarpos*, *L. blepharicarpus*, *L. chloranthus*, *L. cicera*, *L. hierosolymitanus* and *L. hirsutus*, with which *L. sativus* can cross and with which pods are formed. The secondary gene pool includes also species that can cross with the crop species only with use of specialized techniques such as embryo rescue and culture or the use of bridging species. The remaining species of the genus can be considered members of the tertiary gene pool (GP3)' (Sarker *et al.*, 2001).

Proper evaluation, characterization and documentation are an important part of utilizing genetic resources by *Lathyrus* workers. However, in-depth evaluation for phenological, morphological, agronomical and quality characters of available germplasm has yet to be carried out adequately at the national and global level. The Germplasm Resources, Crop Improvement and Agronomy Committee of the International Network for the Improvement of *Lathyrus sativus* and the Eradication of Lathyrism (INILSEL) proposed a list of 16 descriptors to characterize *Lathyrus* genetic resources (Campbell, 1994).

- **Exploitation of diversity**

'Grass pea is nutritionally equivalent with other grain legume species, containing up to 30% crude protein (which is high in lysine), about 60% carbohydrates and 0.6% fat (Hartman *et al.*, 1974). The grass pea is favoured for its ability to mature and produce a yield in times of

drought when other crops have failed. The seed, however, may contain 0.1-2.5% of the water soluble non-protein amino acids ODAP (â-N-oxalyl-á,â diaminopropionic acid) or OAP (l-3-oxalylamino-2-amino propionic acid), which have been found to be neurotoxins, the causative agent of crippling, irreversible neurological disorder, lathyrism (Barrow *et al.*, 1974; Rutter and Percy, 1984; Kaul and Coombes, 1986), which leads to paralysis of the lower limbs. These neurotoxins need to be genetically removed if *Lathyrus* is to gain importance as a food crop (Abd El Moneim and Cocks, 1993). At present, several grass pea - producing countries are involved in the development of very low or toxin-free *L. sativus* varieties (Malek *et al.*, 1996; Tadesse 1997). Additionally, the primary, secondary and tertiary gene pools may play an important role for its improvement. For example, a toxin-free gene has been identified in *L. tingitanus* L., which is being used to develop toxin-free grass pea varieties in China (Zhou and Arora, 1996).

Several species within the genus are cultivated as ornamentals such as sweet pea (*L. odoratus* L.), broad-leaved everlasting pea (*L. latifolius* L.), narrow-leaved everlasting pea (*L. sylvestris* L.) and two-flowered pea (*L. grandiflorus* Sibth. & Smith), and many occur in the wild because of garden escapes (Bagott, 1997). A number of other species, particularly in sect. *Lathyrus*, have potential for the development as new horticultural species (Davis, 1970; Maxted *et al.*, 1990). Due to the potential the genus has as a food, feed and fodder crop, as well as its extensive cultivation as an ornamental, it is necessary to collect and conserve all available cultivars and landraces, as well as the wild species. Table 1.1 above lists those species known to be historically or currently cultivated for agriculture or horticulture.

The genus is well placed to help meet the increasing global demand for animal feed and to provide crops for a diversity of farming systems, particularly when low neurotoxin lines will be available. To prevent genetic erosion and extinction, *Lathyrus* conservation has been

given a priority by the International Plant Genetic Resources Institute since 1985 (Now Bioversity International). Many national programs and international bodies have launched germplasm collection and conservation activities of this under-utilized genus (Sarker *et al.*, 2001). However, to date, an extensive and systematic approach to collect, conserve and evaluate *Lathyrus* has not been adopted. Furthermore, it is necessary to study the genetic diversity of the available collections in order to understand their full utilization potential (Maxted *et al.*, 2003).

6.4.1.2 Taxonomy analysis

The morphological characters used in this study were able to discriminate among most of the sections and to assign the species to their respective sections. Using the characters set and by running both DELTA and Lucid software enable to produce the identification key and the descriptor language to enable determining the taxa. The three DELTA system main files, were constructed from the character list (file - CHARS), the character scores for individual taxa (file - ITMS) and the directive information (file - SPECS) as shown in (Appendices 6.4., 6.5 and 6.6). All data was checked for errors using the standard DELTA error checking directive file (file – CHECK) and any errors encountered corrected. Once the three data files were completed, the description generating directive file (file – TONAT) was used to generate the natural language description and the key generating directive file (file – TOKEY) were used to create natural language descriptions and keys (Appendix 6.7) via the DELTA associated programs CONFOR and KEY respectively.

Lucid Professional is used as a complete system for developing and distributing multimedia tools (keys) for identification, diagnosis and other purposes. As the identification data was initially held in DELTA format, the data was imported to Lucid using the Lucid Translator and then manipulated using Lucid Builder. At this stage the descriptions,

ecogeographic summary, distribution maps, illustrations and photographs were attached to the taxa using Lucid Builder. The Lucid Builder allows to design and build identification or diagnostic keys for any group of organisms, objects or problems, and to illustrate keys with notes, HTML pages, audio files, images and video clips. Character scores imported from DELTA or entered direct into Lucid Builder using a simple point-and-click procedure. Once complete, the interactive key was generated by Lucid Builder and then was run using Lucid Player.

The Lucid Player provides the interface by which users can load and interact with Lucid keys, using text, images, videos and sounds to help select those taxonomic, diagnostic or other features that best describe the particular case being investigated. As the user selects character states, Lucid narrows down the particular options, such as specific taxa or causes of particular symptoms. When the user of a Lucid key is deciding which character states or symptoms best describe the particular specimen or problem of concern, multimedia material, such as line drawings, photographs, videos or sounds, can be used to help make the right decision. Once a specimen has been identified to a particular taxon or a diagnosis made. Lucid keys can provide the user with a full range of multimedia fact sheets, sub keys or links to websites for further information or recommendations. Lucid keys can be built in various languages and use terminology familiar to the user, allowing the package to be used internationally and across a wide range of capabilities.

6.4.2 Conservation field guide conspectus

The genus *Lathyrus* as described earlier by Davis (1970), Kupich (1983) and Maxted (1993) contains about 170 species with a native distribution centered on the Mediterranean

basin and spreading into, west Asia, the Caucasus and Central Asia. The field guide conspectus presented here consists of 76 taxa of genus *Lathyrus* L.:

1. *L. armenus* (Boiss. & A. Huet) Čelak., Oesterr. Bot. Z. 38: 85. 1888.
2. *L. aureus* (Stev.) Brandza *(Steven)* Bornm., Repert. Spec. Nov. Regni Veg. Beih. 89: 217. 1940.
3. *Lathyrus bauhinii* P.A. Genty, Bull. Soc. Dauphin. Échange Pl. Ser. 2. iii. (1892) 90.
4. *L. brachypterus* Čelak., Oesterr. Bot. Z. 38: 47. 1888
5. *L. boissieri* Sirj., Bull. Soc. Bot. Bulgar. vi. 62 (1934)
6. *L. cilicicus* Hayek & Siehe, Ann. Nat. Hofmus. Wien xxviii. 164 (1914).
7. *L. cyaneus* (Stev.) K. Koch, Linnaea 15: 723. 1842
8. *L. czeczottianus* Bässler, Feddes Repert. 72: 91, in adnot. 1966
9. *L. digitatus* (Bieb.) Fiori & Poal., Fl. Italia 2:105. 1900
10. *L. elongatus* (Bornm.) Sirj., Bull. Assoc. Russe Sci. Prague 2:224 (1936). Davis in Notes R.B.G. Edinb. 24:20 (1962)
11. *L. filiformis* (Lam.) Gay, Ann. Sci. Nat., Bot. sér. 4, 8:315. 1857
12. *L. incurvus* (Roth.) Willd., Sp. Pl. 3:1091 (1802)
13. *L. japonicus* Willd., Sp. Pl., ed. 4 [Willdenow] 3(2): 1092. 1802 [1-10 Nov 1802]
14. *L. karsianus* P. H. Davis in Notes R.B.G. Edinb. 24:19,t. 1(1969)
15. *L. laevigatus* (Waldst. & Kit.) Gren., Mém. Soc. Emul. Doubs sér. 3, 10:193. 1865
16. *L. laxiflorus* (Desf.). O. Kuntze in Acta Horti Petrop. 10:185 (1887)
17. *L. laxiflorus* (Desf.). O. Kuntze subsp. angustifolius (Post ex Dinsm) Davis
18. *L. laxiflorus* (Desf.). O. Kuntze subsp. laxiflorus (Desf.) O. Kuntze
19. *L. layardii* J. Ball ex Boiss., Fl. Or. Suppl. 195 (1888)
20. *L. libani* Fritsch in Sitzb. Akad. Wien 104:517 (1895
21. *L. linifolius* (Reichard) Bässler, Feddes Repert. 82(6): 434. 1971
22. *L. niger* (L.) Bernh., Syst. Verz. Pfl.248 (1800)
23. *L. nivalis* Hand.-Mazz. in Ann. Nat. Hofmus. Wien 27:80, t. 2 f. 6 (1913)
24. *L. pallescens* (Bieb.) Koch in Linnaea 15:723 (1841)

25. *L. palustris* L., Sp. Pl. 733 (1753)
26. *L. pisiformis* L., Sp. pl. 2:734. 1753
27. *L. pratensis* L., Sp. Pl. 733 (1753). Ic: Hegi, Ill. Fl. Mittel-Eur. 4(3): t. 171 (1924); Ross-Craig, Draw. Brit. Pl. 7: t. 71 (1954)
28. *L. roseus* Stev. in Mem. Soc. Nat. Mosc. 4:52 (1813)
29. *L. rotundifolius* Willd., Sp. Pl. 3:1088 (1802)
30. *L. satdaghensis* P. H. Davis in Notes R.B.G. Edinb. 29:317 (1969)
31. *L. spathulatus* Čelak. in Ost. Bot. Zeitschr. 38:6 (1888). Davis in Notes R.B.G. Edinb. 24:20-21 (1962)
32. *L. sylvestris* L., Sp. Pl. 733 (1753). Ic. Fl. Germ. 22:t. 211 (1903); Cross-Craig, Draw. Brit. Pl. 7:t. 73 (1954)
33. *L. tingitanus* L., Sp. pl. 2:732. 1753
34. *L. tuberosus* L., Sp. Pl. 732 (1753). Ic:Hegi, Ill. Fl. Mittel-Eur. 4(3):t. 171 (1924); Ross-Craig, Draw. Brit. Pl. 7: t. 72 (1954)
35. *L. tukhtensis* Czecz. in Acta Soc. Bot. Pol. 9:36 (1932); Feddes Rep. Beih. 107:168 (1939)
36. *L. undulatus* Boiss., Diagn. ser. 2(2):41 (1856). Ic: Bot. Mag. 122:t. 74U (1896)
37. *L. variabilis* (Boiss. & Ky.) Maly in Aschers. & Graebn. Syn. Mitteleur. Fl. 6(2):1057 (1910)
38. *L. venetus* (Miller) Wohlf. in Koch, Syn. Fl. Germ. ed. 3, 714 (1892)
39. *L. vernus* (L.) Bernh., Syst. Verz. Plf.247 (1800)
40. *L. amphicarpos* L., Sp. Pl. 2: 729. 1753 [1 May 1753]
41. *L. angulatus* L., Sp. Pl. 2: 731. 1753 [1 May 1753]
42. *L. annuus* L., Demonstr. Pl.24(1753)
43. *L. aphaca* L. Sp. Pl. 729 (1753)
44. *L. basalticus* Rech. fil. Ark. for Bot., I 14 (1951).
45. *L. belinensis* N. Maxted & D.J. Goyder, sp. nov. from Antalya, Turkey is described and illustrated

46. *L. blepharicarpus* Boiss., Diagn. ser. 1(9):126 (1849). Ic:Sbith. & Sm., Fl. Gr. 7:t. 693 (1830).
47. *L. cassius* Boiss., Diagn. ser. 1(9):128 (1849).
48. *L. cicera* L.*,* Sp. Pl. 730 (1753). Ic:Sbith. & Sm/, Fl. Gr. 7:t. 694 (1830); Fiori, Ic. Fl. Ital. t. 2126 (18U)
49. *L. ciliolatus* Sam., (Pl. CLXXIII, n. 1)
50. *L. chloranthus* Boiss., Diagn. ser. 2(6) 67 (1859). Ic: Fl. Azerb. 5:t. 49 (1954).
51. *L. chrysantus* Boiss., Diagn. ser. 1(6):46 (1845). Ic. Mout., Fl. Djebel Druze t. 13 (1953).
52. *L. clymenum* L., Sp. Pl. 732 (1753).
53. *L. gleospermus* Warb. et Eig. Repert. Spec. Nov. XXV 350-2 (1929)
54. *L. gorgoni* Parl. in Gior. Sci. 62:3 (1838).
55. *L. hierosolymitanus* Boiss., Diagn. ser. 1(9):127 (1849).
56. *L. hirsutus* L., Sp. Pl. 732 (1753). Ic:Reichb., Ic. Fl. Germ.22:t. 203 (1903); Jav. & Csap., Ic. Fl. Hung. t. 2U (1932).
57. *L. hirticarpus* Mattatia & Heyn, Isr. J. Bot. 25: 216 (1976).
58. *L. inconspicuus* L., sp. Pl. 730 (1753).
59. *L. lycicus* Boiss., Diagn. ser. 1(9):128 (1849).
60. *L. marmoratus* Boiss. & Bl. in Boiss., Fl Or. 2:606 (1872).
61. *L. nissolia* L., Sp. Pl. 729 (1753).
62. *L. ochrus* (L.) DC. in Lam. & DC., Fl. Fr. 4:578 (1805).
63. *L. odoratus* L. Sp. Pl. 732 (1753).
64. *L. phaselitanus* Hub.-Mor. & Davis in Notes R.B.G. Edinb. 29:318 (1969).
65. *L. pseudocicera* Pamp. in Nuovo Gior. Bot, Ital. n.s. 31:213 (1924).
66. *L. pygmaeus* Gomblaut, Bull. Soc. Bot. de France 90: 42 (1943)
67. *L. sativus* L., Sp. 730 (1753). Ic: Jav. & Csap., Ic. Fl. Hung. t. 2U (1932); Villax, Cult. Pl. Fourr. Medit. Occid. t. 143 (1963).
68. *L. saxatilis* (Vent.) Vis., Fl. Dalm. 3:330 (1852)

69. *L. setifolius* L., Sp. Pl. 731 (1753). Ic: Bonnier, Fl. Comp. Fr., Suisse et Belg. 3:t. 159 (1914); Jav. & Casp., Ic. Fl. Hung. t. 2U (1932)
70. *L. sphaericus* Retz., Obs. Bot. 3:39 (1783). Ic: Bonnier, Fl. Comp. Fr., Suisse et Belg. 3:t. 159 (1914); Jav. & Casp., Ic. Fl. Hung. t. 301 (1932).
71. *L. stenolobus* Boiss., Diagn. ser. 1(9):124 (1849).
72. *L. stenophyllus* Boiss. & Heldr. in Boiss., Diagn. ser. 1(9):126 (1849).
73. *L. tauricola* P. H. Davis in Notes R.B.G Edinb. 29:318 (1969)
74. *L. trachycarpus* (Boiss.) Boiss., Fl. Or. 2:608 (1872).
75. *L. vinealis Boiss. & Noe in Boiss., Diagn. ser. 2(2):42 (1856). Ic: Mout., F. Djebel Druze:t. 11(1953).*
76. *L. woronowii* Bornm. in Monit. Jard. Bot. Tiflis 26:2 (1912)

The ecogeographic conspectus comprises a summary of the ecogeographic information available for these taxa. Where available the following information is provided for each taxon included in the genus:

- Accepted taxon name, author(s), date of publication, where published
- Synonyms for each taxon with author(s), date of publication, where published
- Description
- Geographical distribution (countries from which the taxon recorded) native (derived from personal study and ILDIS, 2010, GRIN, 2011).
- Distribution map
- Line drawing illustration
- Photographs

The following description is provided for *L. aureus* to explain the output of the field guide conspectus. Full set of information related to the taxa of the conspectus also accompanied by the CD with an interactive identification system in Annex1.

***L. aureus* (Stev.) Brandza *(Steven)* Bornm., Repert. Spec. Nov. Regni Veg. Beih. 89: 217. 1940**

Synonymes - *Orobus aureus* Fisch.& C.A. Mey, *Orobus kolenatii* K. Koch, *Orobus orientalis* Boiss.

Description - Perennial, sturdy, erect. Vegetative parts pubescent, usually sparsely, when vegetative parts green. Plant 50–80 cm. Stems terete. Leaflets present, 3-5 pairs per leaf, pinnate. Leaf rachis not laminate, aristate. Leaflets ovate, apex acute, or acuminate, 50–100 mm long, 18–50 mm wide, venation pinnate. Stipules lanceolate, or ovate, base semi-sagittate, margin entire, glabrous. Stipules (10–)20–25(–28) mm long, broader than stem. Stipules longer than petiole. Peduncles (80–)100–140(–150) mm long, more or less equally as long as leaf, or shorter than leaf. Pedicel (4–)5–7(–8) mm long. Flowers (8–)12–25 per inflorescence, concolorous. Corolla gingery-orange. Flower 16–20(–22) mm long. Standard with no conspicuous veins. Wings orange. Calyx glabrescent, or pubescent, 8–12 mm long, tube gibbous, teeth unequal, straight, shorter than tube. Style 4–5 mm, straight, linear. Ovary linear. Legume straight tip, linear, 50–70 mm long, 7–8 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture narrow. Seed surface smooth. Seeds per pod 6–12. Hilum 2–2.5 mm long.

Habitat - Forest margins, and scrubs.

Geographical distribution - Native:

- ASIA-TEMPERATE
  Western Asia: Turkey
  Caucasus: Armenia; Azerbaijan; Georgia; Kyrgyzstan; Russian Federation - Kabardino-Balkaria, Karachay-Cherkessia, Krasnodar, North Ossetia
- EUROPE
  East Europe: Moldova; Ukraine – Crimea.
  Southeastern Europe: Bulgaria; Romania.

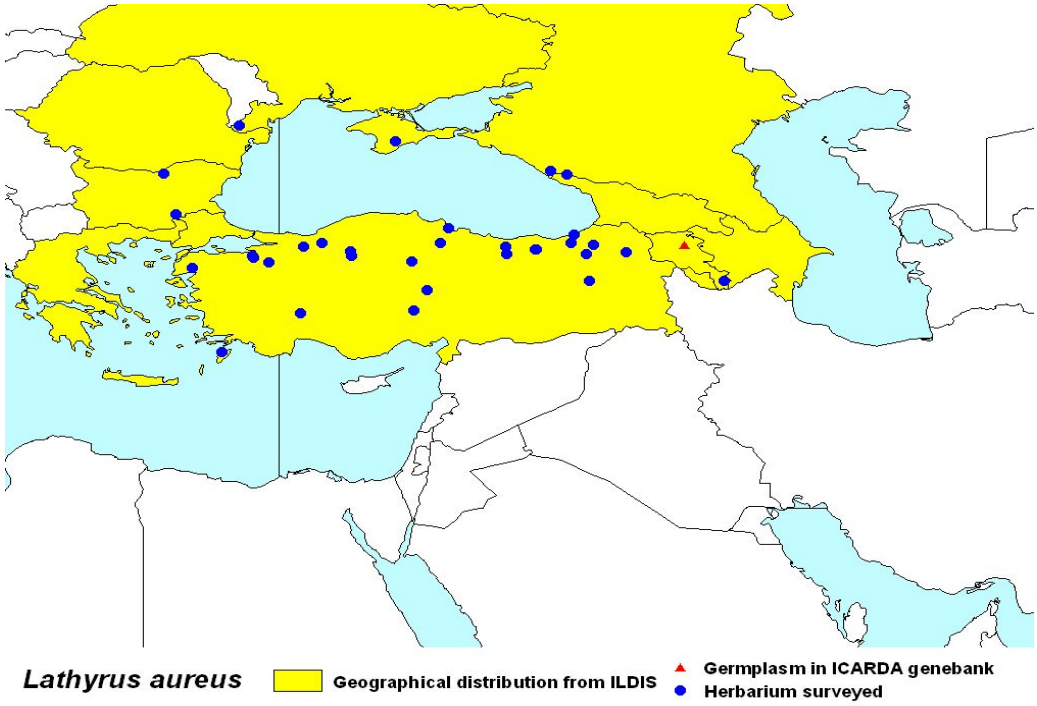

Figure 3.2. Geographical Distribution for *L. aureus* (Stev.) Brandza (*Steven*) Bornm.

Figure 3.3. *L. aureus* (Stev.) Brandza (*Steven*) Bornm.: a habit (x1); b leaflet (x1) c, stipule (x1); d calyx (x2); e flower (x2); f pod (x2); g pod venation and hairiness; h style (x3); i seed (x8); j rooting system (x1).

6.4.3 Taxon description

Based on the morphological observation of the studied herbarium specimens and using the set of characters, taxa descriptions for *Lathyrus* species are provided below .

1. ***L. armenus* (Boiss. & A. Huet) Čelak.**

Perennial herb, slender, erect, glabrous when green. Plant 30–50 cm. Stem terete. Leaflets present; 2 pairs per leaf, subdigitate. Rachis not laminates ends in mucro. Leaflets linear, or linear-lanceolate; 50–80 mm long, 3–6 mm wide, venation parallel. Stipules subulate, or ovate; margin entire; 0.5–1.5 mm wide; shorter than petiole. Peduncle shorter than leaf. Flowers 2–11 per inflorescence, concolorous. Corolla violet. Flower 14–16 mm long. Standard with no conspicuous veins. Wings violet. Calyx 5–7 mm long, teeth unequal, straight, shorter than tube. Style 4 mm long, linear. Ovary linear.Legume linear, 50 mm long, 4 mm wide, glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow.

2. *L. aureus* (Stev.) Brandza *(*Steven*)* Bornm.

Perennial, sturdy, erect. Vegetative parts pubescent, usually sparsely, when vegetative parts green. Plant 50–80 cm. Stems terete. Leaflets present, 3-5 pairs per leaf, pinnate. Leaf rachis not laminate, aristate. Leaflets ovate, apex acute, or acuminate, 50–100 mm long, 18–50 mm wide, venation pinnate. Stipules lanceolate, or ovate, base semi-sagittate, margin entire, glabrous. Stipules (10–)20–25(–28) mm long, broader than stem. Stipules longer than petiole. Peduncles (80–)100–140(–150) mm long, more or less equally as long as leaf, or shorter than leaf. Pedicel (4–)5–7(–8) mm long. Flowers (8–)12–25 per inflorescence, concolorous. Corolla gingery-orange. Flower 16–20(–22) mm long. Standard with no conspicuous veins. Wings orange. Calyx glabrescent, or pubescent, 8–12 mm long, tube gibbous, teeth unequal, straight, shorter than tube. Style 4–5 mm, straight, linear. Ovary linear. Legume straight tip, linear, 50–70 mm long, 7–8 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture narrow. Seed surface smooth. Seeds per pod 6–12. Hilum 2–2.5 mm long.

3. *L. bauhimi* Genty

Perennial, slender, ascending herb. Vegetative parts glabrous when green. Plants 15–50 cm. Stems winged. Leaflets present, 2-4 pairs per leaf , subdigitate. Leaf rachis not laminate, aristate. Leaflets linear, or linear-lanceolate, apex acuminate, 30–60 mm long, 2–6 mm wide, venation parallel. Stipules linear, base sagittate, margin entire, glabrous, 9–12 mm long, 0.5–1.5 mm wide, longer than petiole. Peduncles 35–55 mm long, longer than leaf. Pedicel 3.5–6 mm long. Flowers 4–10 per inflorescence, concolorous. Corolla purplish-pink, or purple. Flower 20–27 mm long. Standard with no conspicuous veins. Wings purple. Calyx glabrous, tube not gibbous, teeth unequal, straight, shorter than tube. Style 3–4 mm, straight, linear, or linear-spathulate. Ovary linear. Legume straight, linear, 45–70 mm long, 4–6 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seed surface smooth. Seeds per pod 9–11.

4. *L. brachypterus* Čelak.

Perennial, sturdy or slender, erect plant. Vegetative parts glabrous, or pubescent, usually sparsely, green. Plants 20–40 cm. Stems terete. Leaflets present, 2-3 pairs per leaf, paripinnate, or subdigitate. Leaf rachis not laminate, end in mucro. Leaflets linear, or linear-oblong, apex acute, 25–55 mm long, (1–)2–7 mm wide, venation parallel. Stipules lanceolate-subulate, stipule base semi-sagittate, margin entire, glabrous, (2.5–) 3–4 mm long, as broad as stem, longer than petiole. Peduncle longer than leaf. Flowers 2–10 per inflorescence, concolorous. Corolla cream, or pale sulphur. Flower (15–)18–25 mm long. Standard with no conspicuous veins. Wings cream. Calyx glabrous, (5–) 6–9 mm long, tube gibbous, teeth unequal, straight, shorter than tube. Style (6.5–) 7–10 mm, straight, linear. Ovary linear. Legume linear, 25–33 mm long, 2–4 mm wide, glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seeds per pod 10–20.

5. *L. boissieri* Sirj.

Perennial, sturdy, erect herb. Vegetative parts glabrous when green. Plants 50–75 cm. Stems angled. Leaflets present, 1-2 pairs per leaf, subdigitate. Leaf rachis not laminate, mucronate. Leaflets linear-elliptic, apex acute, 50–120 mm long, 5–22 mm wide, venation parallel.

Stipules lanceolate, base semi-sagittate, margin entire, glabrous, (1–)2–4(–5) mm long, 1–3 mm wide, longer than petiole. Peduncle shorter than leaf, or longer than leaf. Flowers 7–15 per inflorescence, concolorous. Corolla violet, or pink, or lilac. Flower 14–17 mm long. Standard with no conspicuous veins. Wings pink, or violet. Calyx glabrescent, 5–7 mm long, tube gibbous, teeth unequal, straight, shorter than tube or equal to tube. Style 4–5 , straight, linear-spathulate. Ovary linear. Legume linear, 80 mm long, 8 mm wide, glabrous. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod 7–9.

6. *L. cilicicus* Hayek & Siehe

Perennial, rigid, erect, glabrous, when vegetative parts green. Plants 70–120 cm. Stems terete. Leaflets present 2 pairs per leaf, subdigitate. Leaf rachis not laminate, mucronate, or aristate. Leaflets linear, or linear-lanceolate, apex acute, 80–150 mm long, 3–9 mm wide, venation parallel. Stipules lanceolate-subulate, or lanceolate, or linear, base semi-sagittate, margin entire, glabrous, 0.5–1.5 mm wide longer than petiole. Peduncles 250–280 mm long, longer than leaf. Flowers 5–13 per inflorescence, concolorous. Corolla purple. Flower 25–30 mm long. Standard with no conspicuous veins, apex emarginate. Wings purple. Calyx glabrous, 8–9 mm long, tube not gibbous, teeth equal , straight, shorter than tube, or equal to tube. Calyx lower teeth equal to tube. Style 7, straight, spathulate, or obovate-spathulate. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous.

7. *L. cyaneus* (Stev.) K. Koch

Perennial, slender, erect, or ascending plants. Vegetative parts glabrous when green. Plants 15–30 cm. Stems terete. Leaflets present, (1–)2 pairs per leaf, subdigitate, or pinnate. Leaf rachis not laminate, mucronate. Leaflets linear, or linear-lanceolate, apex acute, 25–60 mm long, 2–6 mm wide, venation parallel. Stipules lanceolate-subulate, or lanceolate, base semi-sagittate, margin entire, glabrous, 2–9(–12) mm long, as broad as stem, more or less equally as long as petiole, or longer than petiole. Peduncle more or less equally as long as leaf, or longer than leaf. Flowers (1–)2–6 per inflorescence, not concolorous. Corolla violet, or lilac-blue. Flower 15–29 mm long. Standard with no conspicuous veins, apex emarginate. Wings blue. Calyx glabrous, 5–7(–8) mm long, teeth equal , or unequal, straight, equal to tube. Calyx lower teeth shorter than tube. Style 4.5–5 mm, twisted, linear-spathulate, or spathulate. Ovary

linear. Legume straight, linear, 35–50(–60) mm long, 5–6 mm wide, glabrous. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow.

8. *L. czeczottianus* Bässler

Perennial, sturdy, erect, or ascending herb. Vegetative parts adpressed pilose, green. Plants (10–) 25–45 cm. Stems terete. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, mucronate. Leaflets lanceolate, apex acute, 15–47 mm long, 3–12 mm wide, venation parallel. Stipules lanceolate-accuminate, base sagittate, margin entire, pubescent, somewhat narrower than leaflet. Peduncle longer than leaf. Flowers 3–7 per inflorescence, concolorous. Corolla pale-lavender, or blue. Flower 17–19 mm long. Standard with no conspicuous veins. Wings blue. Calyx pubescent, 10–14 mm long, tube not gibbous, teeth unequal, straight. teeth longer than tube. Style (3.9–) 4 (–4.1) mm, twisted, linear. Ovary linear. Legume broadly-linear, 35 mm long, 5 mm wide, densely-pilose. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, gland-dotted, or glandular-verrucose. Upper suture narrow. Seed surface papillose, or coarsely-tuberculate.

9. *L. digitatus* (Bieb.) Fiori & Poal.

Perennial, slender, erect, or ascending plant. Vegetative parts glabrous, or pubescent, usually sparsely, when vegetative parts green. Plants (10–)15–40 cm. Stems terete. Leaflets present, (1–)2 pairs per leaf, subdigitate, or sub-sessile. Leaf rachis not laminate, mucronate. Leaflets linear, apex acute, (15–)20–70(–80) mm long, 1–3(–8) mm wide, venation parallel. Stipules lanceolate, base semi-sagittate, margin entire, glabrous, 6–8 mm long, 0.5–1.5 mm wide. Stipules longer than petiole. Peduncles (25–) 40–70(–75) mm long, more or less equally as long as leaf, or longer than leaf. Pedicel (5–) 6–7(–8) mm long. Flowers 3–6(–10) per inflorescence, concolorous. Corolla blue, or purple. Flower 14–20(–30) mm long. Standard with no conspicuous veins, apex emarginate. Wings blue, or purple. Calyx glabrous, 6–9 mm long, tube gibbous, teeth equal , or unequal, straight, shorter than tube. Calyx lower teeth shorter than tube. Style 3–4.5 mm, twisted, linear-spathulate, or spathulate. Ovary linear. Legume straight, linear-sublanceolate. Legume 35–55(–70) mm long, 4.5–6(–9) mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves hairy. Upper suture narrow. Seed surface smooth.

10. *L. elongatus* (Bornm.) Sirj.

Perennial, slender, erect. Vegetative parts glabrous when green. Plants 20–40 cm. Stems terete. Leaflets present, 1 pair per leaf. Leaflets subdigitate. Leaf rachis not laminate. Leaflets linear. Leaflets 70–135 mm long, 1–7 mm wide, venation parallel. Stipules lanceolate-subulate, margin entire, longer than petiole. Peduncle longer than leaf. Flowers 2–7 per inflorescence, concolorous. Corolla blue, or purple. Flower 13–20 mm long. Standard with no conspicuous veins. Wings blue, or purple. Calyx 5–7(–8) mm long, teeth unequal, straight, teeth equal to tube, or longer than tube. Style 4–4.5 mm, straight, spathulate. Ovary linear. Legume linear, 45 mm long, 6 mm wide. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow.

11. *L. filiformis* (Lam.) Gay

Perennial, slender, ascending plant. Vegetative parts glabrous when green. Plants 15–50 cm. Stems winged. Leaflets present, 2–4 pairs per leaf, subdigitate. Leaf rachis not laminate, aristate. Leaflets linear, or linear-lanceolate, apex acuminate, 30–60 mm long, 2–6 mm wide, venation parallel. Stipules linear, base sagittate, margin entire, glabrous, 9–12 mm long, 0.5–1.5 mm wide. Stipules longer than petiole. Peduncles 35–55 mm long, longer than leaf. Pedicel 3.5–6 mm long. Flowers 4–10 per inflorescence, concolorous. Corolla purplish-pink, or purple. Flower 14–22 mm long. Standard with no conspicuous veins. Wings blue, or purple. Calyx tube not gibbous, teeth unequal, straight. Calyx glabrous, teeth shorter than tube. Style 3–4 mm, straight, dilated at apex. Ovary linear. Legume straight, linear, 45–70 mm long, 4–6 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod 9–11.

12. *L. incurvus* (Roth.) Willd.

Perennial, sturdy or slender, decumbent plant. Vegetative parts glabrous, or pubescent, usually sparsely. Vegetative parts glaucous (or glaucescent). Plants 30–100 cm. Stems winged, or terete. Leaflets present, 3–5pairs per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets oblong-elliptic, or oblong-lanceolate, apex obtuse. Leaflets (15–)20–60 mm long, (7–)8–22 mm wide, venation parallel. Stipules lanceolate, or linear, base semi-sagittate, margin entire, glabrous, (5–)8–15(–25) mm long, 1–2 times as broad as stem. Stipules longer than

petiole. Peduncles 20–60 mm long, more or less equally as long as leaf. Pedicel 4–6 mm long. Flowers 3–9(–12) per inflorescence, concolorous. Corolla lilac-blue, or blue, or purple. Flower 10–14(–15) mm long. Standard with no conspicuous veins. Wings blue, or purple. Calyx glabrous, 5–6 mm long, tube gibbous, teeth unequal, straight, teeth equal to tube. Style 3–4 mm, straight, linear. Ovary linear. Legume incurved, linear, 25–35 mm long, 5–6 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved, or glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod 6–8(–11).

13. *L. japonicus* Willd.

Perennial, slender, erect, or ascending or decumbent plant. Vegetative parts glabrous when green. Plants 30–90 cm. Stems terete. Leaflets present 2–6 pairs per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets elliptic, or obovate, or ovate, apex sub-obtuse, or obtuse, (14–)17–40 mm long, (6–)8–33 mm wide. Stipules triangular, base semi-hastate, or sagittate, margin entire, 10–25 mm long, somewhat narrower than leaflet. Stipules shorter than petiole. Peduncles (20–)25–50(–55) mm long. Pedicel 3–5(–6) mm long. Flowers 5–15 per inflorescence, concolorous. Corolla violet, or blue. Flower 14–22 mm long. Standard with no conspicuous veins. Wings blue, or violet. Calyx tube gibbous, teeth unequal, straight, teeth equal to tube. Style (5–)6–7(–9) mm, straight. Ovary linear (rarely). Legume broadly-linear (rarely). Legume 30–50 mm long, (5–)6–8(–10) mm wide, glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seeds per pod 4–8. Hilum (2–)2.5(–3) mm long.

14. *L. karsianus* P. H. Davis

Perennial, slender, or rigid, erect plant. Vegetative parts glabrous when green. Plants 35–60 cm. Stems terete. Leaflets present, 1 pair per leaf, paripinnate. Leaf rachis not laminate, mucronate. Leaflets linear, or linear-lanceolate, apex acute, 30–60 mm long, 2.5–5 mm wide, venation parallel. Stipules lanceolate-subulate, base semi-sagittate, margin entire, glabrous, 2–7(–10) mm long, as broad as stem. Stipules shorter than petiole, or longer than petiole. Peduncles 30–70 mm long, longer than leaf. Pedicel 2–3 mm long. Flowers 5–9 per inflorescence, concolorous. Corolla blue. Flower 17–22(–25) mm long. Standard with no conspicuous veins. Wings blue. Calyx glabrous, 6–8 mm long, tube gibbous, teeth unequal,

straight, shorter than tube. Style 4–5 mm, straight, linear. Ovary linear. Legume straight, linear, 40–60 mm long, 4–5 mm wide, glabrous. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface verrucose. Seeds per pod (7–)8–14(–15). Hilum (1.5–)2–2.5 mm long.

15. *L. laevigatus* (Waldst. & Kit.) Gren.

Perennia, sturdy, erect plant. Vegetative parts glabrous when green. Plants 20–60 cm. Stems ridged. Leaflets present, 2–6 pairs per leaf, pinnate. Leaf rachis not laminate, mucronate. Leaflets linear-elliptic, or elliptic, or oblong, or ovate, or oblong-lanceolate, apex obtuse, 20–100 mm long, 5–45 mm wide, venation pinnate, or reticulate. Stipules lanceolate, or ovate, or suborbicular, base semi-sagittate, margin entire, glabrous, 5–30 mm long, 3–4 times as broad as stem. Stipules shorter than petiole. Peduncles 75–180(–235) mm long, longer than leaf. Pedicel 3–8 mm long. Flowers 2–20 per inflorescence, concolorous. Corolla yellow, or orange. Flower 15–25(–29) mm long. Standard with no conspicuous veins. Wings yellow, or orange. Calyx tube gibbous, teeth unequal, straight. Calyx pubescent, teeth shorter than tube. Style (5–)7–9.5 mm, straight, linear. Ovary linear, or canescent. Legume incurved. Legume linear, or canescent, (45–)60–80(–100) mm long, 6.5–9.5 mm wide, pubescent. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seeds per pod 5–14. Hilum 2.5–4.5 mm long.

16. *L. laxiflorus* (Desf.). O. Kuntze

Perennial, sturdy, ascending or decumbent, or procumbent. Vegetative parts glabrous, or spreading pilose, or villous with soft, spreading hairs, when vegetative parts green. Plants 15–40 cm. Stems terete, or angled. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets elliptic, apex aristate, 10–40 mm long, 4–18 mm wide, venation parallel. Stipules ovate, or lanceolate-ovate, or ovate-accuminate, base semi-hastate, margin entire, glabrous, broader than the leaflet, or as braod as the leaflet. Stipules longer than petiole. Peduncle longer than leaf. Flowers (2–)3–6 per inflorescence. Corolla pale-lavender, or lilac. Flower 15–20 mm long. Standard with no conspicuous veins. Wings blue. Calyx 8–13 mm long, tube gibbous, teeth equal , straight. Calyx glabrous, teeth longer than tube. Calyx lower teeth longer than tube. Style 4–5 mm, straight, oblong. Ovary linear. Legume straight,

broadly-linear. Legume 30–45 mm long, 4–5 mm wide, glabrous, or tomentose. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture narrow. Seed surface smooth. Seeds per pod (9–)10(–11).

17. *L. laxiflorus* (Desf.). O. Kuntze subsp. *angustifolius* (Post ex Dinsm) Davis

Perennial, sturdy, ascending plants, or decumbent, or procumbent plant. Vegetative parts glabrous, or spreading pilose, or villous with soft, spreading hairs, when vegetative parts green. Plants 15–40 cm. Stems terete, or angled. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous, or aristate. Leaflets lanceolate, apex aristate, 10–40 mm long, 4–18 mm wide, venation parallel. Stipules ovate, or lanceolate-ovate, or ovate-accuminate, base semi-hastate, margin entire, glabrous, broader than the leaflet, or as braod as the leaflet. Stipules longer than petiole. Peduncle longer than leaf. Flowers (2–)3–6 per inflorescence, concolorous. Corolla pale-lavender, or lilac. Flower 15–20 mm long. Standard with no conspicuous veins. Wings blue. Calyx glabrous, 8–13 mm long, tube gibbous, teeth equal, straight, longer than tube. Calyx lower teeth longer than tube. Style 4–5 mm, straight, oblong. Ovary linear. Legume straight, broadly-linear, 30–45 mm long, 4–5 mm wide, glabrous, or tomentose. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod (9–)10(–11).

18. *L. laxiflorus* (Desf.). O. Kuntze subsp. *laxiflorus* (Desf.) O. Kuntze

Perennial, sturdy, ascending plants, or decumbent, or procumbent plant. Vegetative parts glabrous, or spreading pilose, or villous with soft, spreading hairs, when vegetative parts green. Plants 15–40 cm. Stems terete, or angled. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, aristate. Leaflets elliptic, or ovate, apex aristate, 10–40 mm long, 4–18 mm wide, venation parallel. Stipules ovate, or lanceolate-ovate, or ovate-accuminate, base semi-hastate, margin entire, glabrous, broader than the leaflet, or as braod as the leaflet. Stipules longer than petiole. Peduncle longer than leaf. Flowers (2–)3–6 per inflorescence, not concolorous. Corolla pale-lavender, or lilac. Flower 15–20 mm long. Standard with no conspicuous veins, apex emarginate. Wings blue. Calyx glabrous, 8–13 mm long, tube gibbous, teeth equal , straight., teeth longer than tube. Calyx lower teeth longer than tube. Style 4–5 mm, straight, oblong. Ovary linear. Legume straight, broadly-linear, 30–45 mm long, 4–5 mm wide, tomentose. Lower suture not ciliate. Mature legume indehiscent.

Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod (9–)10(–11).

19. *L. layardii* J. Ball ex Boiss.

Perennia, sturdy, erect, or ascending plant. Vegetative parts spreading pilose, or villous with soft, spreading hairs, when vegetative parts green. Plants 45–60 cm. Stems terete, or angled. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets elliptic-lanceolate, 20–55 mm long, 3–10 mm wide, venation parallel. Stipules lanceolate-ovate, base sagittate, margin entire. Peduncle longer than leaf. Flowers 5–10 per inflorescence, concolorous. Corolla pale-lavender, or blue. Flower 19–22 mm long. Standard with no conspicuous veins. Wings blue. Calyx 9–12 mm long, teeth unequal, straight, longer than tube. Ovary oblong. Legume oblong-linear, 25 mm long, 4 mm wide, pilose. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous.

20. *L. libani* Fritsch

Perennia, sturdy, erect. Vegetative parts pubescent, usually sparsely, when vegetative parts green. Plants 50–80 cm. Stems terete. Leaflets present, 3–5 pairs per leaf, paripinnate. Leaf rachis not laminate, mucronate, or aristate. Leaflets ovate, apex acute, or acuminate, 50–100 mm long, 18–50 mm wide, venation pinnate. Stipules lanceolate, or ovate, base semi-sagittate, margin entire, glabrous, broader than stem. Peduncle more or less equally as long as leaf, or shorter than leaf. Flowers (8–)12–25 per inflorescence, concolorous. Corolla white. Flower 23–30 mm long. Standard with no conspicuous veins. Wings white. Calyx glabrous, tube gibbous, teeth unequal, teethreflexed, teeth shorter than tube. Style 4–5 mm, straight, linear. Ovary linear. Legume linear, 70–80 mm long, 7–8 mm wide, glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture narrow. Seed surface smooth. Seeds per pod 6–12.

21. *L. linifolius* (Reichard) Bässler

Perennial, sturdy or slender, ascending plant. Vegetative parts glabrous, when green. Plants 15–50 cm. Stems winged. Leaflets present, (1–)2(–4) pairs per leaf, pinnate. Leaf rachis not laminate, aristate. Leaflets linear, or elliptic, apex acute, 10–50(–100) mm long, 1–12(–16) mm wide, venation parallel. Stipules lanceolate, or linear, base semi-sagittate, margin entire,

glabrous, 5–25 mm long, 3–4 times as broad as stem, or broader than stem, or somewhat narrower than leaflet. Stipules longer than petiole. Peduncles (1–)2–5 mm long, longer than leaf. Pedicel (2–)3(–4) mm long. Flowers 2–6 per inflorescence, concolorous. Corolla cream, or blue. Flower 10–16 mm long. Standard with no conspicuous veins. Wings cream, or blue. . Calyx glabrous, tube gibbous, teeth unequal, straight, shorter than tube. Style (3–)4(–5) mm, twisted, linear. Ovary linear. Legume beaked, linear, 25–45 mm long, 4–5 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod 4–10. Hilum 0.2–0.25 mm long.

22. *L. niger* (L.) Bernh.

Perennial, slender, ascending plants. Vegetative parts glabrous when green. Plants 40–75 cm. Stems terete. Leaflets present, 3–5 pairs per leaf, paripinnate. Leaf rachis not laminate, mucronate. Leaflets elliptic, apex subobtuse, 10–30 mm long, 4–13 mm wide, venation pinnate. Stipules lanceolate, base semi-sagittate, margin entire. Peduncles 30–50 mm long, longer than leaf. Flowers 3–8 per inflorescence, concolorous. Corolla blue, or purple. Flower 10–14 mm long. Standard with no conspicuous veins. Wings blue, or purple. Calyx 4.5–6.5 mm long, teeth unequal, straight, shorter than tube. Ovary linear. Legume linear, 40–50 mm long, 5 mm wide, glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod 6–10.

23. *L. nivalis* Hand.-Mazz.

Perennial, sturdy, ascending plants. Vegetative parts glabrous, or pubescent, usually sparsely, when vegetative parts green. Plants 15–25(–30) cm. Stems terete. Leaflets present, 2–4(–5) pairs per leaf, pinnate. Leaf rachis not laminate, mucronate. Leaflets linear, or linear-elliptic, apex acute, 15–36(–40) mm long, 2–5 mm wide, venation parallel. Stipules subulate, or lanceolate, base semi-sagittate, margin entire, pubescent. Stipules (2–)6–7(–9) mm long, as broad as stem. Stipules longer than petiole. Peduncle longer than leaf. Pedicel (2.5–)3 mm long. Flowers 2–4 per inflorescence, concolorous. Corolla violet, or lilac. Flower 20–24 mm long. Standard with no conspicuous veins. Wings blue, or violet. Calyx glabrous, 6–8 mm long, tube gibbous, teeth unequal, straight, shorter than tube. Style 5 mm, straight, linear-spathulate. Ovary oblong. Legume beaked, oblong-linear, 30–35 mm long, 6–7 mm wide,

glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seed surface reticulate, or papillose. Seeds per pod 4–6. Hilum 1–1.5 mm long.

24. *L. pallescens* (Bieb.) Koch

Annual, or perennial, sturdy or slender, erect plants. Vegetative parts glabrous, or pubescent, usually sparsely, when vegetative parts green. Plants 20–40 cm. Stems terete. Leaflets present, 2–3 pairs per leaf , pinnate. Leaf rachis not laminate, aristate. Leaflets linear, apex acute, 22–55(–70) mm long, 1.5–5 mm wide, venation parallel. Stipules subulate, or lanceolate-subulate, base semi-sagittate, margin entire, glabrous, 3–5 mm long, 1 mm wide. Stipules longer than petiole. Peduncle longer than leaf. Pedicel 2–3(–4) mm long. Flowers (2–)4–7 per inflorescence, concolorous. Corolla cream, or pale sulphur. Flower 20–24 mm long. Standard with no conspicuous veins, apex emarginate. Wings cream, or yellow. Calyx glabrescent, or pubescent, 6–8 mm long, tube gibbous, teeth equal , or unequal, straight, shorter than tube. Calyx lower teeth shorter than tube. Style 4–5 mm, twisted, spathulate. Ovary linear. Legume straight, linear, 45–60 mm long, 4 mm wide, glabrous. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seed surface smooth. Seeds per pod 8–15.

25. *L. palustris* L.

Perennial, slender, erect, pubescent, usually sparsely, when vegetative parts green. Plants (40–)60–100(–120) cm. Stems winged. Leaflets present, 3–5 pairs per leaf, pinnate. Leaf rachis not laminate, tendrilous, linear, or oblong-elliptic, or oblong, or lanceolate, apex acute, 20–60(–80) mm long, 3.5–12(–16) mm wide, venation parallel. Stipules lanceolate, or ovate, or lanceolate-ovate, or lanceolate-accuminate, base semi-sagittate, margin entire, or sub-dentate, glabrous, 10–20 mm long, as broad as stem. Stipules more or less equally as long as petiole, or longer than petiole. Peduncle shorter than leaf. Pedicel 2–3 mm long. Flowers (2–)3–7(–8) per inflorescence, concolorous. Corolla purple. Flower 12–15(–20) mm long. Standard with no conspicuous veins. Wings purple. Calyx glabrous, 8–9 mm long, tube gibbous, teeth unequal, straight, shorter than tube, or equal to tube. Style 4–5 mm, straight, linear. Ovary linear. Legume straight, linear, (25–)30–40(–60) mm long, (5–)6–7(–9) mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod (3–)6–12(–20).

26. *L. pisiformis* L.

Perennial, slender, ascending plants. Vegetative parts glabrous when green. Plants 50–100 cm. Stems winged. Leaflets present, 3–5 pairs per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets elliptic, or ovate, apex obtuse. Leaflets 25–60 mm long, (7–)10–30 mm wide, venation pinnate, or parallel. Stipules ovate, or elliptic, base semi-sagittate, margin entire, glabrous, 20–50 mm long, broader than the leaflet, or somewhat narrower than leaflet. Stipules shorter than petiole. Peduncles (35–)50–110(–125) mm long, more or less equally as long as leaf, or shorter than leaf. Pedicel 2–3 mm long. Flowers 8–15(–20) per inflorescence, concolorous. Corolla purplish-pink, or purple. Flower 10–15(–20) mm long. Standard with no conspicuous veins. Wings purple. Calyx glabrous, tube gibbous, teeth unequal, straight, shorter than tube. Style 4–5 mm, straight, linear. Ovary linear. Legume beaked, linear, 40–50 mm long, 4–5 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod 10–20. Hilum 0.125–0.166 mm long.

27. *L. pratensis* L.

Perennial, sturdy or slender, decumbent plants. Vegetative parts glabrous, or pubescent, usually sparsely, when vegetative parts green. Plants 20–50 cm. Stems angled. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis laminate, tendrillous. Leaflets elliptic, or linear-lanceolate, or elliptic-lanceolate, apex acute, 10–40 mm long, 1.5–11 mm wide, venation parallel. Stipules lanceolate-ovate, base sagittate, margin entire, glabrous, broader than the leaflet. Stipules longer than petiole. Flowers 3–10 per inflorescence, concolorous. Corolla yellow. Flower 10–16 mm long. Standard with no conspicuous veins. Wings yellow. Calyx glabrous, 6–9 mm long, tube not gibbous, teeth unequal, straight, teeth equal to tube. Style 3–4 mm, straight, oblong. Ovary oblong. Legume oblong-linear, 20–70 mm long, 5–6 mm wide, glabrous, or tomentose. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod 4–8(–10).

28. *L. roseus* Stev. in Mem. Soc. Nat. Mosc. 4$52 (1813)

Perennial, sturdy or slender, ascending plants. Vegetative parts glabrous when green. Plants 40–60 cm. Stems terete. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, mucronate. Leaflets elliptic, or obovate, or elliptic-orbicular, apex subobtuse. Leaflets 15–45

mm long, 10–30 mm wide, venation pinnate, or reticulate. Stipules lanceolate, base semi-sagittate, margin entire, glabrous, 3–7 mm long, as broad as stem. Flowers 1–4 per inflorescence, concolorous. Corolla pink. Flower 12–19 mm long. Standard with no conspicuous veins. Wings pink. Calyx 5–7 mm long, tube gibbous, teeth unequal, straight. Calyx glabrous, teeth shorter than tube. Style 3–4 mm, twisted, linear-spathulate. Ovary linear. Legume broadly-linear, or linear-sublanceolate. Legume 35–45 mm long, 6–8 mm wide, glabrous. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seed surface smooth. Seeds per pod 5–10.

29. *L. rotundifolius* Willd.

Perennial, sturdy, decumbent plants. Vegetative parts glabrous when green. Plants 100–250 cm. Stems winged. Leaflets present, 1(–2) pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets elliptic, or sub-orbicular, apex obtuse. Leaflets 25–65 mm long, 10–45 mm wide, venation parallel. Stipules lanceolate, or lanceolate-ovate, base semi-sagittate, margin entire, glabrous, 1–2 times as broad as stem. Stipules more or less equally as long as petiole. Peduncle longer than leaf. Flowers 3–13 per inflorescence, concolorous. Corolla pink. Flower 18–25 mm long. Standard with more than 5 conspicuous veins, apex strongly emarginate. Wings pink. Calyx glabrous,7–9 mm long, tube not gibbous, teeth unequal, straight, shorter than tube. Calyx lower teeth shorter than tube. Style 6–7 mm, twisted, arcuate. Ovary linear. Legume linear, 50–70 mm long, 7–10 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves hairy. Upper suture keeled. Seed surface reticulate-rigulose. Seeds per pod 6–10.

30. *L. satdaghensis* P. H. Davis

Perennial, slender or rigid, erect plants. Vegetative parts subadpressed canescent-pubescent, when vegetative parts green. Plants 40–60 cm. Stems terete. Leaflets present, 4–8 pairs per leaf, pinnate. Leaf rachis not laminate, mucronate. Leaflets linear, or linear-lanceolate, apex acute, 30–60 mm long, 2.5–5 mm wide, venation parallel. Stipules lanceolate-subulate, base sagittate, margin entire, glabrous, less than 1/2 as wide as stem. Stipules longer than petiole. Peduncles 30–70 mm long, longer than leaf. Pedicel 6–7 mm long. Flowers 5–9 per inflorescence, concolorous. Corolla blue. Flower 17–22(–25) mm long. Standard with no

conspicuous veins. Wings blue. Calyx glabrous, 6–8 mm long, tube gibbous, teeth unequal, straight, shorter than tube. Style 6–7 mm, straight, linear. Ovary linear, or canescent. Legume beaked, linear, or canescent. Legume 40–60 mm long, 4–5 mm wide, glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow.

31. *L. spathulatus* Čelak.

Perennial, slender, erect plants. Vegetative parts glabrous when green. Plants 20–40 cm. Stems terete. Leaflets present, 2 pairs per leaf, subdigitate. Leaf rachis not laminate, mucronate. Leaflets linear, apex acute, 35–90 mm long, 1–7 mm wide, venation parallel. Stipules lanceolate-subulate, base sagittate, margin entire, glabrous, 1 mm wide. Stipules longer than petiole. Peduncle longer than leaf. Flowers 2–7 per inflorescence, concolorous. Corolla blue, or purple. Flower 13–20 mm long. Standard with no conspicuous veins. Wings blue, or purple. Calyx glabrous, 5–7(–8) mm long, tube gibbous, teeth unequal, straight, shorter than tube, or equal to tube. Style 5–6 mm, straight, spathulate. Ovary linear. Legume linear, 45 mm long, 6 mm wide, glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seeds per pod (9–)10(–11).

32. *L. sylvestris* L.

Perennial, slender, decumbent plants. Vegetative parts glabrous when green. Plants 60–200 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis laminate, tendrillous. Leaflets linear, or lanceolate, or oblong-lanceolate, apex acute, 40–150 mm long, 5–20 mm wide, venation parallel. Stipules subulate, or lanceolate-subulate, base semi-sagittate, margin entire, glabrous, less than 1/2 as wide as stem. Stipules shorter than petiole. Peduncle more or less equally as long as leaf, or shorter than leaf, or longer than leaf. Flowers 3–12 per inflorescence, concolorous. Corolla purplish-pink. Flower 13–20 mm long. Standard with more than 5 conspicuous veins, apex strongly emarginate. Wings blue. Calyx tube not gibbous, teeth unequal, straight. Calyx glabrous, teeth shorter than tube. Calyx lower teeth shorter than tube. Style 4–5 mm, twisted, linear, or arcuate. Ovary linear. Legume linear, 40–80 mm long, 8–10 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface reticulate-rigulose. Seeds per pod (6–)10–15.

33. *L. tingitanus* L.

Annual, sturdy or slender, erect plants. Vegetative parts glabrous when green. Plants 50–100(–180) cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis laminate, tendrillous. Leaflets elliptic, apex acuminate, 40–80 mm long, 15–23 mm wide, venation parallel. Stipules lanceolate, base semi-sagittate, margin entire, glabrous, 12–20(–25) mm long, more or less equally as long as petiole. Peduncles 28–160 mm long, longer than leaf. Pedicel 6–11 mm long. Flowers 1–3(–4) per inflorescence, concolorous. Corolla purple. Flower 20–35 mm long. Standard with no conspicuous veins, apex strongly emarginate, or obtuse. Wings purple. Calyx tube not gibbous, teeth equal , straight. Calyx glabrous, teeth shorter than tube. Calyx lower teeth shorter than tube, or equal to tube, or longer than tube. Style (4.5–)6–8 mm, twisted, spathulate. Ovary oblong. Legume straight, oblong. Legume (65–)70–110 mm long, (7–)8–11 mm wide, glabrous. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture narrowly-winged. Seed surface smooth. Seeds per pod 6–10. Hilum (7–)8–11 mm long.

34. *L. tuberosus* L.

Perennial, sturdy or slender, decumbent plants. Vegetative parts glabrous when green. Plants 30–80 cm. Stems terete. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets elliptic, apex obtuse. Leaflets 10–52 mm long, 3–25 mm wide, venation parallel. Stipules lanceolate, base semi-sagittate, margin entire, glabrous, 6–22 mm long, 1–3 mm wide. Stipules longer than petiole. Peduncle longer than leaf. Flowers 3–9 per inflorescence, concolorous. Corolla pink. Flower 11–15 mm long. Standard with more than 5 conspicuous veins, apex strongly emarginate. Wings pink. Calyx glabrescent , 5–7 mm long, tube gibbous, teeth unequal, straight, teeth equal to tube. Calyx lower teeth equal to tube. Style 6–8 mm, twisted, oblong, or arcuate. Ovary oblong. Legume beaked, oblong-linear, 20–40 mm long, 4–7 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seed surface tuberculate. Seeds per pod 3–6.

35. *L. tukhtensis* Czecz.

Perennial, slender, erect plants. Vegetative parts glabrous, or pubescent, usually sparsely, when vegetative parts green. Plants 15–30 cm. Stems terete. Leaflets present, 1–2 pair per

leaf, subdigitate. Leaf rachis not laminate. Leaflets linear, or linear-oblong. Leaflets 35–65 mm long, 2–9 mm wide, venation parallel. Stipules subulate, or lanceolate, margin entire, more or less equally as long as petiole, or longer than petiole. Peduncle longer than leaf. Flowers 3–12 per inflorescence, concolorous. Corolla blue. Flower 14–17 mm long. Standard with no conspicuous veins. Wings blue. Calyx 5–6.5 mm long, teeth unequal, straight, shorter than tube. Style 4–5 mm, straight, spathulate. Ovary linear. Legume linear, 50–60 mm long, 5–6 mm wide. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow.

36. *L. undulatus* Boiss.

Perennial, slender, decumbent plants. Vegetative parts glabrous when green. Plants 100–250 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets elliptic, or sub-orbicular, apex undulate-margined. Leaflets 25–65 mm long, 10–45 mm wide, venation parallel. Stipules lanceolate-ovate, base semi-sagittate, margin entire, glabrous, 1–2 times as broad as stem. Stipules longer than petiole. Flowers 3–13 per inflorescence, concolorous. Corolla pink. Flower 18–25 mm long. Standard with no conspicuous veins. Wings pink. Calyx glabrous, 7–9 mm long, tube gibbous, teeth unequal, straight, shorter than tube. Style 5–7 mm, twisted, linear. Ovary linear. Legume linear, 50–70 mm long, 7–10 mm wide, glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves hairy. Upper suture narrowly-winged. Seed surface reticulate-rigulose. Seeds per pod 6–10.

37. *L. variabilis* (Boiss. & Ky.) Maly

Perennial, sturdy or slender, ascending plants. Vegetative parts glabrous when green. Plants 15–35 cm. Stems terete. Leaflets present, 2 pairs per leaf, subdigitate. Leaf rachis not laminate, mucronate. Leaflets elliptic, or oblong-elliptic, apex obtuse. Leaflets 20–70 mm long, 5–14 mm wide, venation parallel. Stipules lanceolate-subulate, base sagittate, margin entire, glabrous, 3–5 mm long, 1 mm wide. Stipules longer than petiole. Flowers 2–7 per inflorescence, concolorous. Corolla pink. Flower 20–27 mm long. Standard with no conspicuous veins. Wings pink. Calyx glabrous, (5–)6–9 mm long, tube gibbous, teeth unequal, straight, shorter than tube, or equal to tube. Style 5 mm, straight, oblong, or spathulate. Ovary linear. Legume linear, (55–)60(–65) mm long, (5–)5.5(–6) mm wide,

glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seed surface smooth. Seeds per pod (9–)10(–11).

38. *L. venetus* (Miller) Wohlf.

Perennial, sturdy or slender, erect plants. Vegetative parts glabrous, or pubescent, usually sparsely, when vegetative parts green. Plants 20–40 cm. Stems terete. Leaflets present, 2–3 pairs per leaf , paripinnate. Leaf rachis not laminate, mucronate. Leaflets ovate, apex acute, 35–70 mm long, 15–50 mm wide, venation pinnate. Stipules suborbicular, base semi-sagittate, margin entire, 10–14 mm long, more or less equally as long as leaf, or shorter than leaf. Flowers (6–)10–30 per inflorescence, concolorous. Corolla blue, or purple. Flower 15–18 mm long. Standard with no conspicuous veins. Wings blue, or purple. Calyx 7–10 mm long, tube gibbous, teeth unequal, straight, teeth equal to tube. Ovary linear. Legume linear, 35–60 mm long, 5–8 mm wide. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture narrow. Seed surface smooth. Seeds per pod 8–14.

39. *L. vernus* (L.) Bernh.

Perennial, sturdy or slender, erect. Vegetative parts glabrous, or pubescent, usually sparsely, when vegetative parts green. Plants 20–40 cm. Stems terete. Leaflets present, 2–3 pairs per leaf, paripinnate. Leaf rachis not laminate, mucronate. Leaflets ovate, apex acuminate, 35–70 mm long, 15–35 mm wide, venation pinnate. Stipules ovate-oblong, base semi-sagittate, margin entire. Peduncle more or less equally as long as leaf, or shorter than leaf. Flowers 3–7 per inflorescence, concolorous. Corolla blue, or purple. Flower 15–18 mm long. Standard with no conspicuous veins. Wings blue, or purple. Calyx 7–10 mm long, tube gibbous, teeth unequal, straight, teeth equal to tube. Ovary linear. Legume linear, 35–60 mm long, 5–8 mm wide, glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, eglandular, or glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod 8–14.

40. *L. amphicarpos* L.

Annual, slender, ascending or decumbent herbs. Vegetative parts glabrous when green. Plants 12–50 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis laminate, tendrillous. Leaflets linear, or elliptic, apex acuminate, 10–30 mm long, 2–7 mm wide,

venation parallel. Stipules lanceolate, base semi-sagittate, margin entire, glabrous, (4–)5–17(–22) mm long, 0.5–5 mm wide. Stipules longer than petiole. Peduncle longer than leaf. Pedicel 4–9 mm long. Flowers 1 per inflorescence, concolorous. Corolla violet, or pink. Flower 8–15 mm long. Standard with no conspicuous veins, apex emarginate. Wings pink, or violet. Calyx tube not gibbous, teeth unequal, straight. Calyx glabrous, teeth longer than tube. Calyx lower teeth longer than tube. Style 3.5–4.5 mm, twisted, linear-spathulate, or spathulate. Ovary oblong. Legume beaked, broadly elliptic-oblong, or obovate. Legume 15–30 mm long, 8–10 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods present. Legume valves glabrous, reticulate-nerved. Upper suture narrowly-winged. Seed surface tuberculate. Seeds per pod (1–)2–3(–4). Hilum 1.2–2 mm long.

41. *L. angulatus* L.

Annual, slender, ascending herbs. Vegetative parts glabrous when green. Plants 20–50 cm. Stems angled. Leaflets present, 1 pair per leaf, alternate. Leaf rachis not laminate, tendrillous. Leaflets linear, or lanceolate, apex acute. Leaflet venation parallel. Stipules lanceolate, or linear, base hastate, or semi-hastate, margin entire, glabrous, 1 mm wide, or as broad as stem. Stipules more or less equally as long as petiole. Peduncle longer than leaf. Flowers 1 per inflorescence, concolorous. Corolla purplish-pink, or purple. Flower 8–10 mm long. Standard with no conspicuous veins, apex emarginate (rarely). Wings purple. Calyx tube not gibbous, teeth unequal, straight. Calyx glabrous, teeth longer than tube. Style straight. Style spathulate. Ovary linear. Legume straight, linear, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrowly-winged. Seed surface tuberculate. Seeds per pod 10–12.

42. *L. annuus* L.

Annual, slender, decumbent herbs. Vegetative parts glabrous when green. Plants 20–100 cm. Stems winged. Leaflets present, 1 pair per leaf, paripinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, or linear-lanceolate, apex acute, 60–140 mm long, 2–12(–19) mm wide, venation parallel. Stipules subulate, base semi-sagittate, margin entire, glabrous, 5–25 mm long, 0.5–1.5 mm wide. Stipules shorter than petiole. Peduncle longer than leaf. Flowers 1–6 per inflorescence, concolorous. Corolla yellow, or orange. Flower 12–15(–17) mm long. Standard with no conspicuous veins, apex emarginate. Wings yellow, or orange. Calyx

glabrous, 5–7 mm long, tube not gibbous, teeth unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 4–5 mm, twisted, linear. Ovary linear. Legume straight, oblong-linear, 50–70 mm long, (7–)9–11 mm wide, glabrous. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture canaliculate. Seed surface coarsely-tuberculate. Seeds per pod 6–8. Hilum 1.5 mm long.

43. *L. aphaca* L.

Annual, slender, ascending or decumbent herbs. Vegetative parts glabrous when green. Plants 5–50(–100) cm. Stems terete. Leaflets absent. Leaflets reduced. Leaf rachis not laminate, tendrillous. Leaflets tendrillous, apex absent. Stipules ovate, base semi-hastate, margin entire, glabrous, (5–)10–30 mm long, broader than the leaflet. Pedicel 2–4(–5) mm long. Flowers 1–2 per inflorescence, concolorous. Corolla cream, or pale sulphur, or yellow. Flower (6–)7–13(–16) mm long. Standard with no conspicuous veins, apex emarginate. Wings cream, or yellow. Calyx glabrous, 3–9 mm long, tube not gibbous, teeth equal , straight, longer than tube. Calyx lower teeth longer than tube. Style 3–5 mm, straight, linear. Ovary linear. Legume straight, linear-sublanceolate. Legume 18–35 mm long, 4–6 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod 5–7. Hilum 1–1.5 mm long.

44. *L. basalticus* Rech.

Annual, sturdy or slender, decumbent herbs. Vegetative parts pubescent, usually sparsely, when vegetative parts green. Plants 20–40 cm. Stems winged, or ridged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear-elliptic, or elliptic, apex mucronate, or undulate-margined. Leaflets 5–55 mm long, 1–10 mm wide, venation parallel. Stipules subulate, base semi-sagittate, margin entire, glabrous, shorter than petiole. Peduncles 20–30 mm long. Flowers 1–2 per inflorescence, concolorous. Corolla brick-red. Flower (14.8–)15(–15.2) mm long. Standard with no conspicuous veins, apex emarginate. Wings brick-red. . Calyx pubescent, tube not gibbous, teeth unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 3–5 mm, twisted, oblong. Ovary oblong. Legume beaked, broadly-oblong. Legume 25 mm long, tomentose, or ciliate. Lower suture

not ciliate. Mature legume indehiscent. Amphicarpic pods present. Legume valves hairy. Legume valves tuberculate. Upper suture narrowly-winged. Seed surface reticulate.

45. *L. belinensis* N. Maxted & D.J. Goyder

Annual, slender, ascending or decumbent herbs. Vegetative parts glabrous when green. Plants 50–200 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, or elliptic, or obovate, or oblong-lanceolate, apex mucronate, or obtuse. Leaflets 15–65 mm long, 7–30 mm wide, venation pinnate. Stipules lanceolate, or lanceolate-ovate, base semi-sagittate, margin entire, glabrous, 5–15 mm long, 1–3 mm wide. Stipules shorter than petiole. Peduncles (3–)6–28 mm long. Flowers (1–)3–5 per inflorescence, not concolorous. Corolla orange. Flower 20–26 mm long. Standard with no conspicuous veins, apex strongly emarginate. Wings yellow. Calyx tube not gibbous, teeth unequal, straight. Calyx glabrous, teeth equal to tube. Calyx lower teeth shorter than tube. Style 8–10 mm, twisted, linear. Ovary oblong. Legume oblong. Legume 18–35 mm long, 4–7 mm wide, ciliate. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrowly-winged. Seed surface verrucose. Seeds per pod 2–5(–8). Hilum 1 mm long.

46. *L. blepharicarpus* Boiss.

Annual, slender, ascending plant. Vegetative parts spreading pilose, or villous with soft, spreading hairs, when vegetative parts green. Plants 10–40 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, or elliptic, apex obtuse. Leaflets (7–)10–40 mm long, 2–7 mm wide, venation parallel. Stipules lanceolate, base semi-sagittate, margin entire, glabrous, 5–15 mm long, 2–3 times as broad as stem. Stipules longer than petiole. Peduncles 10–30 mm long, shorter than leaf. Pedicel 3–5 mm long. Flowers 1 per inflorescence, concolorous. Corolla orange, or brick-red. Flower 4–14 mm long. Standard with no conspicuous veins, apex emarginate. Wings orange, or brick-red. Calyx 4.5–7 mm long, tube not gibbous, teeth unequal, straight. Calyx glabrous, teeth longer than tube. Calyx lower teeth longer than tube. Style 4–6 mm, twisted, linear. Ovary oblong. Legume beaked, broadly elliptic-oblong. Legume 20–30 mm long, 10–15 mm wide, ciliate. Lower suture ciliate. Mature legume indehiscent. Amphicarpic pods not present. Upper suture narrowly-winged. Seed surface punctate. Seeds per pod 3–4. Hilum 1 mm long.

47. *L. cassius* Boiss.

Annual, sturdy or slender, ascending plant. Vegetative parts glabrous. Vegetative parts glaucous (or glaucescent). Plants (15–)30–60 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, or linear-lanceolate, 30–70 mm long, 2–9 mm wide, venation parallel. Stipules lanceolate, base semi-sagittate, margin entire, glabrous, 2–15 mm long, 1 mm wide. Stipules shorter than petiole. Peduncles 8–80 mm long, shorter than leaf, or longer than leaf. Pedicel 0.3–1 mm long. Flowers 1–4(–6) per inflorescence, not concolorous. Corolla pink. Flower 9–11(–12) mm long. Standard with no conspicuous veins, apex emarginate. Wings white. Calyx glabrous, 4–5 mm long, tube not gibbous, teeth unequal, straight, teeth equal to tube. Calyx lower teeth shorter than tube, or equal to tube. Style 4–5 mm, straight, or twisted, canaliculate. Ovary linear. Legume straight, oblong-linear, 28–35 mm long, 5–7 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture keeled. Seed surface coarsely-tuberculate, or verrucose. Seeds per pod 5–7. Hilum 1.5 mm long.

48. *L. cicera* L.

Annual, slender, ascending plant. Vegetative parts glabrous, or spreading pilose, or villous with soft, spreading hairs, when vegetative parts green. Plants 15–50 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, or elliptic, or linear-lanceolate, apex acute, 15–95 mm long, 1–9 mm wide, venation parallel. Stipules lanceolate, or lanceolate-ovate, or ovate-accuminate, base semi-sagittate, margin entire, glabrous, 2–3 times as broad as stem. Stipules shorter than petiole. Peduncle longer than leaf. Flowers 1 per inflorescence, concolorous. Corolla brick-red. Flower 12–16 mm long. Standard with no conspicuous veins, apex strongly emarginate. Wings brick-red. Calyx glabrous, tube not gibbous, teeth unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 3.5–5 mm, straight, or twisted, linear. Ovary oblong. Legume beaked, oblong. Legume 25–40 mm long, 8–10.5 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrowly-winged. Seed surface smooth. Seeds per pod 3–5.

49. *L. ciliolatus* Sam.

Annual, slender, decumbent, or prostrate plant. Vegetative parts glabrous when green. Plants 10–20 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, or lanceolate, apex acute, 10–40 mm long, 1–3 mm wide, venation parallel. Stipules subulate, base semi-sagittate, margin entire, glabrous. Flowers 1 per inflorescence, concolorous. Corolla brick-red. Flower 10 mm long. Standard with no conspicuous veins, apex emarginate. Wings brick-red. . Calyx glabrous, tube gibbous, teeth unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 3–6 mm, twisted, spathulate. Ovary canescent. Legume straight, oblong-linear, or oblong, or canescent. Legume 10–20 mm long, 3 mm wide, tomentose. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods present. Legume valves glabrous, reticulate-nerved. Upper suture narrowly-winged. Seed surface reticulate. Seeds per pod 2–3.

50. *L. chloranthus* Boiss.

Annual, sturdy or slender, erect, or decumbent plant. Vegetative parts patently pilose, sometimes densely, when vegetative parts green. Plants 17–70 cm. Stems winged. Leaflets present, 1 pair per leaf–2, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear-elliptic, or elliptic, apex obtuse. Leaflets 20–60 mm long, 7–22 mm wide, venation parallel. Stipules subulate, or lanceolate-subulate, base semi-sagittate, margin entire, pubescent. Stipules 8–20 mm long, 1–3 mm wide. Stipules shorter than petiole. Peduncles 80–160 mm long, longer than leaf. Flowers 1–2(–3) per inflorescence, concolorous. Corolla pale sulphur, or yellow. Flower 15–24 mm long. Standard with no conspicuous veins, apex obtuse. Wings yellow. Calyx glabrescent, 9–11 mm long, tube gibbous, teeth unequal, straightteeth longer than tube. Calyx lower teeth longer than tube. Style 7 mm, twisted, linear. Ovary linear. Legume straight, oblong-linear, 43–50 mm long, 6–9 mm wide, pilose. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves tuberculate. Upper suture narrow. Seed surface papillose, or verrucose. Seeds per pod 5–9.

51. *L. chrysanthus* Boiss.

Annual, sturdy. Growth habit erect plant. Vegetative parts patently pilose, sometimes densely, when vegetative parts green. Plants 30–45(–60) cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear-elliptic, or elliptic, apex

mucronate. Leaflets 40–55 mm long, 9–12 mm wide, venation parallel. Stipules lanceolate, base semi-sagittate, margin entire, pubescent. Stipules (9–)10–11 mm long, 1 mm wide. Stipules shorter than petiole. Flowers 2–4 per inflorescence, concolorous. Corolla yellow. Flower 20–22 mm long. Standard with no conspicuous veins, apex strongly emarginate, or emarginate. Wings yellow. Calyx pubescent, 8 mm long, tube not gibbous, teeth unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 7 mm, twisted, linear. Ovary linear. Legume straight, oblong-linear, (28–)30–32 mm long, (7.5–)8(–8.5) mm wide, pilose. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves hairy. Legume valves gland-dotted. Upper suture narrow. Seed surface papillose. Seeds per pod 6–10.

52. *L. clymenum* L.

Annual, sturdy or slender, decumbent plant. Vegetative parts glabrous when green. Plants 30–80 cm. Stems winged, or terete. Leaflets present, Leaflet pairs per leaf 2–4, pinnate. Leaf rachis laminate, mucronate, or tendrillous. Leaflets linear, or linear-oblong, or oblong, apex mucronate. Leaflets 15–50 mm long, 1.5–7 mm wide, venation parallel. Stipules lanceolate, or ovate, or oblong, base semi-sagittate, margin dentate, or toothed. Stipules as broad as stem. Stipules shorter than petiole, or more or less equally as long as petiole, or longer than petiole. Peduncle more or less equally as long as leaf. Flowers 1–4 per inflorescence, not concolorous. Corolla white, or purple. Flower 16–20 mm long. Standard with no conspicuous veins, apex emarginate. Wings white, or violet. Calyx 5–7 mm long, teeth unequal, straight, shorter than tube. Calyx lower teeth shorter than tube. Style straight. Style spathulate. Ovary linear. Legume beaked, broadly-linear. Legume 59–60 mm long, (7–)9–10 mm wide. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrowly-winged. Seed surface smooth. Seeds per pod 5–6.

53. *L. gleospermus* Warb. et Eig

Annual, slender, ascending plant. Vegetative parts glabrous when green. Stems winged. Leaflets present, 1 pair per leaf–4, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, apex acuminate, 20–50 mm long, 2–5 mm wide, venation pinnate. Stipules lanceolate, or ovate, base semi-sagittate. Stipules shorter than petiole. Peduncles 10 mm long. Flowers 1 per inflorescence, concolorous. Corolla white. Flower 18–20 mm long. Standard with no

conspicuous veins, apex emarginate. Wings white. Calyx tube not gibbous, teeth unequal, straight, shorter than tube. Calyx lower teeth shorter than tube. Style straight. Style spathulate. Ovary linear. Legume beaked, linear, 40–60 mm long, 10–12 mm wide, ciliate. Lower suture not ciliate. Amphicarpic pods not present. Legume valves hairy. Seed surface viscose. Seeds per pod 5–7.

54. *L. gorgoni* Parl.

Annual, slender, decumbent plant. Vegetative parts glabrous when green. Plants 20–60 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets lanceolate, or linear-lanceolate, or elliptic-lanceolate, apex acute, 30–70 mm long, 3–15 mm wide, venation parallel. Stipules lanceolate, or lanceolate-ovate, base semi-sagittate, margin entire, glabrous, 2–3 times as broad as stem. Stipules shorter than petiole, or more or less equally as long as petiole. Peduncles 1–30 mm long, more or less equally as long as leaf, or shorter than leaf. Pedicel 5 mm long. Flowers 1 per inflorescence, concolorous. Corolla gingery-orange. Flower 15–18 mm long. Standard with no conspicuous veins, apex strongly emarginate, or emarginate. Wings orange. Calyx glabrous, 7–9 mm long, tube not gibbous, teeth unequal, teethreflexed, teeth shorter than tube. Calyx lower teeth longer than tube. Style 7–9 mm, twisted, oblong. Ovary linear. Legume straight, oblong-linear, 35–47 mm long, 8–9 mm wide, glabrous, or tomentose. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture keeled. Seed surface smooth. Seeds per pod 5–8. Hilum 1.5 mm long.

55. *L. hierosolymitanus* Boiss.

Annual, slender, decumbent plant. Vegetative parts glabrous when green. Plants 20–100 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, or linear-lanceolate, apex acute, 60–140 mm long, 2–12(–19) mm wide, venation parallel. Stipules subulate, base semi-sagittate, margin entire, glabrous, 5–25 mm long, 0.5–5 mm wide. Stipules shorter than petiole. Peduncles 10–52 mm long, longer than leaf. Flowers 1–6 per inflorescence, concolorous. Corolla orange, or pink. Flower 10–12 mm long. Standard with no conspicuous veins, apex emarginate. Wings orange, or pink. Calyx glabrous, 4–6 mm long, tube not gibbous, teeth unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 3–4 mm, twisted, linear, or linear-spathulate. Ovary linear.

Legume straight, oblong-linear, 50–70 mm long, 5.5–6(–7) mm wide, glabrous. Lower suture not ciliate. Mature legume dehiscent. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture canaliculate. Seed surface ruminate-rugulose. Seeds per pod 6–10.

56. *L. hirsutus* L.

Aannual, or biennial, slender, decumbent plant. Vegetative parts glabrous, or adpressed pilose, when vegetative parts green. Plants (10–)40–60 cm. Stems terete. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear-elliptic, or elliptic, apex mucronate. Leaflets 30–60 mm long, 3–11 mm wide, venation parallel. Stipules subulate, or lanceolate-subulate, base semi-sagittate, margin entire, glabrous, 10–12 mm long, 0.5–1.5 mm wide. Stipules more or less equally as long as petiole. Peduncles 80–90 mm long, longer than leaf. Flowers 1–3 per inflorescence, concolorous. Corolla blue. Flower 10–14 mm long. Standard with no conspicuous veins, apex strongly emarginate. Wings blue. Calyx glabrescent , 4.5–5.5 mm long, tube gibbous, teeth unequal, straight, teeth equal to tube. Calyx lower teeth equal to tube. Style 3–4 mm, twisted, linear. Ovary linear. Legume beaked, oblong-linear, 23–35 mm long, 5.5–7.5 mm wide, pilose. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves hairy. Upper suture narrow. Seed surface verrucose. Seeds per pod 5–7.

57. *L. hirticarpus* Mattatia & Heyn

Annual, slender, ascending or decumbent plant. Vegetative parts pubescent, usually sparsely, when vegetative parts green. Plants 8–50 cm. Stems winged, or angled. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, mucronate, or tendrillous. Leaflets linear-elliptic, or elliptic, apex mucronate, or obtuse. Leaflets 5–50 mm long, 1–10 mm wide, venation parallel. Stipules lanceolate-ovate, base semi-sagittate, margin entire, glabrous, 2–10 mm long, 1–3 mm wide, or 2–3 times as broad as stem. Peduncles 10–40 mm long, more or less equally as long as leaf, or longer than leaf. Pedicel 2–5 mm long. Flowers 1 per inflorescence, concolorous. Corolla brick-red. Flower 10–18(–20) mm long. Standard with no conspicuous veins. Wings brick-red. Calyx glabrous, teeth unequal, reflexed, longer than tube. Calyx lower teeth longer than tube. Style 4–8 mm, twisted, spathulate. Ovary linear. Legume beaked, oblong. Legume 16–28 mm long, 6–10 mm wide. Lower suture not ciliate. Amphicarpic pods not present. Legume valves hairy. Legume valves tuberculate. Upper

suture keeled. Seed surface smooth. Seeds per pod 2–5. Hilum 1.5 mm long.

58. *L. inconspicuus* L.

Annual, slender, erect plant. Vegetative parts glabrous, or pubescent, usually sparsely, when vegetative parts green. Plants 10–35 cm. Stems terete. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous, or aristate. Leaflets lanceolate, or linear-lanceolate, apex acute, 15–60 mm long, 1–7 mm wide. Stipules lanceolate, or lanceolate-accuminate, base hastate, or semi-sagittate, margin entire, glabrous, 0.5–1 mm long, 1 mm wide. Stipules shorter than petiole. Peduncles 10 mm long. Flowers 1 per inflorescence, not concolorous. Corolla pale-lavender. Flower 7–9 mm long. Standard with no conspicuous veins, apex emarginate. Wings white. Calyx glabrous, 4–5 mm long, tube gibbous, teeth equal , straight, longer than tube. Calyx lower teeth longer than tube. Style 2–4 mm, straight, canaliculate. Ovary linear. Legume incurved, linear, 35–50 mm long, 4–5 mm wide, glabrous, or tomentose. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, obscurely-nerved. Upper suture narrow. Seed surface smooth. Seeds per pod 7–11.

59. *L. lycicus* Boiss.

Annual, slender, decumbent plant. Vegetative parts pubescent, usually sparsely. Vegetative parts glaucous (or glaucescent). Plants 20–60 cm. Stems terete. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, mucronate, or tendrillous. Leaflets elliptic, or obovate, apex obtuse. Leaflets 25–50 mm long, 7–25 mm wide, venation parallel. Stipules subulate, base semi-hastate, margin entire, pubescent. Stipules (4–)5(–6) mm long, as broad as stem. Peduncles 50–100(–110) mm long. Flowers 2–3(–6) per inflorescence, concolorous. Corolla pink. Flower 14–18 mm long. Standard with no conspicuous veins. Wings pink. Calyx glabrescent, 6–8 mm long, tube not gibbous, teeth unequal, straight, longer than tube. Style 7 mm, twisted, linear. Ovary linear. Legume oblong-linear, (21–)22–23(–24) mm long, 4.5 mm wide, pilose. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, glandular-verrucose. Upper suture narrow. Seed surface reticulate-rigulose, or coarsely-tuberculate.

60. *L. marmoratus* Boiss. & Bl.

Annual, slender, ascending plant. Vegetative parts glabrous when green. Plants 10–50 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, or linear-lanceolate, apex mucronate. Leaflets 10–40(–50) mm long, 1.5–3 mm wide, venation parallel. Stipules lanceolate, base semi-sagittate, margin entire, glabrous, 1–2 times as broad as stem, or 2–3 times as broad as stem. Stipules longer than petiole. Peduncles 40–60 mm long. Flowers 1 per inflorescence, concolorous. Corolla brick-red. Flower 11–13(–14) mm long. Standard with more than 5 conspicuous veins, apex strongly emarginate. Wings brick-red. Calyx glabrous, 4–7 mm long, tube not gibbous, teeth unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 4–5(–6) mm, twisted, linear. Ovary oblong. Legume beaked, oblong. Legume 20–27 mm long, 6–8 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrowly-winged. Seed surface smooth. Seeds per pod 3–4.

61. *L. nissolia* L.

Annual, slender, erect or ascending plant. Vegetative parts glabrous when green. Plants 15–70(–90) cm. Stems angled. Leaflets present, 1 pair per leaf, phyllodic. Leaf rachis not laminate, aristate. Leaflets linear, or linear-lanceolate, apex acute, (20–)40–100 mm long, 2–6 mm wide, venation parallel. Stipules subulate, or lanceolate-subulate, or lanceolate, or minute, or filiform, base semi-sagittate, margin entire, glabrous, 1–3 mm long, 1 mm wide. Stipules shorter than petiole. Peduncles 20–130 mm long, shorter than leaf, or longer than leaf. Pedicel 1.5–4 mm long. Flowers 1(–2) per inflorescence, concolorous. Corolla pink. Flower (6–)9–15(–18) mm long. Standard with no conspicuous veins, apex emarginate. Wings pink. Calyx glabrous, 4–5 mm long, tube not gibbous, teeth unequal, straight, shorter than tube. Calyx lower teeth longer than tube. Style 2–3 mm, twisted, linear-spathulate, or spathulate. Ovary linear. Legume straight, linear, (30–)32–40(–60) mm long, 2.5–3 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves hairy. Upper suture narrow. Seed surface tuberculate, or verrucose. Seeds per pod 11–16.

62. *L. ochrus* (L.) DC.

Annual, sturdy or slender, decumbent plant. Vegetative parts glabrous when green. Plants 25–

100 cm. Stems winged. Leaflets present, 1–2(–3) pair per leaf, pinnate. Leaf rachis laminate, mucronate, or tendrillous. Leaflets ovate, apex mucronate, 20–45 mm long, 9–12 mm wide, venation parallel, base semi-sagittate. Stipules longer than petiole. Peduncle shorter than leaf. Pedicel 1–3 mm long. Flowers 1 per inflorescence, concolorous. Corolla white, or cream. Flower 14–16 mm long. Standard with no conspicuous veins, apex emarginate with mucro. Wings white, or cream. Calyx 5–8 mm long, teeth unequal, straight, equal to tube. Calyx lower teeth equal to tube. Style straight. Style spathulate. Ovary linear. Legume straight, oblong-linear, or narrowly oblong. Legume 40–50 mm long, 9–12 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrowly-winged. Seed surface smooth. Seeds per pod 5–7. Hilum 2–3 mm long.

63. *L. odoratus* L.

Annual, sturdy or slender, ascending or decumbent plant. Vegetative parts pubescent, usually sparsely, when vegetative parts green. Plants (50–)100–200 cm. Stems winged. Leaflets present, 1 pair per leaf. , paripinnate. Leaf rachis not laminate, tendrillous. Leaflets linear-oblong, or elliptic, or obovate, apex emarginate. Leaflets (20–)35–60 mm long, (7–)12–30 mm wide, venation pinnate, or parallel. Stipules lanceolate, base semi-hastate, or semi-sagittate, margin entire, shorter than petiole. Peduncles 120–125(–150) mm long. Flowers (1–)2–3(–4) per inflorescence, not concolorous. Corolla white, or yellow, or brick-red, or blue. Flower 20–30 mm long. Standard with more than 5 conspicuous veins, apex strongly emarginate. Wings white, or yellow, or brick-red, or blue. Calyx pubescent, teeth unequal, straight, equal to tube, or longer than tube. Calyx lower teeth equal to tube. Style twisted, spathulate, or canaliculate. Ovary linear. Legume beaked, broadly-linear. Legume 40–65 mm long, 9–12 mm wide, pilose. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, tuberculate.

64. *L. phaselitanus* Hub.-Mor. & Davis

Annual, slender, decumbent plant. Vegetative parts glabrous. Vegetative parts glaucous (or glaucescent). Plants 50–100 cm. Stems terete. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear-elliptic, 15–35 mm long, 4–8 mm wide. Stipules subulate, base semi-sagittate, margin entire. Flowers 1–2 per inflorescence,

concolorous. Corolla violet. Flower (19–)20(–21) mm long. Standard with no conspicuous veins. Wings violet. Calyx 10–12 mm long, teeth unequal, straight, longer than tube. Style 11 mm Ovary linear. Legume oblong-linear, 23–35 mm long, 5.5–7.5 mm wide, pilose. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, glandular-verrucose.

65. *L. pseudocicera* Pamp.

Annual, slender, ascending plant. Vegetative parts glabrous when green. Plants 15–50 cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, or elliptic, or linear-lanceolate, apex acute, 15–95 mm long, 1–9 mm wide, venation parallel. Stipules lanceolate, or lanceolate-ovate, or ovate-accuminate, base semi-sagittate, margin entire, pubescent. Stipules 2–3 times as broad as stem. Stipules more or less equally as long as petiole. Peduncle longer than leaf. Flowers 1 per inflorescence, concolorous. Corolla gingery-orange. Flower 12–16 mm long. Standard with more than 5 conspicuous veins, apex strongly emarginate. Wings orange. Calyx glabrous, 7–9 mm long, tube not gibbous, teeth unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 3.5–7 mm, twisted, linear. Ovary oblong. Legume straight, oblong. Legume 25–40 mm long, 8–10.5 mm wide. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, longitudinally-nerved. Upper suture narrowly-winged. Seed surface smooth. Seeds per pod 3–5.

66. *L. pygmaeus* Gomblaut

Annual, slender, ascending or decumbent plant. Vegetative parts glabrous when green. Plants 5–10 cm. Stems terete. Leaflets present, 12–16(–20) pair per leaf, pinnate. Leaf rachis not laminate. Stipules linear, base semi-hastate, pubescent. Flowers concolorous. Corolla pink. Flower 10–12 mm long. Standard with no conspicuous veins. Wings pink. Calyx glabrous, teeth longer than tube. Ovary linear. Legume linear-sublanceolate. Legume 20 mm long. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seed surface reticulate-rigulose, or papillose.

67. *L. sativus* L.

Annual, slender, ascending plant. Vegetative parts glabrous when green. Plants 10–70(–100)

cm. Stems winged. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, or lanceolate, apex mucronate, 20–100 mm long, 1.5–11 mm wide, venation parallel. Stipules lanceolate, or lanceolate-accuminate, base semi-sagittate, margin entire, glabrous, 1–1.5 times as broad as stem. Stipules shorter than petiole. Peduncles 1–40(–45) mm long, longer than leaf. Pedicel 5–8 mm long. Flowers 1 per inflorescence, concolorous. Corolla white, or violet, or blue. Flower 14–20 mm long. Standard with more than 5 conspicuous veins, apex strongly emarginate. Wings white, or blue, or violet. Calyx glabrous, 7–10 mm long, tube not gibbous, teeth unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 5–6 mm, twisted, linear. Ovary oblong. Legume beaked, broadly-oblong. Legume 9–12 mm long, 6–8 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, eglandular. Upper suture broadly winged. Seed surface smooth. Seeds per pod (2–)3–4(–5). Hilum 1.5 mm long.

68. *L. saxatilis* (Vent.) Vis.

Annual, slender, ascending plant. Vegetative parts pubescent, usually sparsely, when vegetative parts green. Plants 7–30 cm. Stems terete. Leaflets present, 1–3 pair per leaf, pinnate. Leaf rachis not laminate, mucronate. Leaflets linear, or oblong, apex mucronate, 12–33 mm long, 0.5–1.5 mm wide. Stipules subulate, base semi-hastate, margin incised. Flowers 1 per inflorescence, concolorous. Corolla cream. Flower 7–8 mm long. Standard with no conspicuous veins. Wings cream. Calyx glabrous, 3 mm long, tube gibbous, teeth unequal, straight, shorter than tube. Style 1–2 mm, twisted, linear. Ovary linear. Legume oblong-linear, 15–22 mm long, 4.5–5.5 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves hairy. Upper suture narrow. Seed surface smooth. Seeds per pod 3–6. Hilum 1.2 mm long.

69. *L. setifolius* L.

Annual, slender, decumbent plant. Vegetative parts glabrous when green. Plants 30–80 cm. Stems winged. Leaflets present, 1 pair per leaf. Leaf rachis not laminate, tendrillous. Leaflets linear, apex acute, 25–75 mm long, 1–3 mm wide, venation pinnate, or parallel. Stipules lanceolate, or lanceolate-accuminate, base semi-sagittate, margin entire, glabrous, 3–10 mm long, 1–1.5 times as broad as stem. Stipules longer than petiole. Peduncles 10–30(–40) mm

long, shorter than leaf. Pedicel 2–8(–10) mm long. Flowers 1 per inflorescence, concolorous. Corolla orange. Flower 6–10 mm long. Standard with no conspicuous veins, apex emarginate. Wings orange. Calyx glabrous, 4–5 mm long, tube not gibbous, teeth equal , or unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 3.6–4.8 mm, straight, linear, or canaliculate. Ovary oblong. Legume oblong. Legume 20–27 mm long, 8–10 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface tuberculate, or papillose. Seeds per pod 2–3. Hilum 1.2 mm long.

70. *L. sphaericus* Retz.

Annual, slender, erect plant. Vegetative parts glabrous when green. Plants 15–50 cm. Stems terete. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous, or aristate. Leaflets linear, apex acute, 25–90 mm long, 0.5–3 mm wide, venation parallel. Stipules subulate, or lanceolate-subulate, base semi-hastate, or semi-sagittate, margin entire, glabrous, 3–13 mm long, as broad as stem. Stipules more or less equally as long as petiole, or longer than petiole. Peduncles 10–120 mm long, longer than leaf. Pedicel 5 mm long. Flowers 1 per inflorescence, concolorous. Corolla brick-red. Flower 8–10 mm long. Standard with no conspicuous veins, apex strongly emarginate. Wings brick-red. Calyx glabrous, 5–6 mm long, tube gibbous, teeth equal , or unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 2–3 mm, straight, linear, or canaliculate. Ovary linear. Legume straight, linear-ensiform. Legume 30–55 mm long, 4 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seed surface smooth. Seeds per pod 5–15. Hilum 1 mm long.

71. *L. stenolobus* Boiss.

Annual, slender, ascending plant. Vegetative parts glabrous when green. Plants 10–30 cm. Stems terete. Leaflets absent. Leaf rachis not laminate, tendrillous. Stipules lanceolate, base sagittate, margin entire, glabrous, 12–22 mm long, 1–3 mm wide, or broader than the leaflet. Flowers 1 per inflorescence, concolorous. Corolla yellow. Flower 7–8 mm long. Standard with no conspicuous veins. Wings yellow. Calyx glabrous, 3.5–4 mm long, teeth equal, straight, longer than tube. Style 2–3 mm, twisted, linear. Ovary linear. Legume linear, 25–30

mm long, 3.5 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous. Upper suture narrow. Seed surface smooth. Seeds per pod 4–6.

72. *L. stenophyllus* Boiss. & Heldr.

Annual, slender, ascending plant. Vegetative parts glabrous when green. Plants 40–70 cm. Stems terete. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, tendrillous. Leaflets linear, apex acute, 15–55 mm long, 0.5–2 mm wide, venation parallel. Stipules lanceolate, base semi-sagittate, margin entire, glabrous, 5–15 mm long, 3–4 times as broad as stem. Stipules shorter than petiole. Flowers 1 per inflorescence, not concolorous. Corolla white, or pink. Flower 12–16 mm long. Standard with 3–5 conspicuous veins, apex strongly emarginate. Wings pink. Calyx glabrous, 7–9 mm long, tube not gibbous, teeth equal , or unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 7–8 mm, twisted, linear. Ovary oblong. Legume beaked, narrowly oblong. Legume 35–45(–50) mm long, 9–10 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, gland-dotted. Upper suture keeled. Seed surface reticulate-rigulose, or ruminate-rugulose.

73. *L. tauricola* P. H. Davis

Annual, slender, ascending or decumbent plant. Vegetative parts glabrous when green. Plants 13–25 cm. Stems ridged. Leaflets present, 1 pair per leaf. Leaf rachis not laminate, mucronate, or tendrillous. Leaflets linear, or elliptic, apex mucronate. Leaflets 20–35 mm long, 1–2 mm wide, venation parallel. Stipules lanceolate, base semi-sagittate, margin entire, glabrous, 3–8 mm long, 1–1.5 mm wide. Peduncles 10–30 mm long. Flowers 1 per inflorescence, concolorous. Corolla yellow. Flower 7–10 mm long. Standard with no conspicuous veins. Wings yellow. Calyx glabrous, 3–4 mm long, tube not gibbous, teeth equal, straight, equal to tube. Style 3–4 mm, twisted, linear. Ovary linear. Legume linear, 2–8 mm long, 4 mm wide, glabrous. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, obscurely-nerved. Seeds per pod (4–)5–8.

74. *L. trachycarpus* (Boiss.) Boiss.

Annual, or biennial, sturdy, erect plant. Vegetative parts glabrous when green. Plants 40–50

cm. Stems terete. Leaflets present, 1 pair per leaf, pinnate. Leaf rachis not laminate, mucronate. Leaflets elliptic, apex obtuse, 40–50 mm long, 10–16 mm wide, venation parallel. Stipules lanceolate, or lanceolate-accuminate, base semi-sagittate, margin entire, glabrous, 7–14 mm long, 0.5–1.5 mm wide. Peduncles 50–90 mm long, longer than leaf. Flowers 3–6 per inflorescence, concolorous. Corolla purplish-pink. Flower (19–)20–21 mm long. Standard with no conspicuous veins. Wings pink. Calyx glabrous, 7–8 mm long, tube not gibbous, teeth equal, straight, longer than tube. Style 7–8 mm, twisted, linear. Ovary oblong. Legume elliptic-oblong. Legume (14–)15(–16) mm long, (7–)8(–8.5) mm wide, densely-pilose. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous, tuberculate. Upper suture narrow. Seeds per pod 1–2(–4).

75. *L. vinealis* Boiss. & Noe

Annual, slender, erect plant. Vegetative parts glabrous when green. Plants 14–40 cm. Stems terete. Leaflets present, 2 pairs per leaf, pinnate. Leaf rachis not laminate, mucronate, or tendrillous, or aristate. Leaflets linear, apex acute, 40–90 mm long, 1–5 mm wide, venation parallel. Stipules subulate, base semi-hastate, or semi-sagittate, margin entire, glabrous, as broad as stem. Stipules more or less equally as long as petiole, or longer than petiole. Peduncle longer than leaf. Flowers 1 per inflorescence, concolorous. Corolla brick-red, or pink. Flower 10 mm long. Standard with no conspicuous veins, apex emarginate. Wings pink, or brick-red. Calyx glabrous, 7–9 mm long, tube not gibbous, teeth equal , or unequal, straight, longer than tube. Calyx lower teeth longer than tube. Style 3–4 mm, straight, linear-spathulate, or canaliculate. Ovary linear. Legume beaked, linear, 40–55 mm long, 5–6.5 mm wide, glabrous. Lower suture not ciliate. Mature legume indehiscent. Amphicarpic pods not present. Legume valves glabrous, reticulate-nerved. Upper suture narrow. Seed surface smooth. Seeds per pod 3–6.

76. *L. woronowii* Bornm.

Annual, slender, ascending or decumbent or procumbent plant. Vegetative parts glabrous, or glaucous (or glaucescent). Plants 8–18 cm. Stems terete. Leaflets present, 1–2 pairs per leaf, pinnate. Leaf rachis not laminate, mucronate. Leaflets elliptic, or ovate, (11–)12(–13) mm long, 5 mm wide. Stipules lanceolate-subulate, base semi-sagittate, margin entire. Peduncle longer than leaf. Flowers 1 per inflorescence, concolorous. Corolla cream, or violet. Standard

with no conspicuous veins. Wings cream, or violet. Calyx glabrous, 5 mm long, teeth unequal, straight, longer than tube. Lower suture not ciliate. Amphicarpic pods not present. Legume valves glabrous.

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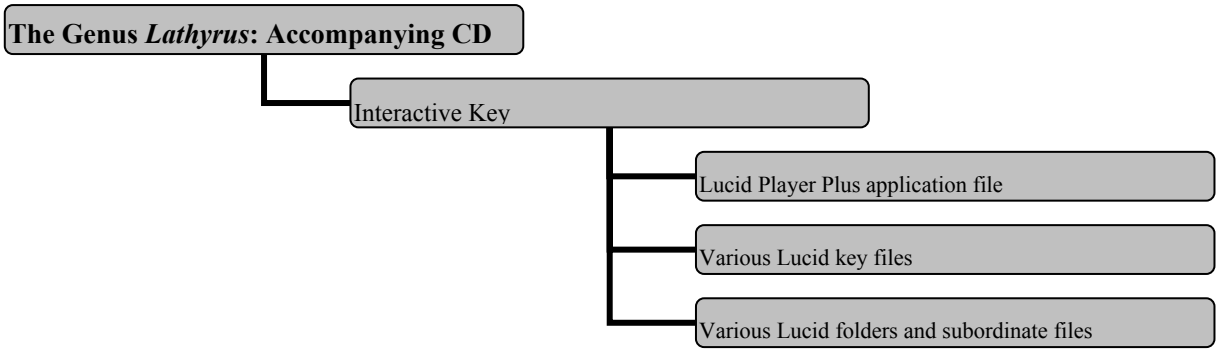
6.6 Appendices

Appendix 6.1. The Genus *Lathyrus*: an Interactive Key

The CD that accompanies the Ecogeographic Study contains the Lucid interactive key for *Lathyrus* species and sub-specific taxa. The various files required to run the key are contained in the subdirectory named Interactive Key as indicated below:



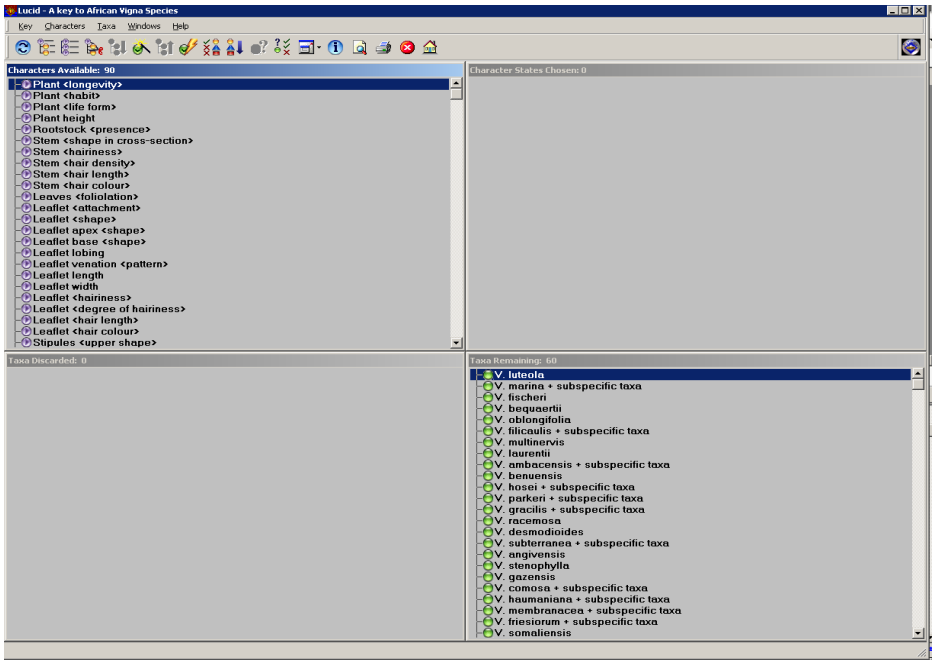
Installing *Lathyrus* Key

The key can either be run from the CD itself or copies into a directory on a hard drive and run from there.

How to Identify *Lathyrus* Specimens (text is adapted from Maslin, 2001)

To start the key:

1. Go to the directory that contains the interactive key.
2. Double-click on the "A key to the genus *Lathyrus*" icon and the front page of the key will appear.
3. Click on the Start key at the top left of the window, you will see a screen divided into four windows, with a menu bar and tool bar.



The four windows display four lists:

- Characters Available lists the characters that you may use to describe your specimen to the key; when you first start the key this will show a list of 90 characters.
- Character States Chosen will list the characters and their states as you select them; when you first start the key this window will be empty.
- Taxa Remaining lists the names of the taxa that 'match' your description; when you first start the key this window shows a list of the entire 60 species that are included in *Lathyrus* data set.
- Taxa Discarded will list all those taxa that do not 'match your description; when you first start the key this window will be empty.

To identify a specimen (i.e. name a *Lathyrus* specimen):

Your aim is to match your unidentified specimen against the species descriptions held in the data set. As your description becomes more and more complete the key will progressively narrow down the list in Taxa Remaining until, hopefully, only one taxon remains - you have identified (in other words, named) the taxon to which your specimen belongs.

Characters and states

To select a character that you have chosen to score click on the name of the character in the Characters Available window and it will open to display its states. A character is any attribute referring to form, structure or behaviour which the taxonomist separates from the whole organism for a particular purpose such as comparison or interpretation. These are distinguished from character states which are the actual representation of that character found in a particular specimen. Thus a character, for example, “Corolla colour”, has multiple character states, yellow, pink, white, blue, purple, etc. Within the context of the interactive key there are two basic sorts of characters, multistate and numeric:

To select states of a character:

Multistate character

Click on the character name, which will ‘open’ the character to show the states, then either double-click the text of the state (e.g. “Corolla colour” in the above example) or drag it with the mouse into the Character States Chosen window; one or more character-states can be chosen in this way. You will now notice that some taxa - those with character-states that do not match your answer - will be moved from Taxa Remaining into Taxa *Discarded*.

As you answer more and more questions the list in Taxa Remaining will get shorter and shorter until, perhaps, only one remains.

Numeric characters

Click on the character name, which will ‘open’ the character to show the states, then double-click the hash (#) symbol to the right of the orange information button (or drag it into Character States Chosen) and a box will pop up into which you can type the measurement: you can enter either a single number or a numeric range (with the two numbers separated by a hyphen [-]). To view other syntax options click on the blue hyperlink at the bottom of the dialogue box.

Apart from plant height, which is measured in metres, all other numeric measurements for the *Lathyrus* data set are recorded in millimetres (but you do not have to type 'mm' into

the box when you record your measurements). It will increase the likelihood of retaining the correct answer in Taxa Remaining if you enter a range of values (e.g. 3-6).

Which characters should you use?

When you first start the key, all 90 characters will be listed in the *Characters Available* window. You can answer questions in any order you wish, so you should be able to make an identification of your specimen based on the characters that are available. Use of dichotomous keys often fails because of the need to assess character states for characters that it is not possible to score on your specimen, e.g. seed characters are difficult to score as they are seldom present with a specimen. However, you can also ask the key itself to help by suggesting what is the appropriate character to use next (see Best and Bingo below) or compare descriptive information of the remaining taxa to see if you can match your specimen that way (see Similarities and Differences below) or scroll through the illustrations or photographs of the taxa remaining and see if your specimen matches any of them (see Slide show below).

The key opens with the full set of characters that are available, but it is also possible to select a particular subset of characters, for example, if you only have vegetative material you may wish to use the vegetative characters alone and this may be achieved by selecting the vegetative character set. To select a particular character set click on characters, then click sets and check the small box to the left of the set name; you can load two or more sets simultaneously by checking more than one box. Now click anywhere outside the sets window and the characters contained in the set(s) you have selected will appear in the *Characters Available* window. The following sets of characters are available:

- *All* - This set contains the entire list of characters which are available for use. This is the default set and when starting a new identification it is generally good practice to load this set and run Best (see below).
- *Fast Find* – This character set comprises the characters that are generally easy to score and which have strong discriminating power.
- *Vegetative* - characters relating to the vegetative characteristics of the plant.
- *Inflorescence* - characters relating to the inflorescences.
- *Flower* - characters relating to flowers.
- *Fruit / legume* - characters relating to the fruit.
- *Seed* - characters specific to seeds (including the hilum and aril).

Using Best

If you have a potential choice over which character to score next and are unsure which to choose you can ask for assistance. You do this by invoking the *Best* or *Bingo* options. Click on the Best button located on the toolbar and all characters in Characters Available will be checked to find those that, on average, will give you the shortest list in Taxa Remaining if you choose one of their states. If you can, answer one of these next. When you use the Best option the programme will either sort the characters, placing those with the strongest discriminating power at the top of the list, or find (and highlight) the next best character to use; you can decide which of these options you require by clicking on Characters then Best Options located on the menu bar.

Using Bingo

The Bingo command also helps you to choose which character is appropriate to use next. Click on the Bingo button located on the toolbar and a window will appear showing

various characters and their states (these will vary depending upon what taxa are left in Taxa Remaining). If your specimens possess any of the character states which are displayed then you will be left with just a single taxon in Taxa Remaining if you double-click that state.

Using Similarities and Differences

Click on the Similarities and Differences button located on the toolbar and you will see a Similarities and Differences tab. Each tab is divided into two panels: the upper one listing the characters and the lower one showing the taxa listed in Taxa Remaining with their character-state scores. Click on a character in the upper panel and the lower panel will display the states scored for that character for each of the remaining taxa. You can then compare the features of your specimen with the character-states for each taxon. Further options available under Similarities and Differences can be accessed at any time via the Lucid Help menu.

Using Slide Show

When you have reduced the number of taxa in Taxa Remaining to a few you can scroll through illustrations of them to see if any match your specimen. To do this click on the Taxa button located on the menu bar, then click on Slide Show and then All Remaining Taxa. Drawings of the remaining taxa will then automatically scroll on-screen (with a 4-second delay between images). You can control the slide show with the buttons located at the upper right-hand corner of the screen.

Starting a new identification

If you wish to restart the key after having identification a specimen then click the Restart button on the menu bar and this will clear both the Character States Chosen and Taxa Discarded windows. When you click this button a small window will appear, and by opening the drop-down list you will see that there are three options available concerning character sets for your new identification session: select one of these options then click on Restart. The characters that then appear in Characters Available will depend upon your selection.

About Lucid

Lucid is an easy to use knowledge management tool that can be used in the production of interactive identification systems. Lucid was developed by the Centre for Biological Information Technology (CBIT) at the University of Queensland. The Lucid system consists of a number of inter-related products that assist with the creation and use of keys (in any language) for any group of organisms. The software has standard system requirements and is available to either download or purchase. You can learn more about Lucid and the software available from the Lucid website: http://www.lucidcentral.com/.

Appendix 6.2. List of species and number of specimens and accessions used in the analysis of the geographic distribution of genus *Lathyrus*.

Taxa	Observed specimens in different herbaria	No. of accessions at ICARDA's genebank	No of geo-referenced observation/accessions
L. amphicarpos	8		8
L. angulatus	76		76
L. annuus	228	74	302
L. aphaca	396	300	696
L. articulatus		78	78
L. armenus	5		5
L. aureus	36		36
L. basalticus	7	5	12
L. bauhinii	14		14
L. bijugus	1		1
L. blepharicarpus	48	47	95
L. boissieri	13		13
L. brachypterus	12		12
L. cassius	38	11	49
L. chloranthus	14	8	22
L. chrysanthus	8	7	15
L. cicera	157	205	362
L. cilicicus	3	5	8
L. ciliolatus	5	7	12
L. cirrhosus	10		10
L. clymenum	390	14	404
L. cyaneus	26	2	28
L. czeczottianus	18		18
L. digitatus	28	1	29
L. elongatus	9		9
L. ensifolius	10		10
L. filiformis	19		19
L. gloeospermus	4	4	8
L. gorgoni	67	68	135
L. grandiflorus	14		14

L. heterophyllus	14		14
L. hierosolymitanus	25	112	137
L. hirsutus	34	42	76
L. hirticarpus	4	1	5
L. humilis	4		4
L. inconspicuus	66	190	256
L. incurvus	15		15
L. japonicus	7		7
L. karsianus	4		4
L. komarovii	2		2
L. laevigatus	14		14
L. latifolius	13	1	14
L. laxiflorus	79		79
L. layardii	3		3
L. libani	4		4
L. linifolius	16		16
L. lycicus	4		4
L. marmoratus	8	24	32
L. membranaceus	3		3
L. mulkak	4		4
L. nervosus	1		1
L. neurolobus	2		2
L. niger	25		25
L. nissolia	26	12	38
L. nivalis	9		9
L. ochrus	168	112	280
L. odoratus		1	1
L. pallescens	12	1	13
L. palustris	6		6
L. pannonicus	29		29
L. phaselitanus	1		1
L. pisiformis	1		1
L. pratensis	63		63

L. pseudocicera	20	76	96
L. pyrenaicus	5		5
L. roseus	33		33
L. rotundifolius	33	1	34
L. satdaghensis	2		2
L. sativus		502	502
L. saxatilis	29		29
L. setifolius	31	8	39
L. spathulatus	29		29
L. sphaericus	40	27	67
L. stenolobus	4		4
L. stenophyllus	15	2	17
L. sylvestris	7		7
L. tauricola	2		2
L. tingitanus	11	7	18
L. trachycarpus	2		2
L. tuberosus	28	2	30
L. tukhtensis	7		7
L. undulatus	4		4
L. variabilis	14		14
L. venetus	18		18
L. vernus	17		17
L. vinealis	14	4	18
L. sp.		27	27
Total	2695	1988	4683

Appendix 6.3. Morphological character set

Abbrev.	Description	States
LF	Life form	1. annual; 2. biennial; 3. perennial
PLSTAT	Plant Status	1. sturdy; 2. slender to sturdy; 3. slender; 4. rigid
GH	Growth habit	1.erect; 2. ascending; 3. prostrate; 4. procombent
Veg. Pub.	Vegetative pubescence	1. glabrous; 2. glabrescent
HRTY	Type of hair	1. glaucous; 2. pilous; 3. villose; 4. no hairs
PL.HT	Plant height/cm.	In cm
STMSH	Stem shape	1. winged; 2. terete; 3. ridgid; 4. angled
LFTST	Leaflet status	1. present; 2. reduced
NLFT/LF	Number of Leaflets per leaf	Number
LFTARR	Leaflet arrangement	1. paripinnate; 2. subdigitate; 3. pinnate; 4. phyllodic; 5. sub-sessile; 6. reduced
LFRAC	Leaf rachis	1. laminate; 2. not laminate
RACEND	Rachis ends in	1. murco; 2. tendril; 3. aristate
LFTSH	Leaflet shape	1. linear; 2. elliptic; 3. oblong; 4. lanceolate; 5. obovate; 6. ovate; 7. sub-orbicular; 8. spatulate; 9. tendrillous
LFTAPSH	Leaflet apex shape	1. mucronate; 2. acute; 3. emarginate; 4. acuminate; 5. subobtuse; 6. obtuse; 7. undulate-margined; 8. aristate; 9. absent
LFTLN	Leaflet length/mm.	mm
LFTWD	Leaflet width/mm.	mm
LFTVN	Leaflet venation	1. pinnate; 2. parallel; 3. reticulate; 4. not applicable
LFTHR	Leaflet hairiness	1. glabrous; 2. glabrescent; 3. pubescent; 4. gland dotted on lower face; 5. not applicable
STPSH	Stipule shape	1. subulate; 2. lanceolate; 3. ovate; 4. oblong; 5. suborbicular; 6. triangular; 7. filiform
STPBS	Stipule base shape	1. hastate; 2. semi-hastate; 3. sagittate; 4. semi-sagittate; 5. variable
STPMRG	Stipule margin	1. entire; 2. dentate; 3. incised; 4. variable
STPPUB	Stipule pubesent	1 glabrous; 2 pubescent
STPLN	Stipule length mm.	mm
STPWD	Stipule width mm.	mm
STPLNPL	Stipule length/petiol length	1. shorter than petiol length; 2. equal to petiol length; 3. longer than petiol length; 4. sub-eqaul to petiol length; 5. not applicable
PEDLLN	Peduncle length/mm.	mm
PDNLNLFL	Peduncle length/leaf length	1. shorter than leaf length; 2. equal to leaf length; 3. longer than leaf length; 4. not applicable
PDCLN	Pedicle length mm.	mm

FLNO	Flowers number	mm
PETCLR	Flower petal colour	1. concolorous; 2. not concolorous
CORCLR	Corolla colour	1. white; 2 .cream; 3. yellow; 4. orange; 5. pink; 6. brick-red; 7. blue; 8. violet; 9. purple
FLLN	Flower length/mm.	mm
STDLN	Standard length/mm.	mm
STDVNN	Standard vein number	1. absent; 2. 3-5 veins; 3. more than 5 veins
STDAPSH	Standard apex shape	1. strongly emarginated; 2. emarginated; 3. emarginated with mucro; 4. obtuse
WNGCLR	Wing colour	1. white; 2 .cream; 3. yellow; 4. orange; 5. pink; 6. brick-red; 7. blue; 8. violet; 9. purple
WNGLN	Wing length/mm.	mm
WNGLMBLN	Wing limb length/mm.	mm
WNGLMBWD	Wing limb width/mm.	mm
WNGCLLN	Wing claw length/mm.	mm
KEELLN	Keel length/mm.	mm
CLXLN	Calyx length/mm.	mm
CLXTHLN	Calyx teeth length/mm.	mm
CLXBLN	Calyx base length/mm.	mm
CLXBSH	Calyx base shape	1. gibbous; 2. not gibbous
CLXTH	Calyx teeth	1. equal; 2. unequal
CLXTHOR	Calyx teeth orientation	1. straight; 2. reflexed
CLXHR	Calyx hairs	1. glabrous; 2. glabrescent; 3. pubescent
CLTHLNTBLN	Calyx teeth length/tube length	1. shorter than tube; 2. equal the tube length; 3. longer than tube
CLXLWTHTBLN	Calyx lowest teeth length/ tube length	1. lowest tooth shorter than tube; 2. lowest tooth equal to tube; 3. lowest tooth longer than tube
STYLN	Style length/mm.	mm
STYCON	Style contortion	1. straight; 2. twisted
STYSH	Style shape	1. linear; 2. oblong; 3. spathulate; 4. canaliculate; 5. arcuate
OVRSH	Ovary shape	1. linear; 2. intermediate; 3. oblong
OVRLN	Ovary length/mm.	mm
OVRWD	Ovary width/mm.	mm
LEGOR	Legume orientation	1. straight; 2. beaked; 3. incurved
LEGSH	Legume shape	1. linear; 2. oblong; 3. canescent
LEGLN	Legume length/mm.	mm
LEGWD	Legume width/mm.	mm

LEGHR	Legume hairiness	1. glabrous; 2. glabrescent; 3. pubescent; 4. tomentose
LEGDEH	Legume at matuirity	1. dehiscent; 2. indehiscent
LEGVLV	Legume valve	1. hairy; 2. not hairy
LEGVLVPTR	Legume valve pattern	1. reticulate-nerved; 2. obscurely-nerved; 3. gland-dotted; 4. tuberculate; 5. longitudinally-nerved; 6. glandular-verrucose; 7. obliquely-nerved; 8. tuberculate-pilose; 9. eglandular; 10. glabrous
UPLEGSUT	Upper legume suture	1. broadly winged; 2. narrowly-winged; 3. not 2-winged
SUTTYP	Suture type	1. keeled; 2. canaculate
SDSURF	Seed surface	1. smooth; 2. tuberculate; 3. reticulate; 4. coarsely-tuberculate; 5. ruminate-rugulose; 6. punctate; 7. verrucose; 8. viscose; 9. pappilose
SDNOPD	Seed number/pod	number
HILLN	Hilum length/mm.	mm
SDDIA	Seed diameter/mm.	
LNSHIL	Relation of lens to hilum	
SDCLR	Seed colour	1. white; 2. yellow; 3. grey; 4. brown; 5. purplish-brown; 6. purple; 7. blackish; 8. dark brown; 9. dark- green
SDSH	Seed shape	1. compressed; 2. round; 3. angular; 4. oval; 5. cubical; 6. globose
LWPDSUT	Lower Suture of pod	1. ciliate; 2. not ciliate
AMPH	Amphicarpic pod	1. yes; 2. no

Appendix 6.4. *CHARACTER LIST

1. Section/
1. Aphaca/
2. Clymenum/
3. Lathyrostylis/
4. Lathyrus/
5. Linearicarpus/
6. Neurolobus/
7. Nissolia/
8. Notolathyrus/
9. Orobastrum/
10. Orobon/
11. Orobus/
12. Pratensis/
13. Viciopsis/

2. Life form/
1. annual/
2. biennial/
3. perennial/

3. Plant Stature/
1. sturdy/
2. slender to sturdy/
3. slender/
4. rigid/

4. Growth habit/
1. erect/
2. ascending/
3. decumbent/
4. prostrate/
5. procombent/

5. Veg. pubescense/
1. glabrous/
2. glabrescent/

6. Vegetative parts/
1. glabrous/
2. glabrescent/
3. pubescent/
4. glaucous/
5. pilous/
6. sub-glabrous/
7. villose/

7. Plant height/
cm. hight/

8. Stem shape/
1. winged/
2. terete/
3. ridged/
4. angled/

9. Leaflet status/
1. present/
2. reduced/

10. Number of Leaflets (Pairs)/
leaflets per leaf/

11. Leaflet arrangement/
1. paripinnate/
2. subdigitate/
3. pinnate/
4. phyllodic/
5. sub-sessile/
6. alternate/
7. reduced/

12. Leaf rachis/
1. laminate/
2. not laminate/

13. Rachis ends in/
1. murco/
2. tendril/
3. aristate/

14. Leaflet shape/
1. linear/
2. linear-elliptic/
3. linear-oblong/
4. elliptic/
5. oblong-elliptic/
6. oblong/
7. lanceolate/
8. lanceolate-linear/
9. obovate/
10. ovate/
11. elliptic-lanceolate/
12. oblong-lanceolate/
13. elliptic-orbicular/
14. sub-orbicular/
15. tendrillous/

15. Leaflet apex shape/
1. mucronate/
2. acute/
3. emarginate/
4. acuminate/
5. subobtuse/
6. obtuse/
7. undulate-margined/
8. aristate/
9. absent/

16. Leaflet length/
mm.long/

17. Leaflet width/
mm.width/

18. Leaflet venation/
1. pinnate/
2. parallel/
3. reticulate/

19. Leaflet hairiness/
1. glabrous/
2. glabrescent/

20. Stipule shape/
1. subulate/
2. lanceolate-subulate/
3. lanceolate/
4. ovate/
5. lanceolate-ovate/
6. ovate-oblong/
7. oblong/
8. suborbicular/
9. triangular/
10. ovate-accuminate/
11. lanceolate-accuminate/
12. minute/
13. filiform/
14. elliptic/
15. linear/

21. Stipule base/
1. hastate/
2. semi-hastate/
3. sagittate/
4. semi-sagittate/

22. Stipule margin/
1. entire/
2. dentate/
3. incised/
4. sub-dentate/
5. toothed/

23. Stipule pubescence/
1. glabrous/
2. pubescent/

24. Stipule length/
mm. length/

25. Stipule width/
1. 1 mm/
2. 0.5-1.5 mm/
3. 1-1.5 mm/
4. 1-3 mm/
5. 0.5-5 mm/
6. broader than the leaflet/

7. 1-2 X as broad as stem/
8. 1-1.5 X as broad as stem/
9. 2-3X as broad as stem/
10. 3-4 X as broad as stem/
11. as broad as stem/
12. broader than stem/
13. as braod as the leaflet/
14. somewhat narrower than leaflet/
15. less than 1/2 as wide as stem/

26. stipule length compare to petiol length/
1. shorter than petiol length/
2. equal to petiol length/
3. longer than petiol length/

27. Peduncle length/
mm. length/

28. Peduncle length compare to leaf length/
1. equal to leafe length/
2. shorter than leaf length/
3. longer than leaf length/
4. sub-eqaul to leaf length/

29. Pedicle length/
mm. length/

30. Flowers number/
per inflorescence/

31. Flower petal colour/
1. concolorous/
2. not concolotous/

32. Corolla colour/
1. white/
2. cream/
3. pale sulphur/
4. yellow/
5. gingery-orange/
6. orange/
7. brick-red/
8. violet/
9. pale-lavender/
10. lilac-blue/
11. blue/
12. purplish-pink/
13. pink/
14. purple/
15. lilac/

33. Flower length/
mm. length/

34. Standard veins number/
1. absent/
2. 3-5 veins/
3. more than 5 veins/

35. Standard apex shape/
1. strongly emarginated/
2. emarginated/
3. emarginated with mucro/
4. obtuse/

36. Wing colour/
1. white/
2. cream/
3. yellow/
4. orange/
5. pink/
6. brick-red/
7. blue/
8. violet/
9. purple/

37. Calyx length/
mm. length/

38. Calyx base length/
mm. length/

39. Calyx base shape/
1. gibbous/
2. not gibbous/

40. Calyx teeth/
1. equal/
2. unequal/

41. Calyx teeth orientation/
1. straight/
2. reflexed/

42. Calyx hairs/
1. glabrous/
2. glabrescent/
3. pubescent/

43. Calyx teeth length ratio to tube length/
1. shorter than tube/
2. equal the tube length/
3. longer than tube/

44. Calyx lower teeth length ratio to tube length/
1. shorter than tube/
2. equal to tube/
3. longer than tube/

45. Style length/
mm./

46. Style contortion/
1. straight/
2. twisted/

47. Style shape/
1. linear/
2. oblong/
3. dialated at apex/
4. linear-spathulate/
5. spathulate/
6. obovate-spathulate/
7. canaliculate/
8. arcute/

48. Ovary shape/
1. linear/
2. oblong/
3. canescent/

49. Legume orientation/
1. straight/
2. beaked/
3. incurved/

50. Legume shape/
1. linear/
2. broadly-linear/
3. linear-sublanceolate/
4. linear-ensiform/
5. oblong-linear/
6. oblong/
7. broadly-oblong/
8. broadly elliptic-oblong/
9. narrowly oblong/
10. elliptic-oblong/
11. canescent/
12. obovate/

51. Legume length/
mm. long/

52. Legume width/
mm. width/

53. Legume hairiness/
1. glabrous/

2. tomentose/
3. pilose/
4. densely-pilose/
5. ciliate/
6. pubescent/

54. Lower suture hairiness/
1. ciliated/
2. not ciliated/

55. Legume at matuirity/
1. dehiscent/
2. indehiscent/

56. Amphicary/
1. amphicarpic pods produced/
2. no amphicarpic pods/

57. Legume valve/
1. hairy/
2. not hairy/

58. Legume valve pattern/
1. reticulate-nerved/
2. obscurely-nerved/
3. gland-dotted/
4. tuberculate/
5. longitudinally-nerved/
6. glandular-verrucose/
7. obliquely-nerved/
8. tuberculate-pilose/
9. eglandular/
10. glabrous/

59. Upper legume suture/
1. broadly winged/
2. narrowly-winged/
3. narrow/
4. keeled/
5. canaliculate/

60. Seed surface/
1. smooth/
2. tuberculate/
3. reticulate/
4. reticulate-rigulose/
5. papillose/
6. coarsely-tuberculate/
7. ruminate-rugulose/
8. punctate/
9. verrucose/
10. viscose/
11. reticulate-rugose/

61. Seed number/ per pod/

62. Hilum length/ mm. long/

63. Seed diameter/ mm. diameter/

64. Seed color/
1. white/
2. brown/
3. dark brown/
4. grey/
5. purplish-brown/
6. blackish/
7. purple/
8. blakish-brown/
9. dark green/

65. Seed shape/
1. compressed/
2. round/
3. sub-globose/
4. globose/
5. subquadrate/
6. angular/
7. sphaerical/
8. roundish/
9. oval/
10. ovoid/
11. cubical/
12. elipsoides/

Appendix 6.5. *ITEM DESCRIPTIONS

#L. armenus Boiss. & Hute) Sirj./
1,3 2,3 3,3 4,1 5,1<rarely> 6,1 7,30-50 8,2 9,1 10,2 11,2 12,2 13,1 14,1/8 16,50-80 17,3-6 18,2 19,1 20,1/4 22,1 25,2 26,1 28,2 30,2-11 31,1 32,8 33,14-16 34,1 36,8 40,2 41,1 43,1 45,4 47,1 48,1 50,1 51,50 52,4 53,1 57,2 59,3

#L. aureus (Stev.) Brandza/
1,11 2,3 3,1 4,1 5,2 6,3 7,50-80 8,2 9,1 10,3-5 11,3 12,2 13,3 14,10 15,2/4 16,50-100 17,18-50 18,1 19,1 20,3/4 21,4 22,1 23,1 24,(10-)20-25(-28) 25,12 26,3 27,(80-)100-140(-150) 28,1/2 29,(4-)5-7(-8) 30,(8-)12-25 31,1 32,5 33,16-20(-22) 34,1 36,4 39,1 40,2 41,1 42,2/3 43,1 45,4-5 46,1 47,1 48,1 49,1 50,1 51,50-70 52,7-8 53,1 55,2 57,2 58,3 59,3 60,1 61,6-12 62,2-2.5 63,(3-)4(-5) 64,6 65,1

#L. bauhimi Genty/
1,3 2,3 3,3 4,2 5,1<rarely> 6,3 7,15-50 8,1 9,1 10,2-4 11,2 12,2 13,3 14,1/8 15,4 16,30-60 17,2-6 18,2 19,1 20,15 21,3 22,1 23,1 24,9-12 25,2 26,3 27,35-55 28,3 29,3.5-6 30,4-10 31,1 32,12/14 33,20-27 34,1 36,9 39,2 40,2 41,1 42,1 43,1 45,3-4 46,1 47,1/4 48,1 49,1 50,1 51,45-70 52,4-6 53,1 55,2 57,2 58,1 59,3 60,1 61,9-11 64,2 65,1

#L. brachypterus Cel./
1,3 2,3 3,2 4,1 5,1<rarely> 6,1/3 7,20-40 8,2 9,1 10,2-3 11,1/2 12,2 13,1 14,1/3 15,2 16,25-55 17,(1-)2-7 18,2 19,1 20,2 21,4 22,1 23,1 24,(2.5-)3-4 25,11 26,3 28,3 30,2-10 31,1 32,2/3 33,(15-)18-25 34,1 36,2 39,1 40,2 41,1 42,1 43,1 45,(6.5-)7-10 46,1 47,1 48,1 50,1 51,25-33 52,2-4 53,1 57,2 58,1 59,3 61,10-20

#L. boissieri Sirj./
1,3 2,3 3,1 4,1 5,1<rarely> 6,1 7,50-75 8,4 9,1 10,1/2 11,2 12,2 13,1 14,2 15,2 16,50-120 17,5-22 18,2 19,1 20,3 21,4 22,1 23,1 24,(1-)2-4(-5) 25,4 26,3 28,2/3 30,7-15 31,1 32,8/13/15 33,14-17 34,1 36,5/8 39,1 40,2 41,1 42,2 43,1/2 45,4-5 46,1 47,4 48,1 50,1 51,80 52,8 53,1 55,1 57,2 59,3 60,1 61,7-9 63,4.5-7 64,2 65,1/9

#L. cilicicus Hayek & Siehe/
1,3 2,3 3,4 4,1 5,1<rarely> 6,1 7,70-120 8,2 9,1 10,2 11,2 12,2 13,1/3 14,1/8 15,2 16,80-150 17,3-9 18,2 19,1 20,2/3/15 21,4 22,1 23,1 25,2 26,3 27,250-280 28,3 29,50-250(-280) 30,5-13 31,1 32,14 33,25-30 34,1 35,2 36,9 39,2 40,1 41,1 42,1 43,1/2 44,2 45,7 46,1 47,5/6 57,2

#L. cyaneus (Stev.) Koch/
1,3 2,3 3,3 4,1/2 5,1<rarely> 6,1/6 7,15-30 8,2 9,1 10,(1-)2 11,2/3 12,2 13,1 14,1/8 15,2 16,25-60 17,2-6 18,2 19,2 20,2/3 21,4 22,1 23,1 24,2-9(-12) 25,11 26,2/3 28,1/3 30,(1-)2-6 31,2 32,8/10 33,15-29 34,1 35,2 36,7 40,1/2 41,1 42,1 43,2 44,1 45,4.5-5 46,2 47,4/5 48,1 49,1 50,1 51,35-50(-60) 52,5-6 53,1 55,1 57,2 59,3

#L. czeczottianus Bassler/
1,12 2,3 3,1 4,1/2 5,1<rarely> 6,3 7,(10-)25-45 8,2 9,1 10,1 11,3 12,2 13,1 14,7 15,2 16,15-47 17,3-12 18,2 19,1 20,11 21,3 22,1 23,2 25,14 28,3 30,3-7 31,1 32,9/11 33,17-19 34,1 36,7 39,2 40,2 41,1 42,3 43,3 45,(3.9-)4(-4.1) 46,2 47,1 48,1 50,2 51,35 52,5 53,4 57,2 58,3/6 59,3 60,5/6

#L. digitatus (Bieb.) Fiori/
1,3 2,3 3,3 4,1/2 5,2 6,1/2/6 7,(10-)15-40 8,2 9,1 10,(1-)2 11,2/5 12,2 13,1 14,1 15,2 16,(15-)20-70(-80) 17,1-3(-8) 18,2 19,1 20,3 21,4 22,1 23,1 24,6-8 25,2 26,3 27,(25-)40-70(-75) 28,1/3 29,(5-)6-7(-8) 30,3-6(-10) 31,1 32,11/14 33,14-20(-30) 34,1 35,2 36,7/9 39,1 40,1/2 41,1 42,1 43,1 44,1 45,3-4.5 46,2 47,4/5 48,1 49,1 50,3 51,35-55(-70) 52,4.5-6(-9) 53,1 55,2 57,1 59,3 60,1

#L. elongatus (Bornm.) Sirj./
1,3 2,3 3,3 4,1 5,1<rarely> 6,1 7,20-40 8,2 9,1 10,1 11,2 12,2 14,1 16,70-135 17,1-7 18,2 19,1 20,2 22,1 26,3 28,3 30,2-7 31,1 32,11/14 33,13-20 34,1 36,7/9 40,2 41,1 43,2/3 45,4-4.5 46,1 47,5 48,1 50,1 51,45 52,6 57,2 59,3

#L. filiformis (Lam.) Gay/
1,3 2,3 3,3 4,2 5,1<rarely> 6,3 7,15-50 8,1 9,1 10,2-4 11,2 12,2 13,3 14,1/8 15,4 16,30-60 17,2-6 18,2 19,1 20,15 21,3 22,1 23,1 24,9-12 25,2 26,3 27,35-55 28,3 29,3.5-6 30,4-10 31,1 32,12/14 33,14-22 34,1 36,7/9 39,2 40,2 41,1 42,1 43,1 45,3-4 46,1 47,3 48,1 49,1 50,1 51,45-70 52,4-6 53,1 55,2 57,2 59,3 60,1 61,9-11

#L. incurvus (Roth.) Willd./
1,11 2,3 3,2 4,3 5,1<rarely> 6,2/3 7,30-100 8,1/2 9,1 10,3-5 11,3 12,2 13,2 14,5/12 15,6 16,(15-)20-60 17,(7-)8-22 18,2 19,1 20,3/15 21,4 22,1 23,1 24,(5-)8-15(-25) 25,7 26,3 27,20-60 28,1 29,4-6 30,3-9(-12) 31,1 32,10/11/14 33,10-14(-15) 34,1 36,7/9 39,1 40,2 41,1 42,1 43,2 45,3-4 46,1 47,1 48,1 49,3 50,1 51,25-35 52,5-6 53,1 55,2 57,2 58,1/10 59,3 60,1 61,6-8(-11) 65,4

#L. japponicus Willd./
1,11 2,3 3,3 4,1/2/3 5,1<rarely> 6,2 7,30-90 8,2 9,1 10,2-6 11,3 12,2 13,2 14,4/9/10 15,5/6 16,(14-)17-40 17,(6-)8-33 19,1 20,9 21,2/3 22,1 24,10-25 25,14 26,1 27,(20-)25-50(-55) 29,3-5(-6) 30,5-15 31,1 32,8/11 33,14-22 34,1 36,7/8 39,1 40,2 41,1 43,2 45,(5-)6-7(-9) 46,1 48,1<rarely> 50,2<rarely> 51,30-50 52,(5-)6-8(-10) 53,1 57,2 59,3 61,4-8 62,(2-)2.5(-3) 63,(4-)5(-6) 64,6 65,1

#L. karsianus P. H. Davis/
1,3 2,3 3,3/4 4,1 5,1<rarely> 6,1 7,35-60 8,2 9,1 10,1 11,1 12,2 13,1 14,1/8 15,2 16,30-60 17,2.5-5 18,2 19,1 20,2 21,4 22,1 23,1 24,2-7(-10) 25,11 26,1/3 27,30-70 28,3 29,2-3 30,5-9 31,1 32,11 33,17-22(-25) 34,1 36,7 39,1 40,2 41,1 42,1 43,1 45,4-5 46,1 47,1 48,1 49,1 50,1 51,40-60 52,4-5 53,1 55,1 57,2 58,10 59,3 60,9 61,(7-)8-14(-15) 62,(1.5-)2-2.5 63,(2-)3 64,2 65,9

#L. laevigatus (Waldst. & Kit.)Gren./
1,11 2,3 3,1 4,1 5,1<rarely> 6,1/3 7,20-60 8,3 9,1 10,2-6 11,3 12,2 13,1 14,2/4/6/10/12 15,6 16,20-100 17,5-45 18,1/3 19,1 20,3/4/8 21,4 22,1 23,1 24,5-30 25,10 26,1 27,75-180(-235) 28,3 29,3-8 30,2-20 31,1 32,4/6 33,15-25(-29) 34,1 36,3/4 39,1 40,2 41,1 42,3 43,1 45,(5-)7-9.5 46,1 47,1 48,1/3 49,3 50,1/11 51,(45-)60-80(-100) 52,6.5-9.5 53,6 55,2 57,2 58,1 59,3 60,U 61,5-14 62,2.5-4.5 63,(4-)4.5(-6.5) 65,12

#L. laxiflorus (Desf.). O. Kuntze/
1,12 2,3 3,1 4,2/3/5 5,2 6,1/2 7,15-40 8,2/4 9,1 10,1 11,3 12,2 13,2 14,4 15,8 16,10-40 17,4-18 18,2 19,1 20,4/5/10 21,2 22,1 23,1 25,6/13 26,3 28,3 30,(2-)3-6 32,9/15 33,15-20 34,1 36,7 39,1 40,1 41,1 42,1 43,3 44,3 45,4-5 46,1 47,2 48,1 49,1 50,2 51,30-45 52,4-5 53,1/2 57,2 58,3 59,3 60,1 61,(9-)10(-11)

#L. laxiflorus (Desf.). O. Kuntze subsp. angustifolius (Post ex Dinsm) Davis/
1,12 2,3 3,1 4,2/3/5 5,2 6,1/2 7,15-40 8,2/4 9,1 10,1 11,3 12,2 13,2/3 14,7 15,8 16,10-40 17,4-18 18,2 19,1 20,4/5/10 21,2 22,1 23,1 25,6/13 26,3 28,3 30,(2-)3-6 31,1 32,9/15 33,15-20 34,1 36,7 39,1 40,1 41,1 42,1 43,3 44,3 45,4-5 46,1 47,2 48,1 49,1 50,2 51,30-45 52,4-5 53,1/2 57,2 59,3 60,1 61,(9-)10(-11)

#L. laxiflorus (Desf.). O. Kuntze subsp. laxiflorus (Desf.) O. Kuntze/
1,12 2,3 3,1 4,2/3/5 5,2 6,1/2 7,15-40 8,2/4 9,1 10,1 11,3 12,2 13,3 14,4/10 15,8 16,10-40 17,4-18 18,2 19,1 20,4/5/10 21,2 22,1 23,1 25,6/13 26,3 28,3 30,(2-)3-6 31,2 32,9/15 33,15-20 34,1 35,2 36,7 39,1 40,1 41,1 42,1 43,3 44,3 45,4-5 46,1 47,2 48,1 49,1 50,2 51,30-45 52,4-5 53,2 55,2 57,2 59,3 60,1 61,(9-)10(-11)

#L. layardii J. Ball ex Boiss./
1,12 2,3 3,1 4,1/2 5,1<rarely> 6,2/7 7,45-60 8,2/4 9,1 10,1 11,3 12,2 13,2 14,11 16,20-55 17,3-10 18,2 19,1 20,5 21,3 22,1 28,3 30,5-10 31,1 32,9/11 33,19-22 34,1 36,7 40,2 41,1 43,3 48,2 50,5 51,25 52,4 53,3 57,2

#L. libani Fritsch/
1,11 2,3 3,1 4,1 5,2 6,2/3 7,50-80 8,2 9,1 10,3-5 11,1 12,2 13,1/3 14,10 15,2/4 16,50-100 17,18-50 18,1 19,1 20,3/4 21,4 22,1 23,1 25,12 28,1/2 30,(8-)12-25 31,1 32,1 33,23-30 34,1 36,1 37,8-12 39,1 40,2 41,2 42,1 43,1 45,4-5 46,1 47,1 48,1 50,1 51,70-80 52,7-8 53,1 57,2 58,3 59,3 60,1 61,6-12 64,6 65,1

#L. linifolius (Reichard)Bassler/
1,11 2,3 3,2 4,2 5,1<rarely> 6,1/6 7,15-50 8,1 9,1 10,(1-)2(-4) 11,3 12,2 13,3 14,1/4 15,2 16,10-50(-100) 17,1-12(-16) 18,2 19,1 20,3/15 21,4 22,1 23,1 24,5-25 25,10/12/14 26,3 27,(1-)2-5 28,3 29,(2-)3(-4) 30,2-6 31,1

32,2/11 33,10-16 34,1 36,2/7 39,1 40,2 41,1 42,1 43,1 45,(3-)4(-5) 46,2 47,1 48,1 49,2 50,1 51,25-45 52,4-5 53,1 55,2 57,2 59,3 60,1 61,4-10 62,.2-.25 63,2-3 64,2 65,5

#L. niger (L.) Bernh./
1,11 2,3 3,3 4,2 5,1<rarely> 6,2 7,40-75 8,2 9,1 10,3-5 11,1 12,2 13,1 14,4 15,5 16,10-30 17,4-13 18,1 19,1 20,3 21,4 22,1 27,30-50 28,3 30,3-8 31,1 32,11/14 33,10-14 34,1 36,7/9 40,2 41,1 43,1 48,1 50,1 51,40-50 52,5 53,1 57,2 58,10 59,3 60,1 61,6-10

#L. nivalis Hand.-Mazz./
1,3 2,3 3,1 4,2 5,1<rarely> 6,2 7,15-25(-30) 8,2 9,1 10,2-4(-5) 11,3 12,2 13,1 14,1/2 15,2 16,15-36(-40) 17,2-5 18,2 19,1 20,1/3 21,4 22,1 23,2 24,(2-)6-7(-9) 25,11 26,3 28,3 29,(2.5-)3 30,2-4 31,1 32,8/15 33,20-24 34,1 36,7/8 39,1 40,2 41,1 42,1 43,1 45,5 46,1 47,4 48,2 49,2 50,5 51,30-35 52,6-7 53,1 55,2 57,2 58,1 59,3 60,3/5 61,4-6 62,1-1.5 63,(2.5-)3-3.5 64,2 65,5

#L. pallescens (Bieb.)/
1,3 2,1/3 3,2 4,1 5,1 6,3 7,20-40 8,2 9,1 10,2-3 11,3 12,2 13,3 14,1 15,2 16,22-55(-70) 17,1.5-5 18,2 19,1 20,1/2 21,4 22,1 23,1 24,3-5 25,1 26,3 28,3 29,2-3(-4) 30,(2-)4-7 31,1 32,2/3 33,20-24 34,1 35,2 36,2/3 39,1 40,1/2 41,1 42,2/3 43,1 44,1 45,4-5 46,2 47,5 48,1 49,1 50,1 51,45-60 52,4 53,1 55,1 57,2 58,1 59,3 60,1 61,8-15

#L. palustris L./
1,11 2,3 3,3 4,1 5,1<rarely> 6,3 7,(40-)60-100(-120) 8,1 9,1 10,3-5 11,3 12,2 13,2 14,1/5/6/7 15,2 16,20-60(-80) 17,3.5-12(-16) 18,2 19,1 20,3/4/5/11 21,4 22,1/4 23,1 24,10-20 25,11 26,2/3 28,2 29,2-3 30,(2-)3-7(-8) 31,1 32,14 33,12-15(-20) 34,1 36,9 39,1 40,2 41,1 42,1 43,1/2 45,4-5 46,1 47,1 48,1 49,1 50,1 51,(25-)30-40(-60) 52,(5-)6-7(-9) 53,1 55,2 56,2 57,2 58,10 59,3 60,1 61,(3-)6-12(-20) 64,8

#L. pisiformis L./
1,11 2,3 3,3 4,2 5,1<rarely> 6,1 7,50-100 8,1 9,1 10,3-5 11,3 12,2 13,2 14,4/10 15,6 16,25-60 17,(7-)10-30 18,1/2 19,1 20,4/14 21,4 22,1 23,1 24,20-50 25,6/14 26,1 27,(35-)50-110(-125) 28,1/2 29,2-3 30,8-15(-20) 31,1 32,12/14 33,10-15(-20) 34,1 36,9 39,1 40,2 41,1 42,1 43,1 45,4-5 46,1 47,1 48,1 49,2 50,1 51,40-50 52,4-5 53,1 55,2 56,2 57,2 59,3 60,1 61,10-20 62,.125-.166

#L. pratensis L./
1,12 2,3 3,2 4,3 5,1<rarely> 6,2/3 7,20-50 8,4 9,1 10,1 11,3 12,1 13,2 14,4/8/11 15,2 16,10-40 17,1.5-11 18,2 19,1 20,5 21,3 22,1 23,1 25,6 26,3 30,3-10 31,1 32,4 33,10-16 34,1 36,3 39,2 40,2 41,1 42,1 43,2 45,3-4 46,1 47,2 48,2 50,5 51,20-70 52,5-6 53,1/2 56,2 57,2 59,3 60,1 61,4-8(-10) 63,2-3 64,2 65,3/10

#L. roseus Stev. Mem. Soc. Nat. Mosc. 4:52 (1813)
1,10 2,3 3,2 4,2 5,1<rarely> 6,1 7,40-60 8,2 9,1 10,1 11,3 12,2 13,1 14,4/9/13 15,5 16,15-45 17,10-30 18,1/3 19,1 20,3 21,4 22,1 23,1 24,3-7 25,11 30,1-4 31,1 32,13 33,12-19 34,1 36,5 39,1 40,2 41,1 42,1 43,1 45,3-4 46,2 47,4 48,1 50,2/3 51,35-45 52,6-8 53,1 55,1 56,2 57,2 58,1 59,3 60,1 61,5-10

#L. rotundifolius Willd./
1,4 2,3 3,1 4,3 5,1 6,1 7,100-250 8,1 9,1 10,1(-2) 11,3 12,2 13,2 14,4/14 15,6 16,25-65 17,10-45 18,2 19,1 20,3/5 21,4 22,1 23,1 25,7 26,2 28,3 30,3-13 31,1 32,13 33,18-25 34,3 35,1 36,5 39,2 40,2 41,1 42,1 43,1 44,1 45,6-7 46,2 47,8 48,1 50,1 51,50-70 52,7-10 53,1 55,2 56,2 57,1 59,4 60,4 61,6-10 63,4 64,2 65,1/8

#L. satdaghensis P. H. Davis/
1,3 2,3 3,3/4 4,1 5,1<rarely> 6,3 7,40-60 8,2 9,1 10,4-8 11,3 12,2 13,1 14,1/8 15,2 16,30-60 17,2.5-5 18,2 19,1 20,2 21,3 22,1 23,1 25,15 26,3 27,30-70 28,3 29,6-7 30,5-9 31,1 32,11 33,17-22(-25) 34,1 36,7 39,1 40,2 41,1 42,1 43,1 45,6-7 46,1 47,1 48,1/3 49,2 50,1/11 51,40-60 52,4-5 53,1 56,2 57,2 59,3

#L. spathulatus Cel./
1,3 2,3 3,3 4,1 5,1<rarely> 6,1 7,20-40 8,2 9,1 10,2 11,2 12,2 13,1 14,1 15,2 16,35-90 17,1-7 18,2 19,1 20,2 21,3 22,1 23,1 25,1 26,3 28,3 30,2-7 31,1 32,11/14 33,13-20 34,1 36,7/9 39,1 40,2 41,1 42,1 43,1/2 45,5-6 46,1 47,5 48,1 50,1 51,45 52,6 53,1 56,2 57,2 59,3 61,(9-)10(-11)

#L. sylvestris L./
1,4 2,3 3,3 4,3 5,1 6,1 7,60-200 8,1 9,1 10,1 11,3 12,1 13,2 14,1/7/12 15,2 16,40-150 17,5-20 18,2 19,1 20,1/2 21,4 22,1 23,1 25,15 26,1 28,1/2/3 30,3-12 31,1 32,12 33,13-20 34,3 35,1 36,7 39,2 40,2 41,1 42,1 43,1 44,1 45,4-5 46,2 47,1/8 48,1 50,1 51,40-80 52,8-10 53,1 55,2 56,2 57,2 59,3 60,4 61,(6-)10-15

#L. tingitanus L./
1,4 2,1 3,2 4,1 5,1 6,4 7,50-100(-180) 8,1 10,1 11,3 12,1 13,2 14,4 15,4 16,40-80 17,15-23 18,2 19,1 20,3 21,4 22,1 23,1 24,12-20(-25) 26,2 27,28-160 28,3 29,6-11 30,1-3(-4) 31,1 32,14 33,20-35 34,1 35,1/4 36,9 37,(6-)7.5-9.5(-11) 38,(4-)4.5(-6) 39,2 40,1 41,1 42,1 43,1 44,1/2/3 45,(4.5-)6-8 46,2 47,5 48,2 49,1 50,6 51,(65-)70-110 52,(7-)8-11 53,1 54,2 55,1 56,2 57,2 58,3 59,2 60,1 61,6-10 62,(7-)8-11 63,3-6 64,2 65,4

#L. tuberosus L./
1,4 2,3 3,2 4,3 5,1 6,1 7,30-80 8,2 9,1 10,1 11,3 12,2 13,2 14,4 15,6 16,10-52 17,3-25 18,2 19,1 20,3 21,4 22,1 23,1 24,6-22 25,4 26,3 28,3 30,3-9 31,1 32,13 33,11-15 34,3 35,1 36,5 39,1 40,2 41,1 42,2 43,2 44,2 45,6-8 46,2 47,2/8 48,2 49,2 50,5 51,20-40 52,4-7 53,1 55,2 56,2 57,2 58,1 59,3 60,2 61,3-6

#L. tukhtensis Czecz./
1,3 2,3 3,3 4,1 5,1<rarely> 6,1/2 7,15-30 8,2 9,1 10,1/2 11,2 12,2 14,1/3 16,35-65 17,2-9 18,2 19,1 20,1/3 22,1 26,2/3 28,3 30,3-12 31,1 32,11 33,14-17 34,1 36,7 40,2 41,1 43,1 45,4-5 46,1 47,5 48,1 50,1 51,50-60 52,5-6 56,2 57,2 59,3

#L. undulatus Boiss./
1,4 2,3 3,3 4,3 5,1<rarely> 6,1 7,100-250 8,1 9,1 10,1 11,3 12,2 13,2 14,4/14 15,7 16,25-65 17,10-45 18,2 19,1 20,5 21,4 22,1 23,1 25,7 26,3 30,3-13 31,1 32,13 33,18-25 34,1 36,5 39,1 40,2 41,1 42,1 43,1 45,5-7 46,2 47,1 48,1 50,1 51,50-70 52,7-10 53,1 56,2 57,1 59,2 60,4 61,6-10

#L. variabilis (Boiss. & Ky.) Maly/
1,3 2,3 3,2 4,2 5,1<rarely> 6,1 7,15-35 8,2 9,1 10,2 11,2 12,2 13,1 14,4/5 15,6 16,20-70 17,5-14 18,2 19,1 20,2 21,3 22,1 23,1 24,3-5 25,1 26,3 30,2-7 31,1 32,13 33,20-27 34,1 36,5 39,1 40,2 41,1 42,1 43,1/2 45,5 46,1 47,2/5 48,1 50,1 51,(55-)60(-65) 52,(5-)5.5(-6) 53,1 56,2 57,2 58,1 59,3 60,1 61,(9-)10(-11)

#L. venetus (Miller) Wohlf./
1,11 2,3 3,2 4,1 5,1<rarely> 6,1/3 7,20-40 8,2 9,1 10,2-3 11,1 12,2 13,1 14,10 15,2 16,35-70 17,15-50 18,1 19,1 20,8 21,4 22,1 24,10-14 28,1/2 30,(6-)10-30 31,1 32,11/14 33,15-18 34,1 36,7/9 39,1 40,2 41,1 43,2 48,1 50,1 51,35-60 52,5-8 56,2 57,2 58,3 59,3 60,1 61,8-14

#L. vernus (L.) Bernh./
1,11 2,3 3,2 4,1 5,1<rarely> 6,1/3 7,20-40 8,2 9,1 10,2-3 11,1 12,2 13,1 14,10 15,4 16,35-70 17,15-35 18,1 19,1 20,6 21,4 22,1 28,1/2 30,3-7 31,1 32,11/14 33,15-18 34,1 36,7/9 39,1 40,2 41,1 43,2 48,1 50,1 51,35-60 52,5-8 53,1 56,2 57,2 58,9/10 59,3 60,1 61,8-14

#L. amphicarpos L./
1,4 2,1 3,3 4,2/3 5,1 6,1 7,12-50 8,1 9,1 10,1 11,3 12,1 13,2 14,1/4 15,4 16,10-30 17,2-7 18,2 19,1 20,3 21,4 22,1 23,1 24,(4-)5-17(-22) 25,5 26,3 28,3 29,4-9 30,1 31,1 32,8/13 33,8-15 34,1 35,2 36,5/8 37,4.5-7(-8) 38,1.5-2.5 39,2 40,2 41,1 42,1 43,3 44,3 45,3.5-4.5 46,2 47,4/5 48,2 49,2 50,8/12 1,
15-30 52,8-10 53,1 54,2 55,2 56,1 57,2 58,1 59,2 60,2 61,(1-)2-3(-4) 62,1.2-2 63,3.5-6.5 64,2 65,12

#L. angulatus L./
1,5 2,1 3,3 4,2 5,1<rarely> 6,1 7,20-50 8,4 9,1 10,1 11,6 12,2 13,2 14,1/7 15,2 18,2 19,1 20,3/15 21,1/2 22,1 23,1 25,1/11 26,2 28,3 30,1 31,1 32,12/14 33,8-10 34,1 35,2<rarely> 36,9 39,2 40,2 41,1 42,1 43,3 46,1 47,5 48,1 49,1 50,1 53,1 55,2 56,2 57,2 59,2 60,2 61,10-12 64,4 65,6

#L. annuus L./
1,4 2,1 3,3 4,3 5,1 6,1/7 7,20-100 8,1 9,1 10,1 11,1 12,2 13,2 14,1/8 15,2 16,60-140 17,2-12(-19) 18,2 19,1 20,1 21,4 22,1 23,1 24,5-25 25,2 26,1 28,3 30,1-6 31,1 32,4/6 33,12-15(-17) 34,1 35,2 36,3/4 39,2 40,2 41,1 42,1 43,3 44,3 45,4-5 46,2 47,1 48,1 49,1 50,5 51,50-70 52,(7-)9-11 53,1 55,1 56,2 57,2 58,3 59,5 60,6 61,6-8 62,1.5 63,4-5 64,2/3 65,3/7

#L. aphaca L./
1,1/4 2,1 3,3 4,2/3 5,1 6,1 7,5-50(-100) 8,2 9,2 11,7 12,2 13,2 14,15 15,9 19,1 20,4 21,2 22,1 23,1 24,(5-)10-30 25,6 29,2-4(-5) 30,1-2 31,1 32,2/3/4 33,(6-)7-13(-16) 34,1 35,2 36,2/3 39,2 40,1 41,1 42,1 43,3 44,3 45,3-5 46,1 47,1 48,1 49,1 50,3 51,18-35 52,4-6 53,1 55,2 56,2 57,2 59,3 60,1 61,5-7 62,1-1.5 63,2-3 64,2/3 65,1/2

#L. basalticus Rech./
1,4 2,1 3,2 4,3 5,2 6,2/5 7,20-40 8,1/3 9,1 10,1 11,3 12,2 13,2 14,2/4 15,1/7 16,5-55 17,1-10 18,2 19,2 20,1 21,4 22,1 23,1 26,1 27,20-30 30,1/2 31,1 32,7 33,(14.8-)15(-15.2) 34,1 35,2 36,6 39,2 40,2 41,1 42,3 43,3 44,3 45,3-5 46,2 47,2 48,2 49,2 50,7 51,25 53,2/5 55,2 56,1 57,1 58,4 59,2 60,3

#L. belinensis N. Maxted & D.J. Goyder/
1,4 2,1 3,3 4,2/3 5,1 6,1/2 7,50-200 8,1 9,1 10,1 11,3 12,2 13,2 14,1/4/9/12 15,1/6 16,15-65 17,7-30 18,1 19,1 20,3/5 21,4 22,1 23,1 24,5-15 25,4 26,1 27,(3-)6-28 30,(1-)3-5 31,2 32,6 33,20-26 34,1 35,1 36,3 39,2 40,2 41,1 42,1 43,2 44,1 45,8-10 46,2 47,1 48,2 50,6 51,18-35 52,4-7 53,5 55,2 56,2 57,2 58,1 59,2 60,9 61,2-5(-8) 62,1

#L. blepharicarpus Boiss./
1,4 2,1 3,3 4,2 5,2 6,5 7,10-40 8,1 9,1 10,1 11,3 12,2 13,2 14,1/4 15,6 16,(7-)10-40 17,2-7 18,2 19,1 20,3 21,4 22,1 23,1 24,5-15 25,9 26,3 27,10-30 28,2 29,3-5 30,1 31,1 32,6/7 33,4-14 34,1 35,2 36,4/6 39,2 40,2 41,1 42,1 43,3 44,3 45,4-6 46,2 47,1 48,2 49,2 50,8 51,20-30 52,10-15 53,5 54,1 55,2 56,2 59,2 60,8 61,3-4 62,1 63,4-5

#L. cassius Boiss./
1,4 2,1 3,2 4,2 5,2 6,1/4 7,(15-)30-60 8,1 9,1 10,1 11,3 12,2 13,2 14,1/8 16,30-70 17,2-9 18,2 19,1 20,3 21,4 22,1 23,1 24,2-15 25,1 26,1 27,8-80 28,2/3 29,.3-1 30,1-4(-6) 31,2 32,13 33,9-11(-12) 34,1 35,2 36,1 39,2 40,2 41,1 42,1 43,2 44,1/2 45,4-5 46,1/2 47,7 48,1 49,1 50,5 51,28-35 52,5-7 53,1 55,2 56,2 57,2 58,3 59,4 60,6/9 61,5-7 62,1.5 63,3.5-4 65,7

#L. cicera L./
1,4 2,1 3,3 4,2 5,2 6,1/3/5 7,15-50 8,1 9,1 10,1 11,3 12,2 13,2 14,1/4/8 15,2 16,15-95 17,1-9 18,2 19,1 20,3/5/10 21,4 22,1 23,1 25,9 26,1 28,3 30,1 31,1 32,7 33,12-16 34,1 35,1 36,6 39,2 40,2 41,1 42,1 43,3 44,3 45,3.5-5 46,1/2 47,1 48,2 49,2 50,6 51,25-40 52,8-10.5 53,1 55,2 56,2 57,2 58,1 59,2 60,1 61,3-5 63,4-6 64,2 65,1/5

#L. ciliolatus Sam./
1,4 2,1 3,3 4,3/4 5,1 6,1 7,10-20 8,1 9,1 10,1 11,3 12,2 13,2 14,1/7 15,2 16,10-40 17,1-3 18,2 19,1 20,1 21,4 22,1 23,1 30,1 31,1 32,7 33,10 34,1 35,2 36,6 39,1 40,2 41,1 42,1 43,3 44,3 45,3-6 46,2 47,5 48,3 49,1 50,5/6/11 51,10-20 52,3 53,2 54,2 55,2 56,1 57,2 58,1 59,2 60,3 61,2-3

#L. chloranthus Boiss./
1,4 2,1 3,2/3 4,1/3 5,2 6,3 7,17-70 8,1 9,1 10,1-2 11,3 12,2 13,2 14,2/4 15,6 16,20-60 17,7-22 18,2 19,2 20,1/2 21,4 22,1 23,2 24,8-20 25,4 26,1 27,80-160 28,3 30,1-2(-3) 31,1 32,3/4 33,15-24 34,1 35,4 36,3 39,1 40,2 41,1 42,2 43,3 44,3 45,7 46,2 47,1 48,1 49,1 50,5 51,43-50 52,6-9 53,3 55,1 56,2 58,4 59,3 60,5/9 61,5-9 63,4 64,2 65,1/8

#L. chrysantus Boiss./
1,4 2,1 3,1 4,1 5,2 6,5 7,30-45(-60) 8,1 9,1 10,1 11,3 12,2 13,2 14,2/4 15,1 16,40-55 17,9-12 18,2 19,2 20,3 21,4 22,1 23,2 24,(9-)10-11 25,1 26,1 30,2-4 31,1 32,4 33,20-22 34,1 35,1/2 36,3 39,2 40,2 41,1 42,3 43,3 44,3 45,7 46,2 47,1 48,1 49,1 50,5 51,(28-)30-32 52,(7.5-)8(-8.5) 53,3 55,2 56,2 57,1 58,3 59,3 60,5 61,6-10

#L. clymenum L./
1,2/4 2,1 3,1/2 4,3 5,1 6,3 7,30-80 8,1/2 9,1 10,2-4 11,3 12,1 13,1/2 14,1/3/6 15,1 16,15-50 17,1.5-7 18,2 19,1 20,3/4/7 21,4 22,2/5 25,11 26,1/2/3 28,1 30,1-4 31,2 32,1/14 33,16-20 34,1 35,2 36,1/8 40,2 41,1 43,1 44,1 46,1 47,5 48,1 49,2 50,2 51,59-60 52,(7-)9-10 55,1 56,2 57,2 59,2 60,1 61,5-6

#L. gleosperma Warb. et Eig./
1,2/4 2,1 3,3 4,2 5,2 6,1/2 8,1 9,1 10,1-4 11,3 12,2 13,2 14,1 15,4 16,20-50 17,2-5 18,1 19,1 20,3/4 21,4 26,1 27,10 30,1 31,1 32,1 33,18-20 34,1 35,2 36,1 39,2 40,2 41,1 43,1 44,1 46,1 47,5 48,1 49,2 50,1 51,40-60 52,10-12 53,5 56,2 57,1 60,10 61,5-7

#L. gorgoni Parl./
1,4 2,1 3,3 4,3 5,1 6,1 7,20-60 8,1 9,1 10,1 11,3 12,2 13,2 14,7/8/11 15,2 16,30-70 17,3-15 18,2 19,1 20,3/5 21,4 22,1 23,1 25,9 26,1/2 27,1-30 28,1/2 29,5 30,1 31,1 32,5 33,15-18 34,1 35,1/2 36,4 39,2 40,2 41,2 42,1 43,1 44,3 45,7-9 46,2 47,2 48,1 49,1 50,5 51,35-47 52,8-9 53,1/2 55,1 56,2 57,2 58,3 59,4 60,1 61,5-8 62,1.5 63,3-5 64,2 65,3/4

#L. hierosolymitanus Boiss./
1,4 2,1 3,3 4,3 5,1 6,1 7,20-100 8,1 9,1 10,1 11,3 12,2 13,2 14,1/8 15,2 16,60-140 17,2-12(-19) 18,2 19,1 20,1 21,4 22,1 23,1 24,5-25 25,5 26,1 27,10-52 28,3 30,1-6 31,1 32,6/13 33,10-12 34,1 35,2 36,4/5 39,2 40,2 41,1 42,1 43,3 44,3 45,3-4 46,2 47,1/4 48,1 49,1 50,5 51,50-70 52,5.5-6(-7) 53,1 55,1 56,2 57,2 58,3 59,5 60,7 61,6-10 65,11

#L. hirsutus L./
1,4 2,1/2 3,3 4,3 5,2 6,1/6 7,(10-)40-60 8,2 9,1 10,1 11,3 12,2 13,2 14,2/4 15,1 16,30-60 17,3-11 18,2 19,1 20,1/2 21,4 22,1 23,1 24,10-12 25,2 26,2 27,80-90 28,3 30,1-3 31,1 32,11 33,10-14 34,1 35,1 36,7 39,1 40,2 41,1 42,2 43,2 44,2 45,3-4 46,2 47,1 48,1 49,2 50,5 51,23-35 52,5.5-7.5 53,3 55,2 56,2 57,1 59,3 60,9 61,5-7 63,3 64,3 65,7

#L. hirticarpus Mattatia & Heyn/
1,4 2,1 3,3 4,2/3 5,2 6,3 7,8-50 8,1/4 9,1 10,1 11,3 12,2 13,1/2 14,2/4 15,1/6 16,5-50 17,1-10 18,2 19,1 20,5 21,4 22,1 23,1 24,2-10 25,4/9 27,10-40 28,1/3 29,2-5 30,1 31,1 32,7 33,10-18(-20) 34,1 36,6 40,2 41,2 42,1 43,3 44,3 45,4-8 46,2 47,5 48,1 49,2 50,6 51,16-28 52,6-10 56,2 57,1 58,4 59,4 60,1 61,2-5 62,1.5 63,4

#L. inconspicuus L./
1,5 2,1 3,3 4,1 5,1 6,1/2/6 7,10-35 8,2 9,1 10,1 11,3 12,2 13,2/3 14,7/8 15,2 16,15-60 17,1-7 19,1 20,3/11 21,1/4 22,1 23,1 24,.5-1 25,1 26,1 27,10 30,1 31,2 32,9 33,7-9 34,1 35,2 36,1 39,1 40,1 41,1 42,1 43,3 44,3 45,2-4 46,1 47,7 48,1 49,3 50,1 51,35-50 52,4-5 53,1/2 56,2 57,2 58,2 59,3 60,1 61,7-11 63,3 64,2 65,1/2

#L. lycicus Boiss./
1,4 2,1 3,3 4,3 5,1 6,4 7,20-60 8,2 9,1 10,1 11,3 12,2 13,1/2 14,4/9 15,6 16,25-50 17,7-25 18,2 19,1 20,1 21,2 22,1 23,2 24,(4-)5(-6) 25,11 27,50-100(-110) 30,2-3(-6) 31,1 32,13 33,14-18 34,1 36,5 39,2 40,2 41,1 42,2 43,3 45,7 46,2 47,1 48,1 50,5 51,(21-)22-23(-24) 52,4.5 53,3 56,2 57,2 58,6 59,3 60,4/6

#L. marmoratus Boiss. & Bl./
1,4 2,1 3,3 4,2 5,2 6,6 7,10-50 8,1 9,1 10,1 11,3 12,2 13,2 14,1/8 15,1 16,10-40(-50) 17,1.5-3 18,2 19,1 20,3 21,4 22,1 23,1 25,7/9 26,3 27,40-60 30,1 31,1 32,7 33,11-13(-14) 34,3 35,1 36,6 39,2 40,2 41,1 42,1 43,3 44,3 45,4-5(-6) 46,2 47,1 48,2 49,2 50,6 51,20-27 52,6-8 53,1 55,2 56,2 57,2 59,2 60,1 61,3-4

#L. nissolia L./
1,7 2,1 4,1/2 5,2 6,2 7,15-70(-90) 8,4 9,1 10,1 11,4 12,2 13,3 14,1/8 15,2 16,(20-)40-100 17,2-6 18,2 19,1 20,1/2/3/12/13 21,4 22,1 23,1 24,1-3 25,1 26,1 27,20-130 28,2/3 29,1.5-4 30,1(-2) 31,1 32,13 33,(6-)9-15(-18) 34,1 35,2 36,5 39,2 40,2 41,1 42,1 43,1 44,3 45,2-3 46,2 47,4/5 48,1 49,1 50,1 51,(30-)32-40(-60) 52,2.5-3 53,1 55,2 56,2 57,1 59,3 60,2/9 61,11-16 63,2.5 64,2

#L. ochrus (L.) DC./
1,2/4 2,1 3,2 4,3 5,1 6,1 7,25-100 8,1 9,1 10,1-2(-3) 11,3 12,1 13,1/2 14,10 15,1 16,20-45 17,9-12 18,2 19,1 21,4 26,3 28,2 29,1-3 30,1 31,1 32,1/2 33,14-16 34,1 35,3 36,1/2 40,2 41,1 43,2 44,2 46,1 47,5 48,1 49,1 50,5/9 51,40-50 52,9-12 53,1 55,2 56,2 57,2 59,2 60,1 61,5-7 62,2-3 63,4-6.5(-7) 64,4/5 65,3

#L. odoratus L./
1,4 2,1 3,2/3 4,2/3 5,2 6,3 7,(50-)100-200 8,1 9,1 10,1 11,1 12,2 13,2 14,3/4/9 15,3 16,(20-)35-60 17,(7-)12-30 18,1/2 19,2 20,3 21,2/4 22,1 26,1 27,120-125(-150) 30,(1-)2-3(-4) 31,2 32,1/4/7/11 33,20-30 34,3 35,1 36,1/3/6/7 40,2 41,1 42,3 43,2/3 44,2 46,2 47,5/7 48,1 49,2 50,2 51,40-65 52,9-12 53,3 55,2 56,2 57,2 58,4 63,4-5 64,6/7 65,5/7
#L. phaselitanus Hub.-Mor. & Davis/
1,4 2,1 3,3 4,3 5,1<rarely> 6,1/2 7,50-100 8,2 9,1 10,1 11,3 12,2 13,2 14,2 16,15-35 17,4-8 19,1 20,1 21,4 22,1 30,1-2 31,1 32,8 33,(19-)20(-21) 34,1 36,8 40,2 41,1 43,3 45,11 48,1 50,5 51,23-35 52,5.5-7.5 53,3 56,2 57,2 58,6 65,7

#L. pseudo-cicera Pamp./
1,4 2,1 3,3 4,2 5,1 6,1/3 7,15-50 8,1 9,1 10,1 11,3 12,2 13,2 14,1/4/8 15,2 16,15-95 17,1-9 18,2 19,1 20,3/5/10 21,4 22,1 23,2 25,9 26,2 28,3 30,1 31,1 32,5 33,12-16 34,3 35,1 36,4 39,2 40,2 41,1 42,1 43,3 44,3 45,3.5-7 46,2 47,1 48,2 49,1 50,6 51,25-40 52,8-10.5 55,2 56,2 57,2 58,5 59,2 60,1 61,3-5 64,1/4/8/9 65,1

#L. pygmaeus Gomblaut Bull./
1,4<rarely> 2,1 3,3 4,2/3 5,1<rarely> 6,3 7,5-10 8,2 9,1 10,12-16(-20) 11,3 12,2 14,1 19,1 21,2 23,2 31,1 32,13 33,10-12 34,1 36,5 42,1 43,3 48,1 50,3 51,20 56,2 57,2 58,1 59,3 60,4/5

#L. sativus L./
1,4 2,1 3,3 4,2 5,2 6,6/7 7,10-70(-100) 8,1 9,1 10,1 11,3 12,2 13,2 14,1/7 15,1 16,20-100 17,1.5-11 18,2 19,1 20,3/11 21,4 22,1 23,1 25,8 26,1 27,1-40(-45) 28,3 29,5-8 30,1 31,1 32,1/8/11 33,14-20 34,3 35,1 36,1/7/8 39,2 40,2 41,1 42,1 43,3 44,3 45,5-6 46,2 7,1 48,2 49,2 50,7 51,9-12 52,6-8 53,1 55,2 56,2 57,2 58,9 59,1 60,1 61,(2-)3-4(-5) 62,1.5 63,6-8 64,1/2 65,1/5/6

#L. saxatilis (Vent.) Vis./
1,13 2,1 3,3 4,2 5,1 6,3 7,7-30 8,2 9,1 10,1-3 11,3 12,2 13,1 14,1/6 15,1 16,12-33 17,.5-1.5 19,1 20,1 21,2 22,3 30,1 31,1 32,2 33,7-8 34,1 36,2 39,1 40,2 41,1 42,1 43,1 45,1-2 46,2 47,1 48,1 50,5 51,15-22 52,4.5-5.5 53,1 55,2 56,2 57,1 59,3 60,1 61,3-6 62,1.2 63,2.5-2.8 64,5

#L. setifolius L./
1,9 2,1 3,3 4,3 5,1 6,1 7,30-80 8,1 9,1 10,1 12,2 13,2 14,1 15,2 16,25-75 17,1-3 18,1/2 19,1 20,3/11 21,4 22,1 23,1 24,3-10 25,8 26,3 27,10-30(-40) 28,2 29,2-8(-10) 30,1 31,1 32,6 33,6-10 34,1 35,2 36,4 39,2 40,1/2 41,42,1 43,3 44,3 45,3.6-4.8 46,1 47,1/7 48,2 50,6 51,20-27 52,8-10 53,1 55,2 56,2 57,2 59,3 60,2/5 61,2-3 62,1.2 63,5 65,3

#L. sphaericus Retz./
1,5 2,1 3,3 4,1 5,1 6,1 7,15-50 8,2 9,1 10,1 11,3 12,2 13,2/3 14,1 15,2 16,25-90 17,.5-3 18,2 19,1 20,1/2 21,2/4 22,1 23,1 24,3-13 25,11 26,2/3 27,10-120 28,3 29,5 30,1 31,1 32,7 33,8-10 34,1 35,1 36,6 39,1 40,1/2 41,1 42,1 43,3 44,3 45,2-3 46,1 47,1/7 48,1 49,1 50,4 51,30-55 52,4 53,1 55,2 56,2 57,2 58,1 59,3 60,1 61,5-15 62,1 63,2-3 64,5 65,4

#L. stenolobus Boiss./
1,1 2,1 3,3 4,2 5,1 6,1 7,10-30 8,2 9,2 10,0 12,2 13,2 19,1 20,3 21,3 22,1 23,1 24,12-22 25,4/6 30,1 31,1 32,4 33,7-8 34,1 36,3 40,1 41,1 42,1 43,3 45,2-3 46,2 47,1 48,1 50,1 51,25-30 52,3.5 53,1 55,2 56,2 57,2 59,3 60,1 61,4-6

#L. stenophyllus Boiss. & Heldr./
1,4 2,1 3,3 4,2 5,1 6,1 7,40-70 8,2 9,1 10,1 11,3 12,2 13,2 14,1 15,2 16,15-55 17,.5-2 18,2 19,1 20,3 21,4 22,1 23,1 24,5-15 25,10 26,1 30,1 31,2 32,1/13 33,12-16 34,2 35,1 36,5 39,2 40,1/2 41,1 42,1 43,3 44,3 45,7-8 46,2 47,1 48,2 49,2 50,9 51,35-45(-50) 52,9-10 53,1 55,2 56,2 57,2 58,3 59,4 60,4/7

#L. tauricola P. H. Davis/
1,5 2,1 3,3 4,2/3 5,1<rarely> 6,2 7,13-25 8,3 9,1 10,1 12,2 13,1/2 14,1/4 15,1 16,20-35 17,1-2 18,2 19,1 20,3 21,4 22,1 23,1 24,3-8 25,3 27,10-30 30,1 31,1 32,4 33,7-10 34,1 36,3 39,2 40,1 41,1 42,1 43,2 45,3-4 46,2 47,1 48,1 50,1 51,2-8 52,4 53,1 56,2 57,2 58,2 61,(4-)5-8

#L. trachycarpus (Boiss.) Boiss./
1,4 2,1/2 3,1 4,1 5,1<rarely> 6,1 7,40-50 8,2 9,1 10,1 11,3 12,2 13,1 14,4 15,6 16,40-50 17,10-16 18,2 19,1 20,3/11 21,4 22,1 23,1 24,7-14 25,2 27,50-90 28,3 30,3-6 31,1 32,12 33,(19-)20-21 34,1 36,5 39,2 40,1 41,1 42,1 43,3 45,7-8 46,2 47,1 48,2 50,10 51,(14-)15(-16) 52,(7-)8(-8.5) 53,4 56,2 57,2 58,4 59,3 61,1-2(-4)

#L. vinealis Boiss. & Noe/
1,5 2,1 3,3 4,1 5,1 6,1 7,14-40 8,2 9,1 10,2 11,3 12,2 13,1/2/3 14,1 15,2 16,40-90 17,1-5 18,2 19,1 20,1 21,2/4 22,1 23,1 25,11 26,2/3 28,3 30,1 31,1 32,7/13 33,103 34,1 35,2 36,5/6 39,2 40,1/2 41,1 42,1 43,3 44,3 45,3-4 46,1 47,4/7 48,1 49,2 50,1 51,40-55 52,5-6.5 53,1 55,2 56,2 57,2 58,1 59,3 60,1 61,3-6 63,3-4 64,8 65,5/8

#L. woronowii Bornm./
1,5 2,1 3,3 4,2/3/5 5,1<rarely> 6,1/4 7,8-18 8,2 9,1 10,1/2 11,3 12,2 13,1 14,4/10 16,(11-)12(-13) 17,5 19,1 20,2 21,4 22,1 28,3 30,1 31,1 32,2/8 34,1 36,2/8 40,2 41,1 42,1 43,3 56,2 57,2

Appendix 6.6. Specifiaction file.

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*NUMBER OF CHARACTERS 65
*MAXIMUM NUMBER OF STATES 15
*MAXIMUM NUMBER OF ITEMS 76

*CHARACTER TYPES  7,RN 10,RN 16,RN 17,RN 24,RN
27,RN 29,RN 30,RN 33,RN 37,RN 38,RN 45,RN 51,RN
52,RN 61,RN 62,RN 63,RN

*NUMBERS OF STATES  1,13 2,3 3,4 4,5 6,7 8,4
11,7 13,3 14,15 15,9 18,3 20,15 21,4 22,5 25,15
26,3 28,4 32,15 34,3 35,4 36,9 42,3 43,3 44,3
47,8 48,3 49,3 50,12 53,6 58,10 59,5 60,11 64,9
65,12

*DEPENDENT CHARACTERS
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Appendix 6.7. General key to *Lathyrus* taxa of the Mediterranean Basin, Caucasus, Centra and West Asia region

4.1.1 Key 5. Confirmatory characters

Characters: 59 indata, 44 included, 27 in key.
Items: 76 in data, 76 included, 121 in key.
Parameters: Rbase = 1.40 Abase = 2.00 Reuse = 1.01 Varywt =.80
Characters included: 1–5 7–8 10–14 17–21 23–24 26 29–30 32–34 36–41 43–47 50–57
Character reliabilities: 1–59,5.0

1. Wings white 2
Wings cream 6
Wings yellow 10
Wings orange 14
Wings pink 16
Wings brick-red 22
Wings blue 25
Wings violet 38
Wings purple 44

2(1). Legume linear 3
Legume broadly-linear 4
Legume oblong-linear 5
Legume broadly-oblong ..... ***L. sativus* L.**
Legume narrowly oblong ..... ***L. ochrus* (L.) DC.**

3(2). Style linear ..... ***L. libani* Fritsch**
Style spathulate ..... ***L. gleosperma* Warb. et Eig.**
Style canaliculate ..... ***L. inconspicuus* L.**

4(2). Leaflets paripinnate; Vegetative parts pubescent, usually sparsely; Leaf rachis not laminate; Leaflet apex emarginate ..... ***L. odoratus* L.**
Leaflets pinnate; Vegetative parts glabrous; Leaf rachis laminate; Leaflet apex mucronate ..... ……***L. clymenum* L.**
5(2). Leaf rachis laminate; Style spathulate; Vegetative parts green; Growth habit decumbent ..... ……***L. ochrus* (L.) DC.**
Leaf rachis not laminate; Style canaliculate; Vegetative parts glaucous (or glaucescent); Growth habit ascending ..... ***L. cassius* Boiss.**

6(1). Leaflets paripinnate ..... ***L. brachypterus* Cel.**
Leaflets subdigitate ..... ***L. brachypterus* Cel.**
Leaflets pinnate 7
Leaflets reduced ..... ***L. aphaca* L.**

7(6). Calyx teeth shorter than tube 8
Calyx teeth equal to tube ..... ***L. ochrus* (L.) DC.**
Calyx teeth longer than tube ..... ***L. woronowii* Bornm.**
8(7). Leaflet apex mucronate; Stipule base semi-hastate; Legume oblong-linear; Rachis mucronate ..... ……..***L. saxatilis* (Vent.) Vis.**
Leaflet apex acute; Stipule base semi-sagittate; Legume linear; Rachis aristate 9

9(8). Growth habit erect; Style spathulate; Stems terete; Legume straight..... ***L. pallescens* (Bieb.)**
Growth habit ascending; Style linear; Stems winged; Legume beaked . ***L. linifolius* (Reichard)Bassler**

10(1). Legume linear 11

Legume broadly-linear ..... ***L. odoratus* L.**
Legume linear-sublanceolate ..... ***L. aphaca* L.**
Legume oblong-linear 13
Legume oblong ..... ***L. belinensis* N. Maxted & D.J. Goyder**
Legume canescent ..... ***L. laevigatus* (Waldst. & Kit.)Gren.**

11(10). Calyx teeth shorter than tube 12
Calyx teeth equal to tube ..... ***L. tauricola* P. H. Davis**
Calyx teeth longer than tube ..... ***L. stenolobus* Boiss.**

12(11). Stems terete; Rachis aristate; Leaflet apex acute; Stipules 1 mm. wide ..... ***L. pallescens* (Bieb.)**
Stems ridged; Rachis mucronate; Leaflet apex obtuse; Stipules 3-4 times as broad as stem .....
……***L. laevigatus* (Waldst. & Kit.)Gren.**

13(10). Stipules 1 mm. wide ..... ***L. chrysanthus* Boiss.**
Stipules 0.5-1.5 mm. wide ..... ***L. annuus* L.**
Stipules 1-3 mm. wide ..... ***L. chloranthus* Boiss.**
Stipules broader than the leaflet ..... ***L. pratensis* L.**

14(1). Stipules 0.5-1.5 mm. wide ..... ***L. annuus* L.**
Stipules 0.5-5 mm. wide ..... ***L. hierosolymitanus* Boiss.**
Stipules 1-1.5 times as broad as stem ..... ***L. setifolius* L.**
Stipules 2-3 times as broad as stem 15
Stipules 3-4 times as broad as stem ..... ***L. laevigatus* (Waldst. & Kit.)Gren.**
Stipules broader than stem ..... ***L. aureus* (Stev.) Brandza**

15(14). Legume oblong-linear ..... ***L. gorgoni* Parl.**
Legume oblong ..... ***L. pseudo-cicera* Pamp.**
Legume broadly elliptic-oblong ..... ***L. blepharicarpus* Boiss.**

16(1). Legume linear 17
Legume broadly-linear ..... ***L. roseus* Stev. in Mem. Soc. Nat. Mosc. 4$52 (1813)**
Legume linear-sublanceolate 20
Legume oblong-linear 21
Legume broadly elliptic-oblong ..... ***L. amphicarpos* L.**
Legume narrowly oblong ..... ***L. stenophyllus* Boiss. & Heldr.**
Legume elliptic-oblong ..... ***L. trachycarpus* (Boiss.) Boiss.**
Legume obovate ..... ***L. amphicarpos* L.**

17(16). Stipules 1 mm. wide 18
Stipules 1-3 mm. wide ..... ***L. boissieri* Sirj.**
Stipules 1-2 times as broad as stem 19
Stipules as broad as stem ..... ***L. vinealis* Boiss. & Noe**
18(17). Stems terete; Leaflets subdigitate; Rachis mucronate; Leaflet apex obtuse .....
…… ***L. variabilis* (Boiss. & Ky.) Maly**
Stems angled; Leaflets phyllodic; Rachis aristate; Leaflet apex acute ..... ***L. nissolia* L.**

19(17). Leaflet apex obtuse; Style arcuate; Stipules more or less equally as long as petiole; Standard with more than 5 conspicuous veins ..... ***L. rotundifolius* Willd.**
Leaflet apex undulate-margined; Style linear; Stipules longer than petiole; Standard with no conspicuous veins ..... ***L. undulatus* Boiss.**
20(16). Calyx teeth shorter than tube; Stipule base semi-sagittate; Stipules glabrous; Life form perennial .....
……***L. roseus* Stev. in Mem. Soc. Nat. Mosc. 4$52 (1813)**
Calyx teeth longer than tube; Stipule base semi-hastate; Stipules pubescent; Life form annual .....
……..***L. pygmaeus* Gomblaut Bull.**

21(16). Stipules 1-3 mm. wide; Legume valves reticulate-nerved ..... ***L. tuberosus* L.**
Stipules 0.5-5 mm. wide; Legume valves gland-dotted ..... ***L. hierosolymitanus* Boiss.**

Stipules as broad as stem; Legume valves glandular-verrucose ..... ***L. lycicus* Boiss.**

22(1). Legume linear ..... ***L. vinealis* Boiss. & Noe**
Legume broadly-linear ..... ***L. odoratus* L.**
Legume linear-ensiform ..... ***L. sphaericus* Retz.**
Legume oblong-linear ..... ***L. ciliolatus* Sam.**
Legume oblong 23
Legume broadly-oblong ..... ***L. basalticus* Rech.**
Legume broadly elliptic-oblong ..... ***L. blepharicarpus* Boiss.**
Legume canescent ..... ***L. ciliolatus* Sam.**

23(22). Ovary linear ..... ***L. hirticarpus* Mattatia & Heyn**
Ovary oblong 24
Ovary canescent ..... ***L. ciliolatus* Sam.**
24(23). Leaflet apex mucronate; Stipules longer than petiole; Standard with more than 5 conspicuous veins .....
……***L. marmoratus* Boiss. & Bl.**
Leaflet apex acute; Stipules shorter than petiole; Standard with no conspicuous veins ..... ***L. cicera* L.**

25(1). Legume linear 26
Legume broadly-linear 34
Legume linear-sublanceolate ..... ***L. di*gitatus (Bieb.) Fiori**
Legume oblong-linear 37
Legume broadly-oblong ..... ***L. sativus* L.**
Legume canescent ..... ***L. satdaghensis* P. H. Davis**

26(25). Leaflets paripinnate 27
Leaflets subdigitate 28
Leaflets pinnate 31

27(26). Stipules lanceolate-subulate ..... ***L. karsianus* P. H. Davis**
Stipules lanceolate ..... ***L. nig*er (L.) Bernh.**
Stipules ovate-oblong ..... ***L. vernus* (L.) Bernh.**
Stipules suborbicular ..... ***L. venetus* (Miller) Wohlf.**

28(26). Stipules subulate ..... ***L. tukhtensis* Czecz.**
Stipules lanceolate-subulate 29
Stipules lanceolate 30
Stipules linear ..... ***L. filiformis* (Lam.) Gay**

29(28). Flowers concolorous; Style straight ..... ***L. spathulatus* Cel.**
..... ***L. elongatus* (Bornm.) Sirj.**
Flowers not concolorous; Style twisted ..... ***L. cyaneus* (Stev.) Koch**

30(28). Flowers concolorous; Calyx teeth shorter than tube; Style straight ..... ***L. tukhtensis* Czecz.**
Flowers not concolorous; Calyx teeth equal to tube; Style twisted ..... ***L. cyaneus* (Stev.) Koch**

31(26). Rachis mucronate 32
Rachis tendrillous 33
Rachis aristate ..... ***L. linifolius* (Reichard) Bassler**

32(31). Vegetative parts glabrous; Stipule base semi-sagittate; Stipules as broad as stem; Flowers not concolorous ..... ***L. cyaneus* (Stev.) Koch**
Vegetative parts subadpressed canescent-pubescent; Stipule base sagittate; Stipules less than 1/2 as wide as stem; Flowers concolorous ..... ***L. satdaghensis* P. H. Davis**

33(31). Vegetative parts glaucous (or glaucescent); Leaf rachis not laminate; Leaflet apex obtuse; Stipules 1-2 times as broad as stem ..... ***L. incurvus* (Roth.) Willd.**

Vegetative parts green; Leaf rachis laminate; Leaflet apex acute; Stipules less than 1/2 as wide as stem ……. . ***L. sylvestris* L.**

34(25). Leaflet apex acute ..... ***L. czeczottianus* Bassler**
Leaflet apex emarginate ..... ***L. odoratus* L.**
Leaflet apex subobtuse ..... ***L. japponicus* Willd.**
Leaflet apex obtuse ..... ***L. japponicus* Willd.**
Leaflet apex aristate 35

35(34). Leaflets elliptic 36
Leaflets lanceolate ..... ***L. laxiflorus* (Desf.). O. Kuntze subsp. *angustifolius* (Post ex Dinsm) Davis**
Leaflets ovate ..... ***L. laxiflorus* (Desf.). O. Kuntze subsp. *laxiflorus* (Desf.) O. Kuntze**

36(35). Rachis tendrillous ..... ***L. laxiflorus* (Desf.). O. Kuntze**
Rachis aristate ..... ***L. laxiflorus* (Desf.). O. Kuntze subsp. *laxiflorus* (Desf.) O. Kuntze**

37(25). Calyx teeth shorter than tube ..... ***L. nivalis* Hand.-Mazz.**
Calyx teeth equal to tube ..... ***L. hirsutus* L.**
Calyx teeth longer than tube ..... ***L. layardii* J. Ball ex Boiss.**

38(1). Calyx teeth shorter than tube 39
Calyx teeth equal to tube 41
Calyx teeth longer than tube 42

39(38). Style linear ..... ***L. armenus* Boiss. & Hute) Sirj.**
Style linear-spathulate 40
Style spathulate ..... ***L. clymenum* L.**

40(39). Stems terete; Leaflets pinnate; Stipules pubescent; Stipules as broad as stem .. ***L. nivalis* Hand.-Mazz.**
Stems angled; Leaflets subdigitate; Stipules glabrous; Stipules 1-3 mm. wide ..... ***L. boissieri* Sirj.**

41(38). Leaflets subdigitate; Stipules 1-3 mm. wide; Legume linear; Stems angled..... ***L. boissieri* Sirj.**
Leaflets pinnate; Stipules somewhat narrower than leaflet; Legume broadly-linear; Stems terete.....
……..***L. japponicus* Willd.**

42(38). Stipules subulate ..... ***L. phaselitanus* Hub.-Mor. & Davis**
Stipules lanceolate-subulate ..... ***L. woronowii* Bornm.**
Stipules lanceolate 43
Stipules lanceolate-accuminate ..... ***L. sativus* L.**

43(42). Leaf rachis laminate; Leaflet apex acuminate; Stipules 0.5-5 mm. wide; Stipules longer than petiole ....

…… ***L. amphicarpos* L.**
Leaf rachis not laminate; Leaflet apex mucronate; Stipules 1-1.5 times as broad as stem; Stipules shorter than petiole ..... ***L. sativus* L.**

44(1). Leaflets paripinnate 45
Leaflets subdigitate 46
Leaflets pinnate 50
Leaflets sub-sessile ..... ***L. digitatus* (Bieb.) Fiori**
Leaflets alternate ..... ***L. angulatus* L.**

45(44). Leaflet apex acute; Stipules suborbicular ..... ***L. venetus* (Miller) Wohlf.**
Leaflet apex acuminate; Stipules ovate-oblong ..... ***L. vernus* (L.) Bernh.**
Leaflet apex subobtuse; Stipules lanceolate ..... ***L. niger* (L.) Bernh.**

46(44). Stems winged 47

Stems terete 48

47(46). Style linear ..... ***L. bauhimi* Genty**
Style dilated at apex ..... ***L. filiformis* (Lam.) Gay**
Style linear-spathulate ..... ***L. bauhimi* Genty**

48(46). Plants slender 49
Plants rigid ..... ***L. cilicicus* Hayek & Siehe**

49(48). Stipules lanceolate-subulate; Style straight; Legume linear; Legume valves glabrous ***L. spathulatus* Cel.** ..... ***L. elongatus* (Bornm.) Sirj.**
Stipules lanceolate; Style twisted; Legume linear-sublanceolate; Legume valves hairy .....
……...***L. digitatus* (Bieb.) Fiori**

50(44). Leaflet apex acute ..... ***L. palustris* L.**
Leaflet apex acuminate ..... ***L. tingitanus* L.**
Leaflet apex obtuse 51

51(50). Vegetative parts glaucous (or glaucescent); Growth habit decumbent; Stipules longer than petiole; Calyx teeth equal to tube ..... ***L. incurvus* (Roth.) Willd.**
Vegetative parts green; Growth habit ascending; Stipules shorter than petiole; Calyx teeth shorter than tube ..... ***L. pisiformis* L.**

Annex 1. Field guide CD

CHAPTER SEVEN

GENERAL DISCUSSION

7.1 Novel Classification of *Lathyrus* L. species

In this study, the combined use AFLP molecular markers and morphological characters allowed to discriminate well the sections *Aphaca, Clymenum, Lathyrostylis, Orobus, Pratensis* and to some extent *Orobastrum*. *Nissolia* and *Linearicarpus* sections formed the same cluster, and Sect. *Lathyrus* showed high diversity calling for more in-depth analysis of this section as one of its sub-clusters was very distant from the other *Lathyrus* sub-clusters and from the other *Lathyrus* sections. While all the species were separated using morphological characters, AFLP method provided specific markers at the level of sections and species, and have allowed to confirm and add species in the secondary genepool of grass pea.

7.2 Conservation Strategy for *Lathyrus* L. species

This study has allowed to update and enrich the global Lathyrus database available at ICARDA. Ecogeographic study used the information of seed accessions from major *Lathyrus* collections around the world, and added the information on *Lathyrus* specimens from eight herbaria from Europe. The information was used to define areas for new and targeted collections, to mainly focus on wild relatives of major *Lathyrus* cultivated species. It will be also rewarding to assess the status of the existing collections in terms of viability and genetic integrity. For *in situ* conservation, this study was able to define hotspot areas for species richness of different *Lathyrus* section, and indicated the need to establish more genetic reserves in the Fertile Crescent to contribute to the conservation of *Lathyrus* diversity along

with other wild relatives of forage and pastoral legumes and other plant species. Both *ex situ* and *in situ* approaches should be used. Opportunities for on-farm conservation of *Lathyrus* landraces should be more investigated, as no efforts are undertaken in this regard. *In situ* conservation under natural habitats and on-farm conservation of *Lathyrus* diversity will require a holistic community-driven approach with appropriate low-cost technology options, add-value technologies, alternative sources to improve the income of the custodians of agrobiodiversity or biodiversity in general, institutional arrangements and enabling policies and legislations. In addition, increased public awareness and national, regional, and international support to biodiversity rich areas within the benefit sharing mechanisms included in CBD and International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). More funding from the Global Environment Facility and from the ITPGRFA benefit sharing fund need to be directed to promote both *in situ* and *ex situ* conservation of landraces and wild relatives of *Lathyrus* and other crops of global importance, in the countries in need. Developmental projects should also consider promoting the conservation of agrobiodiversity through the support of the livelihoods of local communities directly involved in their maintenance. In addition, marketing of products from local agrobiodiversity should be encouraged at national and international markets. Regional collaboration is needed among neighboring countries to establish trans-boundary genetic reserves to conserve wild relatives and native species and their ecosystems. Several areas on the bordures between Lebanon and Syria, between Syria and Turkey could be rewarding for conservation of crop wild relatives of many plant species including *Lathyrus*. ICARDA and other international and regional organizations could play an important role in promoting the conservation of dryland agrobiodiversity in general and *Lathyrus* genepools in particular.

7.3 Utilization Strategy for *Lathyrus* L. species

This work has allowed to confirm the species in the genepools of grass pea and to recommend *L. odoratus* in the secondary genepool. Focused Identification of Germplasm Strategy was introduced by this study to select a subset for heat and drought tolerance. Similar analysis could be done to target best bet subsets for traits of global importance such as low ODAP content. Diversity analysis using environmental layers could also be use to select species and accessions within species that are better adapted to heat, salinity and extreme drought to be used in breeding or in rehabilitation of degraded ecosystems. This type of research is needed more to adapt to the adverse effects of climate change hitting most parts of Africa and Asia, and even Europe. Documentation of traditional knowledge and collaboration with advanced institutions could investigate other uses of *Lathyrus* species including in preventing and curing illnesses for humans and animals. ICARDA *Lathyrus* breeding program should investigate more the genetic resources of cultivated and wild species to transfer genes of low ODAP content which could bring back interest in cultivation and use of *Lathyrus* species.

7.4 Recommendations from Research

This work allowed to identify areas to be strengthened including:

- The need to maintain and update the global *Lathyrus* genetic resources database now available at ICARDA. This can be followed by the development of a comprehensive *Lathyrus* crop registry, allowing to identify overlaps and duplicates among various collections, towards efficient conservation and sustainable use of *Lathyrus* genetic resources. ICARDA-Genetic Resources Section, as stated in the global *Lathyrus* conservation strategy should take the lead

to promote more coordination of the efforts of conservation and use of *Lathyrus* genetic resources;

- More information is needed from herbaria around the world and from genebanks not yet included in the *Lathyrus* global database to gain more precision in the distribution of to help target *in situ* and *ex situ* conservation and collecting efforts. This might need also access to more accurate environmental layers and more phenotypic and genotypic information. All this information will guide future collecting missions either to fill the gaps based on geographic coverage or to target useful adaptive traits right at the planning of the missions;

- *Lathyrus* collections were characterized by the number of accessions they are holding. Another important step is needed, mainly for genebank with limited facilities for proper handling, to assess the viability of the accessions, as reported by the *Lathyrus* global conservation strategy, several collections require urgent attention for their regeneration;

- While morphologic characters will continue to be the main method for taxonomic characterization, the use of molecular markers could facilitate the identification of species when specific markers are identified for specific taxon. These markers could also be used to study further the species in the genepools of different *Lathyrus* cultivated species. The relevance of new DNA molecular techniques need to be further investigated. Morphologic characters and DNA molecular markers could be supported by cytogenetic studies to gain knowledge about the phylogenetic relationships among different *Lathyrus* species and to increase the use of wild relatives in grass pea improvement;

- It might be rewarding to develop collaborative actions with national research institutions in Central, West Asia and North Africa region (CWANA) and in South Asia and Sub-Saharan Africa to assess the trends and threats to biodiversity/agrobiodiversity in general

and crop wild relatives in particular. In this regards, ICARDA should continue to monitor biodiversity of crop wild relatives in the sites identified within the GEF-funded, ICARDA-coordinated project on "conservation and sustainable use of dryland agrobiodiversity in Jordan, Syria, Palestine and Lebanon". ICARDA has continued the ecogeographic surveys in 2009 and 2011. This will be extended to assess the species diversity within the existing protected areas to answer the question of to what extent the existing genetic reserve are contributing to the conservation of crop wild relatives including for *Lathyrus*;

- To promote efficient conservation of *Lathyrus* genetic resources, more research is needed to elucidate the reproduction system of some *Lathyrus* species as several studies are reporting high cross-pollination rates even for grass pea. ICARDA is already applying regeneration and multiplication under isolation cages to ensure the maintaining of genetic integrity of *Lathyrus* accessions;

- To promote efficient use of *Lathyrus* genetic resources in grass pea improvement, more characterization and evaluation data should flow back to the *Lathyrus* Global database to have enough information to be able to derive subsets for different valuable traits sought by the users using the FIGS approach or other trait-oriented selection approaches. The introgression of useful genes from *Lathyrus* species in the secondary and tertiary genepools will require the development of strong pre-breeding research within ICARDA and other leading grass pea breeding programs;

- The "*Lathyrus* Field Guide" developed within this study should be further pursued to include more species and more recent information. This field guide should be used in training modules for taxonomic identification of *Lathyrus*.

7.5 Critique of Methodologies Used

The limited information (passport, environment, evaluation) on the herbaria specimens and seed accessions in the Global *Lathyrus* Database has reduced the sample size used throughout the study. However, the study has introduced several new methodologies such as:

- AFLP markers to help in the taxonomic identification of *Lathyrus* species, however, although all the six AFLP primers combinations were highly polymorphic, the study could gain more in precision if larger number of AFLP primer combinations were used. The use of other DNA markers methods such as SSR and any other method which can saturate the genomes of *Lathyrus* could be highly rewarding. DNA markers can not substitute for morphologic characters agreed upon by taxonomists, but can ease the identification in case of availability of specific DNA markers for given species;

- Use of Structure program for the genetic diversity analysis, could allow more information on the phylogenetic relationships among *Lathyrus* sections and species if more molecular markers are available;

- Use of MaxEnt, PowerCore and R-language platform programs to derive subsets for the original collections, capturing most of the variability. The study was constraint to use only climatic variable due to the limited evaluation and characterization data available. PowerCore was adapted to help in selecting core subsets, knowing that this approach requires information at allele level or evaluation data. The study has introduced also the Focused identification of Germplasm Strategy for selecting best bet for heat and drought tolerance, with the same limitation of limited evaluation data. Both MaxEnt and FIGS and also R-Language Platform facilitated approaches require a two stage development to more accuracy in selecting best bet.

Our study used only climatic data, because of limited information on characterization and evaluation.

7.6 Recommendations for further studies

As stated above, there are many aspects of research which deserve further investigations:

- Research is needed to assess the *Lathyrus* species diversity and threats in the regions identified as hot spots for various *Lathyrus* sections, and mainly to find out if the existing protected areas are serving the purpose of conserving wild relatives of *Lathyrus* and other crops of global importance;
- Research on assigning weights to different morphological characters will improve the taxonomic identification of species;
- Need to confirm the AFLP markers specific to *Lathyrus* sections and extend the analysis to the species taxon level, and need to use other DNA molecular techniques to gain more insights in taxonomic identification and in studying phylogenetic relationships among different taxa;
- *L. odoratus* was suggested as a member of the grass pea genepool 2. It will be good to assess its crushability and its chromosome and genomic affinities using appropriate cytogenetic methods;
- Upon enriching the *Lathyrus* database with evaluation information, FIGS approach and other approaches could be used to select best bet sets for adaptive traits which accuracy will be checked by evaluation of the selected accessions for the sought trait;

7.7 General conclusions

This work has allowed me to extend my knowledge to new areas including use of molecular markers in diversity analysis, use of several statistical programs and in applying Arc-View GIS, DIVA- GIS, FloraMap tools in studying the geographic distribution of species and in selecting core sets. This work has contributed to the efforts of conservation and sustainable use of *Lathyrus* genetic resources through:

- The compilation of a more comprehensive database on *Lathyrus* which is maintained at ICARDA for further use and enrichment with evaluation information; This information will enable students and future researchers to identify the gaps in the existing collections, to conduct traits-guided collecting missions and to spot areas for *in situ* conservation efforts;
- Identification in *ex situ* collections mainly for *Lathyrus* wild relatives and spotting areas for *in situ* conservation efforts of different *Lathyrus* sections, with the area around Tel Kalakh and Kalaa Al-Hosn in Syria as hot spot for priority *Lathyrus* species;
- Use of morphological characters and the produced field guide will continue to facilitate the taxonomic identification of *Lathyrus* species, but the use of molecular markers could add more information on the phylogenetic relationships among species;
- Core subsets were developed using different methods, and FIGS approach was used to derive the first heat and drought tolerance subset to be evaluated in the future.