

CONSERVATION AND USE OF CROP WILD RELATIVES IN MALAWI

by

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GENERAL ABSTRACT

The increase in global population comes along with increased demand for food supply and this brings in the urgency to increase the availability of genetic diversity to develop productive crop varieties. Crop wild relatives (CWR) provide such diversity and must be conserved if the global population is to benefit from it. Malawi plans to develop a national conservation strategy that guides systematic conservation of priority CWR. This PhD study contributes to this through development of a national CRW checklist and an inventory. The checklist has 446 taxa while the inventory comprises of 277 taxa priority to Malawi. The prioritisation was based on potential use in crop improvement, threat levels and endemicity. The study further analysed in situ and ex situ conservation gaps, projected impact of climate change on taxa richness and distribution in the next 50 years. Huge conservation gaps exist in Malawi with only three taxa conserved under ex situ and none is conserved in situ. Taxa outside protected areas (PAs) will be most impacted by climate change than taxa in PAs and these may need to be collected. Six PAs identified with broad range of the diversity should be condisered for active in situ conservation. The study also used simple sequence repeats (SSRs) markers to identify drought tolerance among Oryza accessions from Malawi Plant Genetic Resources Centre to promote use of these in crop improvement. The information generated will guide formulation of national conservation strategy and action plan that provides a road map for CWR conservation in Malawi.

ACRONYMS

APPSA: Agricultural Productivity Programme for Southern Africa

- CBD: Convention on Biological Diversity
- CIMMYT: International Maize and Wheat improvement Center
- **CWR: Crop Wild Relatives**
- DNA: Deoxyribonucleic Acid
- EAD: Environmental Affairs Department
- FAO: Food and Agriculture Organization of the United Nations
- **GBIF:** Global Biodiversity Information Facility

GCMs: General Circulation Models

- **GRIN:** National Genetic Resources Institute
- IPCC-AR: Intergovernmental Panel on Climate Change Assessment Report
- ITPGRFA: International Treaty on Plant Genetic Resources for Food and Agriculture
- IUCN: International Union for Conservation of Nature
- MG: Malawi Government
- MPGRC: Malawi Plant Genetic Resources Centre
- NBSAP: National Biodiversity Strategy and Action Plan
- NSO: National Statistic Office
- PAs: Protected Areas
- PCR: Polymerase Chain Reaction
- RCP: Representative Concentration Pathways
- SADC: Southern Africa Development Community
- SDGs: Sustainable Development Goals
- SSRs: Simple Sequence Repeats
- UN: United Nations
- **UNEP: United Nations Environment Programme**
- USD: United States Dollar

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To God be the glory, honour today and forever more! Amen!

DECLARATIONS

This Thesis represents original ideas of the author and with guidance of the supervisors. It has not been submitted to any other institution for the award of any form of certificate. Any piece of work or thesis section modified by supervisors or internal and or external examiners is acknowledged. All figures adapted from other sources were acknowledged. Below are the additional declarations for the workp presented in this Thesis;

Chapter 2: Development of a prioritised checklist of crop wild relatives for conservation in Malawi

Nolipher Khaki Mponya^{1&2}, Zacharia L. K. Magombo ³, Lawrent Pungulani¹, Joana Magos Brehm² and Nigel Maxted²

This chapter was published with African Crop Science Journal with DOI: <u>https://dx.doi.org/10.4314/acsj.v28i2.12</u>. The content presented here is similar to the paper published. The two co-authors (Zacharia Magombo and Lawrent Pungulani) were involved in species prioritization being the heads of national conservation institutions.

Chapter 3: *In situ* and *ex situ* conservation gap analyses of crop wild relatives from Malawi

Nolipher Khaki Mponya^{1,2*}, Tembo Chanyenga³, Joana Magos Brehm² and Nigel Maxted² This chapter was published with Genetic Resources and Crop Evolution Journal with DOI: 10.1007/s10722-020-01021-3. Contents are similar to what is presented here with exception of additional Tables included in this Thesis. Dr. Tembo Chanyenga commented on reserve selection for *in situ* conservation of crop wild relatives him having knowledge of the status of protected areas in Malawi.

Chapter 4: Climate change impact and conservation of crop wild relatives in Malawi.

Nolipher Khaki Mponya^{a &b}, Joana Magos Brehm^b & Nigel Maxted ^b

This chapter will be submitted for publishing with Genetic Resources and Crop Evolution. Contents of the manuscript to be submitted are similar to what is presented herein.

Prof. Nigel Maxted

Nolipher Khaki Mponya

Date: 30/01/2021

CHAPTER 1

INTRODUCTION

1.1 General Background

The demand and access to nutritious food has increased as 690 million people go hungry everyday and will continue to grow for the next thirty years as the world population triples by 2050 (UN, 2019). Increase in global population comes along with habitat fragmentation and extinction, overexploited environment and permanent diminishment of the base resource (UN, 2015). Sustainable supply of crop diversity plays a vital role in crop improvement and ensuring that future food demands are met (FAO, 2019, UN, 2019).

Maintenance and continuous supply of crop diversity is crucial especially in this era when the genetic base of most crop varieties is significantly narrowed due to continuous selection processes (Jarvis *et al.*, 2008). The diversity (variability of novel genes and alleles) will help improve crop's resilience to thrive better in adverse climatic conditions, which in most cases is characterized by the outbreaks of pests and diseases and occurrences of droughts and floods (FAO, 2012; Kaur *et al.*, 2018). Such resilience can also enable crop production in marginalized areas such as in salty and waterlogged conditions (FAO, 2015; FAO, 2018). Eventually this will result into improved crop performance, high yields and increase in food supply.

More to this challenge is added need to reduce the impacts of climate change, which most often overturns the performance of agricultural production and its associated ecosystems (FAO, 2019). With projected increase in global warming (IPCC, AR, 2019), the impact of climate change is expected to be felt more than before. In Sub Saharan Africa countries, the impacts will be greatest and mitigating action is required if the impact is not to be devastation (FAO, 2019). Crop wild

relatives harbour genes that could be tapped to improve crop's resilience to biotic and abiotic constraints and climate change adaptation of not only farmers by the entire food supply chain.

1.2. Definition of Crop Wild Relatives (CWR)

CWR are defined as plant species closely related to cultivated plants and these include their ancestors (Maxted *et al.*, 2006). Depending on their level of closeness to cultivated plants, CWR were classified into taxonomic and genetic pool groups. For instance, CWR that are taxonomically and genetically close to the cultivated plants are grouped into Taxon group 1 (TG1) and Primary gene pool (GP1b) respectively, and those on the next level as TG 2 and GP2, TG3 and GP3 and TG4) (Maxted *et al.*, 2006) see figures 1.1 and 1.2. CWR in TG1 and GP1b can cross with cultivated plants with no barriers and the resulting F1 generation is fertile while gene introgression in the other groups is possible through embryo rescuing or gene cloning (Hajjar and Hodgkin, 2007, Harlan and de Wet, 1971).

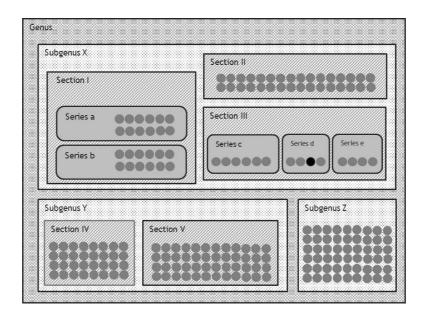


Figure 1. 1. Taxomic group concept as regards to classification of crop wild relatives. Figure adatpted from Maxted and Vincent 2021.

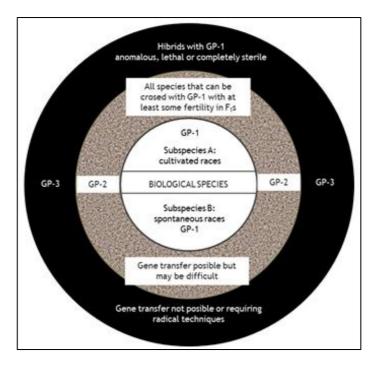


Figure 1. 2. Gene pool concept as regards to classification of crop wild relatives at gene pool level. Figure adatpted from Maxted and Vincent 2021.

Crop domestication history indicates that most cultivated crops were once in the wild and became domesticated through selection processes. (Maxted and Kell, 2009; Harlan and de Wet, 1971). In the past, use of CWR in crop improvement met with challenges of transfer of undesirable genes and sterility (Hajjar and Hodgkin 2007). Repeated backcrossing solved the challenge associated with transfer of undesirable genes while development and continued use of modern gene introgression technologies dealt with issues of incompatibility of the crosses and sterility (Hajjar and Hodgkin 2007). Further, the existence of these technologies added more value to CWR in crop improvement since transfer of a desirable gene is possible at all genetic or taxonomic levels (Jarvis *et al.*, 2008). Unlike landraces, CWR received less conservation attention primarily because of little understanding of their importance (Maxted *et al.*, 2011). No

wonder that most of the CWR were passively conserved globally (Castañeda *et al.*, 2016) and even now are not put under active conservation in many countries and this exposes them to a number of threats. Sustainable and systematic conservation of CWR will enable the global community to benefit from the CWR diversity.

1.3. Diversity and Use of CWR

Most diversity of CWR is found in the centres of crop origin also known as Vavilov centres named after Nikolai Ivanovich Vavilov who was among the first botanists to link diversity in crops to the centres where crops originated (Phillips, 2017, Freek, 1994). Diversity was also observed to occur in centres of domestication and non-diversity centres (Maxted, 2007, Maxted and Vincent, 2021). Vavilov centres and centres of crop diversification (Figure 1.3) fall in China (1), India and Sri lanka (2), Indo-Malayan (2a), Central Asia (3), Neat Eastern (4), Mediterranean (5), Abyssinia (6), Mesoamerican (7), South America (8), Ciloe, Chile , Brazil and Paraguayan region (8a and 8b), and West and East region of United States of America (9 and 9a), Coastal west Africa (10), East Africa (11) and Northern Australia (12), (Maxted and Vincent, 2021).

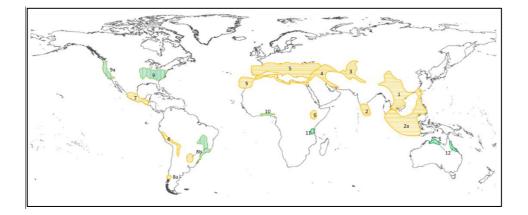


Figure 1. 3. World map showing distribution of Vavilov centres of crop origin and diversification. Map adapted from Maxted and Vincent 2021. (Orange horizontal hatched areas are original Vavilov centre and green vertical hatched areas are additional centres; Numbers refer to Vavilov Centre standard notation with additional number added for additional centres identified).

In African, most the diversity of CWR was observed in Coastal West, East Africa and this diversity is related to main food crops and such include; Rice, Sorghum, Pearl and Finger millets and Cowpeas (Maxted and Vincent, 2021). These crops are widely cultivated in the continent and makes this CWR diversity of high value for conservation. Common areas of occurrence of CWR include nature reserves, natural habitats, agricultural landscapes (abandoned and cultivated land) and in any area with conducive environment for their occurrence (Maxted and Kell, 2009).

Use of CWR in breeding dates back to 1800's with sugarcane being one of the early crops benefiting from the diversity in CWR (Ramdoyal and Badaloo, 2002). The potential of CWR to improve traditional landraces resilience to climate change and making them food secure continued to unfold with discovery of more desirable genes such as pest and diseases resistant (FAO, 2005). This contributed to the development of disease resistant and high yielding varieties of wheat, tomatoes, soya and maize that helped cushion the global food supply (FAO, 2005). Presence of genes that improve nutrition status of the crops (Global Crop Trust, 2016; Maxted and Kell, 2009) have potential to improve food nutritional quality through breeding.

For instance, nutritional studies by CIMMYT on some wheat wild species revealed that other species had higher levels of zinc (1.8 times more) and iron (1.5 times more) compared to the cultivated wheat varieties. Wild relative of wheat, *Triticum turgidum* var. *dicoccoides* from East Mediterranean was used to boost protein content in bread and a durum wheat (UNEP, 2004). *Triticum araraticum* Jakubz from Armenia is high in protein content compared to the cultivated wheat varieties (CIMMYT, 2004; UNEP, 2004). *Glycine soja* Siebold & Zucc. for example,

boosted protein content in soybean (UNEP, 2004). The diversity in CWR has been so vital in improving crop diversification and this has increased availability of different food types (FAO, 2012) and must therefore be sustainably conserved. Dietary diversification is so essential to human nutrition and health and availability of such genes is crucial in improvement of these crops of global economic and food security importance. This is of particular importance especially, when global projected population undernourishment keeps on increasing and when the number of under five children being malnourished is so high at present (UNICEF, 2016, World Food Programme, 2020).

An account of how CWR improved biotic and abiotic stress resistance is reported in Harlan and de Wet, 1971; Hajjar and Hodgkin, 2007; Maxted and Kell, 2009. For example, rice wild relatives *Oryza bathii, Oryza longistaminata and Oryza punctata, Oryza glaberrima, Oryza brachyantha, Oryza eichingeri* and *Oryza rufipogon* have genes for drought, salt, aluminium toxicity and cold tolerance, where as *Oryza nivara* has genes resistant to grassy stunt virus (Kiambi *et al.,* 2009). Grassy stunt virus resistant genes isolated from *Oryza nivara*, contributed to the development of rice resistant varieties in central India, which helped, stabilize rice yields across Asia region and this saved over US\$1billion in Asia region (http://www.economist.com/news/international/216 64194-wild-ancestors-worlds-most-important-crops-could-help-avert-devastating).

In America, blight resistant genes isolated from wild maize were used to combat corn blight in Mexican maize in 1970s (Maxted and Kell, 2009). Genes resistant to powdery mildew in wheat (*Triticum aestivum* L.), fusarium and nematode resistance genes were also isolated from wild tomato and used to improve these traits in the related crops (Maxted and Kell, 2009). Nematodes and fusarium resistant genes saved about US\$250 million in tomato industry (Castañeda *et al.*,

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2016) and use of late blight resistant genes from wild potato *Solanum bulbocastanum* was estimated to save over US\$ 400 million which United States of America (USA) incur annually due to late blight (UNEP, 2004). *Triticum boeoticum* Bois, a wheat wild relative was reported to contain genes resistant to fungal diseases that could be used to improve cultivated wheat varieties (Migui and Lamb, 2003). Wild America grape rootstocks provided resistance to grapes in Europe (Walker *et al.*, 2017) which were heavily attacked by an aphid like insect (*Phylloxera*) and genes of cold tolerance were also found in *Vitis amurensis*, wild relative of grapes (Reynolds, 2015).

Use of pests and diseases resistant crop varieties have a positive bearing on the environment as there will be reduced use of harmful pests control chemicals (non-biodegradable pesticides) that commonly pollute the environment (The Global Crop Trust, 2016). Reduced use of chemicals means safe and healthy environment (clean air, water, food *etc*) that improves human and biodiversity (including ecosystem processes) functioning (Ikeda *et al.*, 2016, The Global Crop Trust, 2016). As useful sources of desired genes of economic importance for crop improvement, CWR have potential to address food insecurity, environmental and health concerns in addition to boosting the economy. For instance, Pricewaterhouse Coopers (PwC) projected an increase in the economic value of use of CWR in crop improvement from US\$40 to US\$120 billion and thus for improving productivity and resilience alone of 29 global priority crops (PwC, 2013).

The availability of modern breeding techniques; embryo rescuing, soma clonal embryogenesis and gene cloning (genetic engineering) where a desirable gene is cloned into a mediator or transporter e.g. bacterial phage or plasmids will significantly improve the use of CWR in crop improvement although it will require substantial amount of investments (Kaur *et al.*, 2018). With the ability to transfer useful genes of sexually incompatible plants species, this will allow use of 7

distantly related plants with novel genes hence placing CWR on an important role in crop improvement (Meilleur and Hodgkin, 2004).

1.4 Threats to CWR

CWR like many other wild plants are faced with a number of threats and major to these include those influenced by human activities and threats related to climate change. All these lead to either loss of habitat or reduced number of species.

1.4.1. Direct human influenced threats

Human influenced threats include but not limited to changing agricultural farming practices, unsustainable harvesting or over exploitation, uncontrolled bushfires, infrastructural developments and increase in human population (Maxted and Kell, 2009; Kell *et al.*, 2013; UN, 2015). For instance, forestry bushfires in the USA destroyed about 4.9 million hectares of crops for the past years with this damage valued at US\$ 689 million (FAO, 2015). It was therefore likely that CWR occurring in these habitats were destroyed too.

Demographic dynamics have also a negative impact on survival and future availability of CWR. Increase in human population puts pressure on land for settlement and cultivation and brings in with high demand for food (FAO, 2015). This leads to habitat degradation, fragmentation, destruction as well as over exploitation of natural resources (Hunter and Heywood 2012). The consequence is loss or reduced diversity of CWR, wild plants and related biodiversity. Infrastructure development without guidance of environmental impact assessments may lead to unconsciously loss of the diversity of CWR and that of useful plants. The implication of such infrastructural development is disappearing of such CWR and associated biodiversity due to disturbance of their biosphere. Involvement of botanists, taxonomists and plant conservation

authorities in Environmental Impact Assessment processes in the proposed sites could ensure that potential habitats for CWR are maintained and taxa rescued before any developmental actions are undertaken.

Maxted and Kell (2009) and Hunter and Heywood (2012) cited introduction of invasive alien species, predominant use of improved varieties, war and political instability and in availability of conservation plan and strategies as other threats to continued survival of CWR.

1.4.2. Indirect human influenced threats (Climate change)

Threats related to climate change include natural disasters such as floods, droughts, pest and diseases outbreaks (UNEP, 2004). Future impact of climate change as expressed by increase in global warming, is expected to affect biodiversity in general by either reducing its genetic diversity and or its distribution due changes in ecological processes (Ikeda *et al.*, 2016, Scheffers *et al.*, 2016). A slight change in temperatures has a great impact on the structural and composition of species in an ecosystem with extreme temperatures causing species to undergo evolutionary adaptation (Cornell and Lawton, 1992). In the end, these may lead to species speciation, migration and extinction (Kraft *et al.*, 2011).

Lane *et al.*, 2007 projected a reduction in species richness of CWR of cowpeas; peanuts and potato in Mexico come 2050 due to impact of climate change. This impact will lead to the extinction of 14 species of CWR of potato and three species of wild cowpea. Ureta *et a l.* (2011) projected a reduction in distribution of Maize wild species between 2030 and 2050. Again, Contreras-Toredo (2018) investigated climate change impact projections on CWR indicated that, a relative diversity of CWR will be significantly impacted with climate change in Mexico and this will cause taxa migration. In Norway, climate change is expected to negatively impact on

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habitats causing a shift in taxa distribution due to change in habitat suitability (Phillips *et al.*, 2016).

Although such kind of studies have not been done in Southern Africa Development Community (SADC), the situation could be similar to the current Sub Saharan Africa countries where climate change is expected to have impact on biodiversity and environmental in general (Serdeczny *et al.*, 2016). FAO, (2015) estimates US\$400 billion as damage from natural disasters between 2003 and 2013 for developing countries (Africa, Latin America and Asia) with agricultural sector alone accounting for 84% of the loss. Although the impacts of climate change are complex and random, Southern Africa is consistently projected to be more vulnerable to climate change impact (ITPGRFA, 2009) calling for a policy support system.

In Malawi, climate change is expected to increase more stress on the environment and associated biodiversity (Nigel *et al.*, 2019). Unfortunately, Malawi continues to experience the negative impact of climate change that further threatens its biodiversity (Government of Malawi, 2010, Nigel *et al.*, 2019). For example, the damage by cyclone Idai in March 2019 on crops alone is estimated at US\$ 11.1 million and left about 87,000 people displaced and food insecure (Government of Malawi, 2019). Climate change was projected to increase number of warm days in the near future and this will lead to drying up of soils hence negatively affecting crop production (Mittal *et al.*, 2017, England *et al.*, 2018). This scenario requires among other interventions, development of resilient crop varieties. Further, Malawi has experienced a significant loss of natural habitats due to deforestation; land degradation, increased demand for settlement land and expansion of agricultural area and the current state of deforestation is so alarming requiring urgent actions (Nigel *et al.*, 2019; Government of Malawi, 2010).

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As part of disaster preparedness and management in mitigating climate change impact (reducing green gas emissions, increasing carbon sink), Malawi is aligning its policies to regional and global climate change policies on mitigating the impact of climate change (Department of Forestry, 2015). For instance, the enaction of the Environmental Management Act (EMA) 2017 provides key strategic actions that aim at reducing environmental degradation (Nigel *et al.*, 2019). Malawi's approach is to employ measures with the ability to reduce and adapt the impact of climate change (Government of Malawi, 2019). Most vital measures are those with long-term impact, which include re afforestation, crop diversification and use of climate smart agricultural technologies reflected in the national development priorities (Department of Forestry, 2015).

National Agricultural Policy considers among others crop diversification and development of resilient crop varieties as sustainable measures in management of climate change impact (Government of Malawi, 2016).

1.5. The need to conserve CWR

There is increased threats on CWR due to climate change, change in land use and habitat destruction (Jarvis *et al.*, 2008). The demand to use CWR diversity in crop improvement is on the rise due to increased demand for food (FAO, 2019). To increase food availability, there is a need to improve crop productivity and this shall require diversity from CWR (Maxted *et al.*, 2011; Dulloo, 2012). CWR remain the reliable sources of genes for future crop improvement programmes as they have adapted and developed unique diversity that cannot be obtained in the landraces and other crop varieties (Dempewolf *et al.*, 2014). Therefore, for their continued availability in the future, CWR must be sustainably conserved and national conservations institutes should make deliberate efforts to conserve these.

Crop gene pool diversity is a potent asset for climate change adaptation, food and nutritional security as well as development of resilient ecosystems (FAO, 2012; Kaur *et al.*, 2018). Wide genetic variations is needed for plant adaptation and their survival in harsh conditions (Olsen and Gross, 2008). The synthetic account of the second global plan of action for Plant Genetic Resources for Food and Agriculture (PGRFA) adopted from FAO second global plan of action (2nd GPA) recognises CWR as important contributors to local economies, food security and environmental health and encourages generation of conservation strategies and formation of *in situ* conservation networks of CWR (FAO, 2012).

Acknowledging the significance of CWR on economy, food and nutrition security, the global community advocates for their urgent and active conservation (CBD, 2000; ITPGRFA, 2009; FAO, 2012; UN, 2015b). The Aichi Biodiversity Strategic goal C; Targets 11, 12, 13 (<u>https://www.cbd.int/sp/targets/</u>) and Target 2.5 of the United Nations Sustainable Development goal number 2 have specific biodiversity strategic actions that promote sustainable conservation of plant genetic resources including CWR to check on their genetic erosion (UN, 2015b). However, the state of CWR conservation is still low globally.

Maxted *et al.* (1997) and Khoury *et al.* (2010) noted low representation of CWR diversity under *ex situ* conservation. Vincent *et al.* (2013) led the first step into sustainable conservation of global priority CWR through prioritization and production of an inventory of the available genetic resources of CWR in international genebanks in order to promote their use. Castañeda *et al.* (2016) did global assessments of conservation status of global priority taxa and found out that even taxa related to crops of global importance were not conserved under *ex situ.* Although this could partly be contributed to different conservation approaches adopted and practiced by 12

different conservation authorities, it could also mean that there is low *ex situ* representation of CWR diversity at national genebanks as it is a common practice that national PGR is duplicated with regional and global genebanks. These results mirror the conservation status of CWR in Malawi. This means that access to use such materials for crop improvement is limited and that this diversity is at threat of being lost if measures to safeguard it are not put in place.

1.6. Conservation techniques for CWR

A number of conservation techniques are recognized in PGRFA and these are grouped into two; *in situ* and *ex situ* conservation techniques.

1.6.1. In situ conservation of CWR

In situ conservation refers to all forms of conservation of taxa in their natural ecosystems in order to allow for continued evolution of the species (Maxted *et al.*, 1997c) and this forms a major conservation technique of CWR having aproxmately 90% of CWR not actively conserved globally (Maxted and Kell 2009). This technique allows for continuation of natural evolutionary processes (Maxted *et al.*, 2011), which lead to generation of additional diversity for the continued survival of the species (FAO, 2012). It also involves the maintenance and recovery of the viable species populations (CBD, 1992). Key aspects in *in situ* conservation include; site identification and defining management actions of the target species and the sites (Maxted *et al.*, 1997). With increasing need to breed for resilient crop varieties due to climate change, *in situ* conservation becomes one of the reliable techniques to generate the needed diversity in crop improvement. In the synthetic account of global plan of action by FAO, (2012), *in situ* conservation is considered a priority component in conservation of genetic resources and FAO encourages for development

of regional and global *in situ* conservation networks to enhance conservation capacity. Some notable forms of *in situ* conservation include;

i. Genetic reserves conservation: This involves conservation of CWR in their natural habitats ie in forestry reserves, parks, botanic gardens and other natural protected areas (Maxted and kell, 2009; Arora and Paroda, undated). The main emphasis in genetic reserve conservation is to actively conserve and protect certain amount of species diversity in designated areas by putting deliberate efforts to manage and periodically monitor the population dynamics of the target taxa (Matxed *et al.*, 1997b). It is considered one of the most cost effective conservation technique in that its maintenance and success does not necessarily require more financial inputs but rather the management practices of the site (Hunter and Heywood, 2011). This means that, before any designation, a site management and action plan should be reviewed.

ii. On farm conservation is a form of *in situ* conservation practiced by farmers where the crops and associated CWR are conserved on farm (Altieri and Merrick, 1987) in areas to which they are adapted and developed some distinctive characteristics (Maxted *et al.*, 1997b; Joshi and Upadhya, 2019). Sometimes this may also be refered to as conservation by use, is more applicable to conservation of crop landraces, and most practiced in traditional farming systems (Maxted *et al.*, 1997b). This technique allows for natural co-existence of the crops and associated wild or weedy plants hence allowing natural crossing. In this technique, the diversity is generated through random crossing among the mixed varieties of a landrace. The diversity is maintained through natural selection and deliberate planting of a mixture of seeds in the subsequent growing season.

iii. **Home garden conservation** is another form of *in situ* conservation where an individual farmer with a purpose of home consumption as well as maintenance of those traditional crops and related wild species (Maxted *et al.*, 1997b) grows a small population of different crops on small piece of land (Hogkin, 2001). In the case of Malawi, these are neglected crops but preferred by farmers for some other reasons. Although not often recognised, home gardening may provide additional diversity (Maxted *et al.*, 1997b) if this technique is formally recognised and encouraged among farmers.

1.6.2 Ex situ conservation technique of CWR

Ex situ conservation refers to conservation of components of biological diversity outside their natural environment (CBD, 1992). These include seeds, plant tissue, plant organ or the whole plant (Maxted and Kell, 2009). *Ex situ* conservation involves site mapping, collection and conservation of the colleted material outside its natural environment (Maxted, 1997c). Since conservation of plant genetic resources (PGR) started, *ex situ* conservation formed a major conservation technique of crop accessions held in national seed gene banks (FAO, 2012). FAO reports over 7.4 million accessions held under *ex situ* globally (FAO, 2012). Conserving CWR *ex situ* provides backup to the diversity in the wild and facilitates use of the CWR in crop improvement (Hunter and Heywood, 2012). With sufficient data about the distribution, diversity and knowledge about the taxa's reproductive cycle it should be possible to increase number of CWR accessions under *ex situ* conservation. More important to this technique is the need to maintain the indigenous information and ecological data associated with the collected samples otherwise the conserved samples become useless. Other forms of *ex situ* conservations include field gene banks, tissue culture, DNA banks and cryopreservation (FAO, 2013; Dulloo *et al.*,

2006; Reed *et al.*, 2004). Field genebank allows for conservation of vegetative propagated plants and plants with low seed dormancy for the target taxa (Saad and Ramanatha, 2001; FAO, 2013). Cryo-preservation is a technique that allows conservation of tissues and organelles at lower temperatures and commonly in liquid nitrogen (Reed *et al.*, 2004). This involves arresting of cell development and growth processes in the plant tissues and organs. Use of cryo-preservation in conservation has been limited due to technical and economic challenges faced by most national gene banks (FAO, 2012).

1.7. National approaches to CWR conservation

Two approaches are recognised in national conservation of CWR; Floristic approach and Monographic approach and simply refer to the scope of the conservation strategy being considered for the target taxa (Magos Brehm *et al.*, 2017). Maxted *et al.* (2011) define Floristic approach, as "conservation of taxa in a defined geographic area be it at sub regional, regional or the entire country level". As opposed to the Floristic approach, the monographic approach refers to conservation of taxa of a particular crop gene pool perceived as of value for conservation and may be carried out at any geographic scale (Maxted *et al.*, 2013). The floristic approach is applied at national, province, county or subcounty and targets all taxa occurring in that particular geographical area and monographic approach commonly targets the entire eco geographic distribution where CWR is native or known to occur (Maxted, 2003). Because of this, Floristic approach enables capturing of all taxa occurring in a given location but may not capture the whole diversity of the taxa observed in that particular area as diversity is dependent on geographic distribution of the target taxa (Maxted, 1997b). However, the success of both conservation approaches depend on the quality and quantity of data to be used, capacity and scope of the lead institution to develop the inventory and to do the conservation itself (Maxted *et al.*, 2011). A combination of both approaches give the complete information about the taxa, which could be useful in the development of the national conservation strategy (Maxted *et al.*, 2013). Although the responsibility of the conservation of the taxa rests in the hands of the national institution, it is recommended that whatever conservation approach and technique adopted for the conservation of CWR, consideration should also be paid to regional and global conservation priorities (Maxted *et al.*, 2011). This will ensure complementarity of conservation efforts in addition to ensuring there is interface among conservation strategies of different components of PGR (Maxted *et al.*, 2013).

1.8 Flora of Malawi

Malawi is endowed with diversity of natural resources. It is home to about 6000 plant species excluding 200 bryophytes (Msekandiana and Mlangeni, 2002). Out of these, 237 species are known to be endemic or near endemic to Malawi (Hyde *et al.*, 2020) and 26 taxa known to be endemic or near endemic to Malawi (globally these taxa, are known to occur in Malawi only) are also threatened globally (Hyde *et al.*, 2020b). Vascular plants species are estimated at 3765 with 50 among the list of endemic species (Hyde *et al.*, 2020). Malawi falls in the Zambezia region making the flora of Malawi to be included in the Flora of Zambesiaca (Mwafongo *et al.*, 2010; Hyde *et al.*, 2018). The Zambeziaca region covers areas of the Zambezi River basin which include; Botswana, Malawi, Mozambique, Zambia, Zimbabwe and Caprivi Strip and is rich in plant species diversity (Mwafongo *et al.*, 2010) and most species diversity is captured in protected areas (PAs) (Department of Forestry, undated).

Malawi has a total area of 118,484 km² and about 5650km² is under agriculture and 23,677km² (25% of the land) is under forest cover (Government of Malawi, 2018). About 22, 857km² is under Miombo woodlands while the remainder under plantations (Government of Malawi, 2018). Protected area planet data base identified 131 sites as PAs in Malawi and these include; National Parks (5), Forest Reserves (118), Wildlife Reserves (4) conservation area (1), world heritage site (1), Ramsar Site, wetland of international importance (1) and UNESCO-MAB Biosphere Reserve (1) (UNEP-WCMC (2020)). Out of 131 PAs, only 99 are formally registered and those recognised out of these, include, 87 Forest Reserves, 5 National Parks, 4 Game Reserves and 3 Sanctuaries (Government of Malawi, 2018). Gazetted as early as 1900's, only 19 PAs have management plans and management effectiveness evaluations (UNEP-WCMC (2020); Department of Forestry, undated). About 63% of the forest cover is located on customary land and is managed by traditional leaders and the communities (Department of Forestry, undated).

Most of these PAs are characterized with typical Brachystegia woodlands with wide grassy areas, ferns and epiphytic orchids most common among forest reserves (Department of Forestry, undated). Because Malawi is relatively rich in biodiversity, these PAs serve as tourists attraction sites (Government of Malawi, 2018). Malawi reserve areas have been reliable catchment areas of many waters bodies and sustaining different ecosystems and providing large proportion of carbon sink (Environmental Affairs Department, 2015; FAO, 2016).

Through community based natural resources management groups, communities have benefited from the ecosystems services the forest reserves offer (Department of Forestry, undated). However, due to unsustainable harvesting of the natural resources with 2.3% annual deforestation rate, there has been a rapid decline of the forest cover (Mauambeta *et al.*, 2010). To avert this, a 18

number of initiatives in line with Aichi target 11 to contain the rate at which natural resources are utilized were implemented such as development of forests national inventory, forestry landscape restorations and development of forest cover maps and forest monitoring system (Department of Forestry, 2015). This significantly contributed to sustainable management of forest and its associated ecosystem in Malawi (Government of Malawi, 2018). Some PAs like Nyika National Park in the North, borders North Luangwa National Park on Zambia side creating a Malawi –Zambia transfrontier conservation area that covers 6494km² (UNEP-WCMC, 2020;) and this provides an opportunity for regional *in situ* conservation network.

1.9. Current Conservation Status of CWR in Malawi

Malawi endorsed its commitment to conservation of plant genetic resources in early 1990's through establishment of the national plant genetic resources centre; officially known as the Malawi Plant Genetic Resources Centre (MPGRC). This was formed as part of the Southern Africa Development Community (SADC) Plant Genetic Resources Conservation network. It became a member of the Food and Agriculture Organization of the United Nations (FAO) in 1965 and later ratified to international bodies that advocate for conservation of plant genetic resources that include the Convention on Biological Diversity (CBD) in 1994 and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) in 2004. Apart from meeting the obligation to global biodiversity conservation bodies, Malawi recognises the need to conserve PGRFA (Mponya *et al.*, 2020).

For the past two and half decades, MPGRC's focus was on collection and conservation of crop diversity and during this period, MPGRC managed to collect and conserve under *ex situ* approximately 5,600 collections from 1,121 plant species. Most (80%) of which being crop

landraces, 19.5% being forests genetic resources and fodder crops and $\leq 0.5\%$ being wild relatives. Advancement in collections methods of crops and vast knowledge of where crops are cultivated aided such huge collection. On the other hand, lack of information on CWR prevented collection and their conservation.

Malawi is rich in plant diversity currently having an estimate of about 5,017 native plant species (Hyde et al., 2018). With four unique agro ecologies zones and having such a diversity of native species, occurrence of a high diversity of CWR is expected. FAO, (1996) state of plant genetic resources report for Malawi give similar assertions of occurrence of a diversity of CWR species. However, there is in adequate and sketchy information of the type, number and range distribution of these taxa and this limited exploration and systematic conservation of the same. Malawi registers a number of crop wild relatives such as Oryza longistaminata A.Chev.& Roehr., O.barthii A.Chev., Coffea mufindiensis Bridson subsp. pawekiana (Bridson) etc. These CWR have potential for crop improvement in traits such as drought tolerance, pests and disease resistance (Mponya et al., 2020, Hajjar and Hodgkin, 2007) and these could be sources of genes in the development of resilient varieties. Malawi has a number of breeding programmes that could benefit from these traits and presence of CWR of major food crops like rice, sorghum, cowpea and important cash crops such as cotton, coffee and sugarcane provides an opportunity for improvement of these crops. At present, CWR are reported to be used directly by farmers and the rural communities as food from the wild and are also harvested for medicinal purposes but there is no documentation on use in breeding programmes.

In order to facilitate use of these CWR and safeguard this treasure, Malawi needs to collect the diversity of such potential CWR taxa, put it under *ex situ* conservation, and promote active *in* 20

situ conservation of the taxa. Nevertheless, due to lack of proper documentation on the distribution of the crop wild relatives, in availability of the national taxa checklist and inventory, it has been until now practically impossible to conserve these CWR taxa under *ex situ* and *in situ*. Malawi will need to develop a national inventory, analyze conservational gaps *in situ* and *ex situ* to guide the formulation of the conservation actions as well as demonstrate the importance of conserving such germplasm under *ex situ*.

Currently, there is no systematic and active conservation of CWR in Malawi. Apart from National Biodiversity Strategy and Action Plan (NBSAP) (Environmental Affairs Department, 2015) that guides general biodiversity conservation in Malawi, there is no specific strategy on conservation of CWR. Until recently, there has been no deliberate efforts by countries including Malawi to have policies and strategies put in place for conservation of CWR. Most existing conservation strategies lacked interface and this has left issues of conservation hanging (Maxted, 2003) calling for a standalone conservation strategy. Although the government recognises the need to conserve these resources, in the absence of such a conservation strategy, sustainability cannot be guaranteed. Development of a prioritized national checklist is one of the main conservation planning stages that guides other processes (Magos Brehm *et al.*, 2017).

From their study, Khoury, (2010) and Castañeda *et al.* (2016) recommend development of national checklists to guide *ex situ* collections and for systematic and sustainable conservation on the same. Maxted *et al.* (2007) and Magos Brehm *et al.* (2017) recommend for development of national action plans and conservation strategies. Malawi plans to develop a national conservation strategy that will guide systematic conservation of CWR under *in situ* and *ex situ*. However, for sustainability and relevance, the formulation of the strategy should be guided by 21

scientific based evidence to help design better management options and effective conservation actions. Malawi required conducting studies on CWR to unveil its status and identify conservation gaps to inform decision in the development of the national conservation strategy. The strategy will facilitate systematic conservation as well as use of priority CWR. With the availability of inventory with a list of CWR potential for crop improvement, MPGRC will use this to lobby for their use by breeders and other users. The national strategy will have a road map on *in situ* and *ex situ* conservation actions for an individual taxon or a group of taxa. It is also envisaged that, the national conservation strategy will help communicate Malawi's conservation plan in a more defined and organised manner to different stakeholders.

1.10. Study Objectives

The overall objective of this PhD study was to contribute to systematic and sustainable conservation and use of CWR in Malawi by guiding the formulation of the national conservation strategy of CWR in Malawi and specifically to

- i. Develop a national prioritized checklist of crop wild relatives for their immediate conservation (Chapter 2)
- ii. Analyze *in situ* and *ex situ* conservation gaps of the priority taxa (Chapter 3).
- Model mid- and long-term impact of the future climate change on the priority taxa and identify taxa most impacted by climate change across different climatic scenarios (Chapter 4)
- **iv.** Establish the distribution of drought tolerance genes from *Oryza* accessions (wild species and cultivated) conserved at MPGRC in order to promote its use and for further exploration in rice improvement (Chapter 5).

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CHAPTER 2

DEVELOPMENT OF AN INVENTORY OF CROP WILD RELATIVES FOR CONSERVATION IN MALAWI

ABSTRACT

The national increase in human population in Sub-Saharan Africa (SSA) demands for more food; while increase in the impact of climate change, demands for resilient agricultural production systems, and both call for improved agricultural productivity. Plant breeders will need adaptive traits to improve crop productivity and resilience. Crop wild relatives (CWR) have the potential to offer the much needed diversity for crop improvement, but their diversity is inadequately conserved. Lack of knowledge about their occurrence in Malawi, limits their systematic conservation and utilisation. Developing a CWR national inventory helps to define conservation priorities and actions. The objective of this study was to match checklists of crop genera and national flora, using their taxonomic and genetic relatedness information. This resulted into the first comprehensive annotated checklist of 446 CWR taxa in Malawi, which was prioritized by a set of criteria previously agreed with national stakeholders based on socio-economic importance of the related crop, potential use of the wild relative in crop improvement and threat status. The inventory comprises of 277 CWR taxa, identified as priority for conservation in Malawi; of which 78% were native. These belong to 54 genera and are related to 56 food, fodder, spices and beverage crops; and include taxa related to crops of regional and global importance. Eighty-seven taxa of highest priority for conservation were further identified, 12.6% of which have confirmed uses in crop improvement on pests and disease resistance, drought tolerance and yielding ability. The inventory will facilitate effective conservation and availability of these taxa for their use in crop improvement

Key Words: Annotated checklist, national inventory, systematic conservation

2.1. INTRODUCTION

Crop wild relatives (CWR) have potential for contributing to improved global food and economic security in that they are donors of adaptive genes for crop improvement (Harlan and de Wet, 1971; Hajjar and Hodgkin, 2007). Maxted et al. (2006) defined CWR as wild plant species, genetically close to cultivated plants. The use of CWR in improving crop adaptation to abiotic and biotic stresses dates back to 1800's (Ramdoyal and Badaloo, 2002). Evidence of gene transfer from CWR to cultivated plants was reported in a number of studies (Hajjar and Hodgkin, 2007; Maxted and Kell, 2009; Ishimaru et al., 2010), and therefore, the need to manage the diversity in CWR and make it available and accessible to plant breeders at all levels is inevitable. The need to conserve CWR is also recognised in global instruments such as Global Plan of Action of the Food and Agriculture Organisation of the United Nations (FAO, 2012); the Sustainable Development Goals 2, sub-item 2.5 and 15 sub items 15.4, 15.5 and 15.6 (UN, 2015). It is also echoed in Aichi targets on Biodiversity Strategic goal C, Targets 11, 12 and 13 (https://www.cbd.int/ sp/targets/), the Convention on Biological Diversity (CBD), Global Strategy on Plant Conservation (GSPC) (CBD, 2000), the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, 2009) and the joint notification of the CBD/ITPGRFA/CGRFA/ Bioversity (CBD, 2019) that countries like Malawi are a party to. The national gene bank of Malawi manages the gene pool diversity of local crops, in an effort to improve utilisation of genetic diversity in the national breeding programmes. But due to limited resources, conservation thus far, has only covered landraces, excluding CWR diversity. The FAO (1996) state of the country report on plant genetic resources indicates the occurrence of some CWR in Malawi, but to use these in crop improvement programme requires effective conservation (Dempewolf et al., 2014). The objective of this study was to develop a national 35

inventory of CWR based on their native status, national and global distribution (rarity and/or endemicity), threat status, potential use in crop improvement, and importance of related crop to facilitate their conservation and use.

2.2. MATERIALS AND METHODS

2.2.1. Crop wild relatives general checklist

A floristic approach was used in the development of Malawi's CWR checklist. The procedure followed those outlined in the Interactive Toolkit for CWR Conservation Planning (Magos Brehm et al., 2017). First, a crop genera checklist was compiled, with information from Flora of Malawi, using cultivated plant families (Hyde et al., 2018); and useful plants in Malawi (edible and cultivated) (Williamson, 2005). Crops of global economic importance from Annex 1 of the Plant Treaty (ITPGRFA, 2009), crops listed in FAOSTAT (FAOSTAT, 2016); crops from national agricultural production estimates and a crop checklist from the Malawi Plant Genetic Resources Centre (MPGRC) accession database were also used. The crop genera checklist included crops cultivated and those not cultivated in Malawi, but were of regional and global importance and have wild relatives occurring in Malawi. The main reason for including crops not cultivated in Malawi was to capture CWR diversity that under pins the Southern Africa Development Community (SADC) and global food security (ITPGRFA, 2009; Allen et al., 2019). Second, a national flora checklist was compiled with data from global and national databases, which included The Royal Botanic Gardens-Kew (2017), Global Biodiversity Information Facility (GBIF, 2017), the Flora of Malawi (Hyde et al., 2018), National Herbarium and Botanic Gardens of Malawi, Useful plants of Malawi (Williamson, 2005) and MPGRC as these maintain collections of wild species of Malawi. For herbarium specimens, the process

involved image capture, digitization and taxonomic name check. The Plant List was the main reference for taxomic name check (The Plant List, 2013).

Finally, the national flora checklist was matched against the crop genera checklist to produce a national CWR checklist (Figure 2.1). The checklist was annotated with crop commodity groups' information that included main use of related crop such as food, fodder, beverage, oil and food and fiber crops based on the Department of Agricultural Research Services (DARS) System, with reference to FAO (n.d.) crop commodity groups' classification. Information about related crops, gene pool and taxon group concepts were sourced from the USDA, Agricultural Research Service, National Plant Germplasm System (2018) and the Harlan de Wet inventory (Vincent et al., 2013) guided by definition and classification of CWR (Harlan and de Wet, (1971) and Maxted et al. (2006). National and global distributions were sourced from Flora of Malawi (Hyde et al., 2018) and GRIN Taxonomy (USDA, Agricultural Research Service, National Plant Germplasm System, 2018) and Red List threat status information sourced from plants red list data sources (Raimondo et al., 2009; IUCN, 2018) provided aproxy indication of the taxa threat status at regional and global levels. The Plant List (2013), the USDA, Agricultural Research Service, National Plant Germplasm System (2018) and Wiersema and León (2016) were instrumental in sorting out species nomenclature and synonyms. The checklist was then compared with the inventory of priority CWR of the SADC region (Allen et al., 2017; Allen et al., 2019) developed through the SADC Crop Wild Relatives project (http://www.cropwild relatives.org/), to ensure that taxa of SADC regional importance were captured.

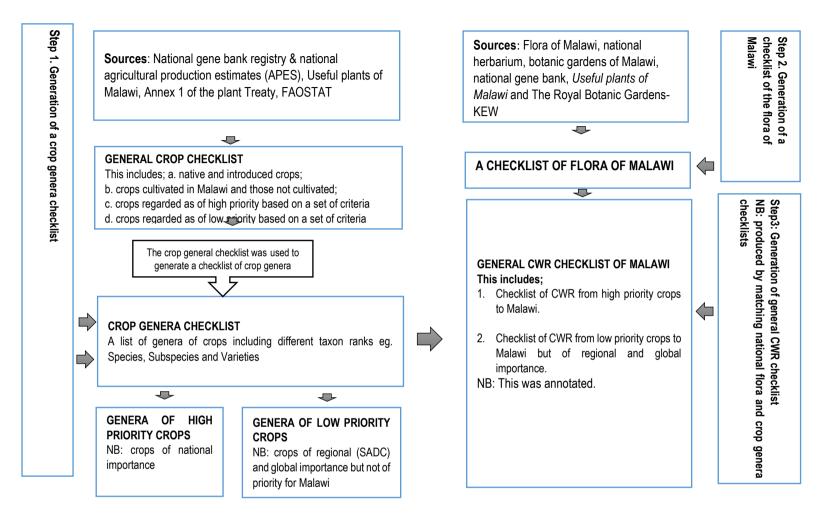


Figure 2.1: Processes in generation of Crop Wild Relatives (CWR) checklist for Malawi

2.2.2. Prioritization of the CWR checklist to develop a national inventory of CWR

The prioritization process involved relevant national stakeholders, and was carried out in two steps (Figure 2.1); namely (i) defining crops regarded as of high priority for Malawi and their wild relatives; and (ii) prioritization of the wild relatives of crops of low priority to Malawi (Figure 2.2). The process is as described below. It is worth noting that CWR of low priority to Malawi are of SADC region and global importance and that makes them of value for conservation.

2.2.3. Defining crops of priority for Malawi and their wild relatives

National stakeholders were involved in identifying crops that are priority to food security in Malawi. This was during a National Stakeholders (Suplementary Table 1) Consultative Workshop held on the 19th October 2017 at Silver Sands Resort in Salima, Malawi. Experts included those from the fields of plant breeding, crop production (field and horticultural crops), pasture agronomy, taxonomy, statistics, natural resources conservation and those responsible for national plant genetic resources conservation. Stakeholders were involved in order to bring in relevance of the checklist to the users and to encourage ownership and use of the priority checklist in national conservation and utilisation of CWR.

The selection of the crops regarded as priority to Malawi were based on (Suplementary Table 2);

Crop economic importance: role of the crop as foreign exchange earner and ability to boost local economy based on national economic analysis reports (FAO, 2019)

Food security: main food and fodder crops with multiple uses (used raw, processed and its by-product) and that are utilised in the country across seasons measured by production quantity and foreign exchange value (FAO, 2019).

Climate change adaptation: crops known to be adaptable to extreme weather conditions; in Malawi e.g. sorghum is associated with drought tolerance.

Nutritional value: crops mainly regarded as of high nutritional content and are readily available to the majority of the population in Malawi.

Medicinal value: crops with benefits to human health.

Potential for value addition: crops with potential for commercialisation

Crops were matched against each criterion outlined above, and those that qualified for one or more of the six criteria were regarded as of high importance to Malawi; hence of high priority and therefore their CWR were also regarded as priority for conservation in Malawi.

2.2.4. Prioritization of the wild relatives of crops of low priority to Malawi

Prioritization of the wild relatives of low priority crops to Malawi was carried out in order to capture CWR taxa of SADC region and global importance but were threatened, with the aim of rescuing them. Prioritization criteria used were a combination from those suggested by Hunter and Heywood (2011), as well as those used to prioritise Jordan vascular plants species (Magos Brehm *et al.*, 2016). Five criteria outlined below were selected for the prioritization. Additional information such as taxon nativeness, threat status, geographic distribution and gene pool and taxonomic groups in relation to the CWR prioritization criteria was gathered. Taxa that qualified for one or more criteria below were selected as priority for conservation.

Taxon native status. Taxa known to be native to Malawi or introduced to the country and adapted to local conditions, but not invasive, the native species were prioritized.

Taxon national distribution. This was based on the taxon range distribution within the country based on number of regions of occurrence. Taxa with wide range distribution had a chance of surviving than those with restricted range distribution; and these may be rare or endemic hence, were given higher priority for conservation.

Taxon global distribution. This refers to worldwide distribution of the taxon. The likelihood of losing taxa with restricted geographic distribution due to localised threats and climate change impacts is high compared to those with a wide range distribution; and hence the former must receive more conservation attention than the latter. This was categorised as: (i) endemic to Malawi; (ii) occurring in Malawi plus two countries in the SADC region; (iii) endemic to the SADC region, (iv) occurring in all tropical African countries and outside Africa. For this, priority was given to taxa endemic to Malawi.

Potential use of taxon in crop improvement. Taxonomic and genetic relatedness of taxon to the crop based on taxon and gene pool group concepts determines how easily these wild relatives can be used for crop improvement (Harlan and de Wet, 1971; Maxted *et al.*, 2006). GRIN taxonomy (USDA, Agricultural Research Service, National Plant Germplasm System., 2018), the Harlan and de Wet CWR inventory (Vincent *et al.*, 2013) and literature (Plaza *et al.*, 2014) were the sources of the required information. For taxa whose gene pools were not explicitly documented, the taxon group concept proposed by Maxted *et al.* (2006) was used to assign species taxonomic groups based on the classification information about the taxa.

This was done by matching CWR taxa with the genus, subgenus, and species and/or series of its cultivated taxa (Figure 1.1) based on a general definition of a CWR. Species that fall in TG1b, TG2 and TG3 and those in GP1b and GP2, regardless of their native and the assigned global or national threat status were considered of high priority for conservation as they have highest potential use in crop improvement (Harlan and de Wet, 1971; Maxted *et al.*, 2006)

Taxon threat status. Level of threat of the wild relative based on the Global IUCN Red Listing found on:http://www.iucnredlist.org, and South African plants (Raimondo *et al.*, 2009). South Africa red listing results were used because no recent threat assessments on vascular plants have been done in Malawi. Moreover, Malawi's 2002 species red listing (Dombo *et al.*, 2012), included only one taxon for CWR. South Africa was an alternative due to its record of high diversity of flora in the SADC region, and its assessments included substantial number of species. Therefore, to have an overview of species threat levels within the SADC region and at global level, red listing results by IUCN and South Africa were used as proxy indicator for the threat status of Malawi's CWR species. Species that were Critically Endangered (CR), Endangered (EN), Vulnerable (VU) and Near threatened (NT) were of high priority for conservation regardless of their taxonomic, gene pool concept and national priority category.

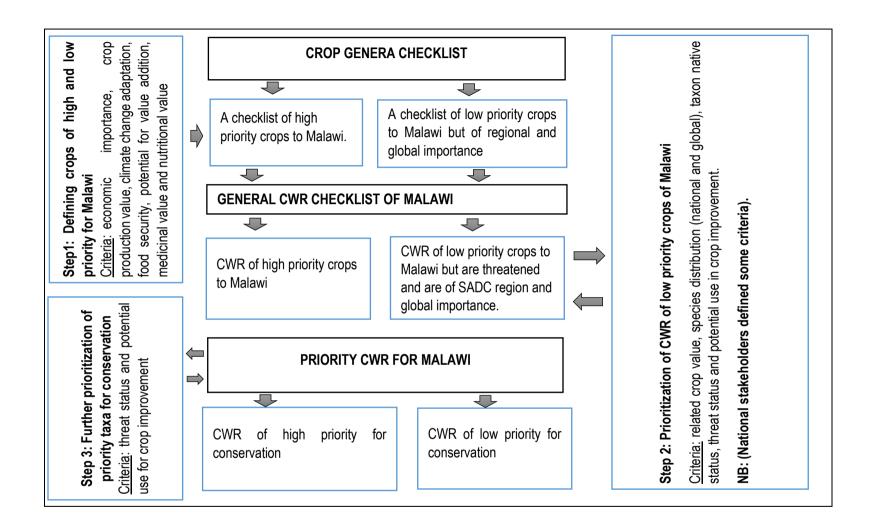


Figure 2.2: Steps in defining an inventory of CWR for conservation in Malawi

2.3. RESULTS

2.3.1. The general CWR checklist

The crop genera checklist had 131 crops (103 cultivated and 28 not cultivated in Malawi) and these are from 117 genera (Table 2.1). The plant occurrence data used had 1,173 taxa from the flora of Malawi; and after matching against the crop genera checklist, 446 taxa were identified as CWR related to 76 crops, belonging to 68 genera within 22 families. Out of the 446 CWR, 74.7% of the taxa were native to Malawi. The largest numbers of taxa were recorded in the families of Poaceae (133), Leguminosae (83), Lamiaceae (42), Convolvulaceae (34) and Solanaceae (33). About 60% of the CWR in the checklist were related to food crops, 26% to fodder crops, and 8% to crops in the category of spices; while 6% belonged to confectionery, fiber and oil seed crops. Eight and half percent of 446 taxa had been assessed in the IUCN Red List of Threatened Species and the South African Plants Red Listing (Raimondo *et al.*, 2009) included 34.8% of 446 taxa and 4.3% of which was assessed at both global level and in South Africa. Threat assessments data from South Africa and IUCN revealed that eight taxa were threatened.

Crop	Genus	Cultivation status in Malawi	Priority level
Acorn Squash	Cucurbita	С	LP
Adzuki Bean	Vigna	С	LP
Air yam	Dioscorea	С	LP
Almond	Prunus	С	LP
Amaranth	Amaranthus	C/W	HP
Apple	Malus	С	LP
Apricot	Prunus	С	LP

Table 2.1: Crops and crop genera used to generate a general checklist of crop wild relatives occurring in Malawi with their cultivation status and priority levels based on the prioritization criteria

Сгор	Genus	Cultivation status in Malawi	Priority level
Asparagus	Asparagus	С	HP
Bambara Groundnut	Vigna	С	HP
Banana	Musa	С	HP
Barley	Hordeum	NC	HP
Beet	Beta	С	LP
Black Mustard	Brassica	С	LP
Black pepper	Piper/Peperomia	С	LP
Blue berries	Vaccinium	С	LP
Bread fruit	Treculia	С	LP
Breadfruit/Jackfruit	Artocarpus	C/W	LP
Cabbage	Brassica	С	LP
Cacao	Theobroma	NC	LP
Cardamom	Aframomum	С	LP
Carrot	Daucus	С	LP
Cashew	Anacardium	С	HP
Cassava	Manihot	С	HP
Castor oil	Ricimus	W	LP
Cat's whiskers	Cleome	C/W	LP
Centro	Centrosema	С	LP
Cherry	Prunus	С	LP
Chickpea	Cicer	С	LP
Cinnamon	Cinnamomum	С	HP
Coco yam/taro	Colocasia	С	LP
Cocoyam	Xanthosoma	С	LP
Coffee	Coffea	С	HP
Common bean	Phaseolus	С	HP
Cotton	Gossypium	С	HP
Cowpea	Vigna	С	HP
Crotalaria	Crotalaria	NC	LP
Cucumber	Cucumis	С	LP
Desmodium	Desmodium	C/W	LP
Eggplant	Solanum	С	LP
Faba Bean	Vicia	С	HP
Finger Millet	Eleusine	С	HP
Fish bean	Tephrosia	C/W	LP
Garden peas	Pisum	C	LP
Garlic	Allium	C	LP
Ginger	Zingiber	C	HP
Gourds	Lagenaria	C	LP
		-	45

Сгор	Genus	Cultivation status in Malawi	Priority leve
Grape	Vitis	С	LP
Grapefruit	Citrus	С	LP
Green grams	Vigna	С	LP
Groundnut	Arachis	С	HP
Hyacinth beans	Lablab	С	LP
Leek	Allium	С	LP
Lemon	Citrus	С	LP
Lentil	Lens	С	LP
Lettuce	Lactuca.	С	LP
Livingstone potato	Plectranthus	C/W	LP
Macadamia	Macadamia	С	HP
Macrotyloma	Macrotyloma	C/W	LP
Maize	Zea	С	HP
Mango	Mangifera	С	LP
Melon	Cucumis	С	LP
Millet (Panicum)	Panicum	С	LP
Millet (Setaria)	Setaria	С	LP
Moringa (Drumstick tree)	Moringa	С	HP
Mustard	Brassica	С	HP
Oat	Avena	NC	LP
Okra	Hibiscus	С	LP
Olive	Olea	NC	LP
Onion	Allium	С	LP
Orange	Citrus	С	LP
Papaya	Carica	С	LP
Peach	Prunus	С	LP
Pear	Pyrus	С	LP
Pearl Millet	Pennisetum	С	HP
Pepper	Capsicum	С	LP
Pigeon Pea	Cajanus	С	HP
Pineapple	Ananas	С	LP
Plum	Prunus	С	LP
Potato	Solanum	С	HP
Pumpkin	Cucurbita	С	LP
Purple bush bean	Macroptilium	C/W	LP
Quinoa	Chenopodium	С	LP
Rape	Brassica	С	HP
Raspberry	Rubus	С	LP

Сгор	Genus	Cultivation status in Malawi	Priority level
Rhodes grass	Chloris	С	HP
Rice	Oryza	С	HP
Rye	Secale	NC	LP
Safflower	Carthamus	NC	LP
Sesame	Sesamum	С	HP
Sorghum	Sorghum	С	HP
Soybean	Glycine	С	HP
Spinach	Spinacia	С	LP
Strawberry	Fragaria	С	LP
Sugarcane	Saccharum	С	HP
Sunflower	Helianthus	С	LP
Sweet potato	Ipomoea	С	HP
Tea	Camellia	С	HP
Teff	Eragrostis	С	LP
Tobacco	Nicotiana	С	HP
Tomato	Lycopersicon	С	LP
Turnip	Brassica	С	LP
Urd Bean/Mung bean	Vigna	С	LP
Velvet beans	Мисипа	C/W	LP
Vetch	Vicia	С	LP
Water melon	Citrullus	С	LP
Water Yam	Dioscorea	С	LP
Wheat	Triticum	С	LP
White Guinea Yam	Dioscorea	С	LP
Yellow Yam	Dioscorea	С	LP
Milkvetch	Astragalus	NC	Annex 1 IT (LP)
Jack bean	Canavalia	NC	Annex 1 IT (LP)
Scorpion vetch	Coronilla	NC	Annex 1 IT (LP)
Alpine sweetvetch	Hedysarum	NC	Annex 1 IT (LP)
Grasspea	Lathyrus	NC	Annex 1 IT (LP)
Lespedeza (all varieties)	Lespedeza	NC	Annex 1 IT (LP)
Trefoil	Lotus	NC	Annex 1 IT (LP) Annex 1 IT
Lupin	Lupinus	NC	(LP)

Сгор	Genus	Cultivation status in Malawi	Priority level
Alfalfa	Medicago	NC	Annex 1 IT (LP)
Melilot,	Melilotus	NC	Annex 1 IT (LP)
Common sainfoin	Onobrychis	NC	Annex 1 IT (LP)
Bird's-foot	Ornithopus	NC	Annex 1 IT (LP)
African mesquite, iron tree	Prosopis	NC	Annex 1 IT (LP)
Puero, Tropical Kudzu	Pueraria	NC	Annex 1 IT (LP)
Clovers	Trifolium	NC	Annex 1 IT (LP)
Broomsedge	Andropogon	NC	Annex 1 IT (LP)
Crested wheatgrass	Agropyron	NC	Annex 1 IT (LP)
Redtop	Agrostis	NC	Annex 1 IT (LP)
Meadow foxtail	Alopecurus	NC	Annex 1 IT (LP)
False oat-grass	Arrhenatherum	NC	Annex 1 IT (LP)
Grass, Orchard	Dactylis	NC	Annex 1 IT (LP)
Blue fescue	Festuca	NC	Annex 1 IT (LP)

IT are crops of global importance according to the International Treaty on Plant Genetic Resources for Food and Agriculture. LP = Low priority, HP = High priority, C = Cultivated, NC = Not cultivated, C/W = Cultivated but also occur in the wild

2.3.2. Prioritized crop wild relatives

Out of 131 crops used to generate crop genera, 33 crops were identified as of high priority based on their role in food (including feed) and nutrition security, climate change adaptation and their economic importance and potential for value addition. However, only 24 crops had CWR occurring in Malawi, and these had 158 CWR taxa. Forty-one CWR taxa from this group had potential use for crop improvement. For the 98 low priority crops, only 37 crops had CWR occurring in Malawi. In total, these 37 low priority crops registered occurrence of 288 CWR taxa, of which after prioritization, 119 taxa were identified as priority crops. From this category, thirty-two CWR taxa had potential use in crop improvement, four taxa were endemic to Southern Region of Malawi, one taxa was threatened at global level, and the rest were not assessed but were selected based on their national distribution status.

2.3.3. The National inventory

The national inventory had 277 priority CWR taxa (from both high and low priority crops), and were from 54 genera related to 56 crops across 19 plant families. Most of them were in the families of Leguminosae (79), Poaceae (74), Convolvulaceae (34) and Solanaceae (33); while the rest of the families had less than 20 taxa. About 78% of the taxa in the national inventory were related to crops that were rated as of high value in terms of food, feed and nutritional security, economic importance and potential for value addition and adaptation to climate change. Examples of such crops included coffee, cotton, cowpeas, rice, sorghum, sugarcane, asparagus, black pepper, sweet potato and cassava (Tables 2.2 and 2.3). A total of 164 CWR taxa in the inventory were related to crops of global importance (Vincent *et al.*,

2013), 34 taxa were also included as priority in the SADC region (Allen *et al.*, 2017; 2019), and 21 CWR taxa were priority in Malawi, the SADC region and at global level (Table 2.2). Out of the 277 taxa, 78% were native, 5.8% were introduced to Malawi, and the status of 45 (16.3%) taxa was not specified (Table 2.2). Although results reveal that several priority taxa had a restricted range distribution within Malawi, 87% of 277 taxa occurred in more than one country.Within Malawi, Southern region reported the highest diversity of priority taxa (48) that did not occur in other regions; followed by Northern (34) and Central region (12).

About 25.6% of the taxa occurred across the country, and the remainder occurred in one or two regions. It was also noted that *Coffea mufindiensis* Bridson subsp. pawekiana (Bridson) and C. arabica L. wild types were endemic to Southern Region, and C. mufindiensis Hutch ex Bridson subsp. lundaziensis and Setaria grandis Stapf were near endemic and only found in the Northern Region. Other endemic species included *Eragrostis fastigiata* Cope., E. sylviae Cope. and Plectranthus mandalensis Baker only known from Southern Region of Malawi. Prioritization of CWR taxa by threat status and the potential use of the wild relative for crop improvement revealed that 34 taxa were assessed for threat status at global level. Of these, 29 taxa were Least Concern (LC), one taxon was assessed as Data Deficient (DD) [Vigna hosei (Craib) Backer], and four taxa were threatened and these included the wild populations of Coffea arabica L. and C.salvatrix Swynnerton & Phillipson assessed as Endangered (EN), Prunus africana (Hook.f.) Kalkman, and C.ligustroides S. Moore as Vulnerable (VU). South African Red List assessments covered 106 CWR taxa of the national inventory; three species were reported threatened and these included Siphonochilus aethiopicus (Schweinf.) B.L.Burtt assessed as Critically Endangered (CR), Prunus Africana

(Hook.f.) Kalkman, and *Oryza longistaminata* A. Chev. & Roehras Vulnerable (VU), and the remaining 103 taxa as Least Concern (LC). In terms of potential use for crop improvement, 73 taxa have potential use for crop improvement and 69 taxa were found to be in GP1b and GP2, and eleven taxa have verified use in crop improvement (Table 2.3). Based on these, 87 CWR taxa were then categorised as of high priority; while 190 are low priority for conservation in a scenario where resources for conservation are limited.

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
Amaranth	Amaranthus dubius Mart. ex Thell.*	N	N	native
	A. graecizans L. subsp. Silvestris (Vill.) Brenan*	Ν	Ν	native
	A. hybridus L	Ν	Ν	native
Asparagus	Asparagus laricinus Burch.	Ν	Y	native
	A. asparagoides (L.) Druce	Ν	Y	native
	A. buchananii Baker	Ν	Y	native
	A. migeodii Sebsebe	Ν	Y	native
	A. pendulus (Oberm.) Fellingham & N.L. Mey.	Ν	Y	native
	A. psilurus Welw. ex Baker	n	Y	native
	A. suaveolens Burch.	n	Y	unknown
	A. virgatus Baker	n	Y	native
	A. africanus Lam. Var. africanus	n	Y	native
	A. africanus (Baker) Sebsebe var. puberulus	n	Y	native
	A. racemosus Willd.	n	Y	unknown
	A. saundersiae Baker	n	Y	native
	A. schroederi Engl.	n	Y	native
	A. setaceus (Kunth) Jessop	n	Y	native
Bambara groundnut	Vigna hosei (Craib) Backer var. pubescens	у	Y	native
-	V. luteola (Jacq.) Benth.	n	Y	native
	V. oblongifolia A.Rich.	n	Y	native
	V. fischeri Harms	n	Y	unknown
	V. heterophylla A.Rich. subsp.ambacensis	n	Y	native
	V. racemosa (G.Don) Hutch. & Dalziel	n	Y	unknown

Table 2.2: Priority crop wild relatives for conservation in Malawi and their native status

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
Banana	Ensete ventricosum (Welw.) Cheesman	n	Y	native
	Musa livingstonianum (J.Kirk) Cheesman	n	Y	unknown
Bitter melon	Momordica foetida Schumach.*	у	Ν	native
	Coccinia adoensis (A. Rich.) Cogn.*	n	Ν	unknown
	Momordica boivinii Baill.	n	Ν	native
	M. friesiorum (Harms) C. Jeffrey	n	Ν	native
Black pepper	Peperomia exigua (Blume) Miq.	n	Ν	native
	P. retusa (L.f.) A. Dietr.	n	Ν	native
	Piper capense L. fil. subsp.capense **	n	Ν	native
	P. capense L. fil. var.brachyrhachis *	n	Ν	native
	P. umbellatum L.	n	Ν	native
Blue berry	Vaccinium exul Bolus **	n	Ν	native
Cardamom	Aframomum alboviolaceum (Ridl.) K. Schum.	n	Ν	native
	A. albiflorum Lock	n	Ν	native
	A. alboviolaceum (Ridl.) K. Schum.	n	Ν	native
	A. angustifolium (Sonn.) K. Schum.	n	Ν	native
	<i>A. zambesiacum</i> (Baker) K. Schum. subsp. <i>Zambesiacum</i>	n	Ν	unknown
Cassava	Manihot glaziovii Müll. Arg.**	n	Y	introduced
Chinese/Indian mastard, Rape	Brassica juncea (L.) Czern. **	n	Y	introduced
Clover	<i>Trifolium polystachyum</i> Fresen. var. <i>psoraleoides</i> Welw. ex Hiern	n	Y	native
	T. pseudostriatum Baker f.	n	Y	native
	T. rueppellianum Fresen. var. rueppellianum	n	Y	native
	T. semipilosum Fresen	n	Y	native
	T. simense Fresen.	n	Y	native

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
	T. usambarense Raub.	n	Y	native
Coffee	Coffea arabica L. wild types **	n	Ν	native
	C. eugenioides S.Moore *	у	Ν	unknown
	C. ligustroides S.Moore*	n	Ν	unknown
	<i>C. mufindiensis</i> Hutch. ex Bridson subsp. <i>Mufindiensis</i> *	у	Ν	unknown
	<i>C. mufindiensis</i> Hutch ex Bridson subsp. <i>australis</i> *	У	Ν	native
	C. mufindiensis Hutch ex Bridson subsp. lundaziensis *	у	N	native
	C. mufindiensis Hutch ex Bridson subsp. pawekiana *	У	N	native
	C. salvatrix Swynnerton & Phillipson.*	У	Ν	native
Cotton	Gossypium barbadense L.**	У	Ν	unknown
Cowpeas	Vigna comosa Baker	n	Y	native
	V. phoenix Brummitt	n	Y	native
	V. scabra (L.f.) Sond subsp.scabra	n	Y	unknown
	V. schimperi Baker	n	Y	native
	V. unguiculata (E.Mey.) Marechal & al. subsp.tenuis	у	Y	native
	V. adenantha (G.Mey.) Marechal & al	n	Y	unknown
	V. antunesii Harms	n	Y	native
	V. frutescens A.Rich. subsp.frutescens	n	Y	native
	V. gazensis Baker f.	n	Y	native
	V. nuda N.E.Br.	n	Y	native
	<i>V. unguiculata</i> (L.) Walp. subsp. <i>unguiculata</i> var. <i>spontanea</i> **	n	Y	native
	V. unguiculata (L.) Walp. subsp. pawekiae*	У	Y	native

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
	V. unguiculata (L.) Walp.subsp. pubescens*	у	Y	unknown
	V. unguiculata (L.) Walp.subsp.stenophylla **	у	Y	unknown
	V. unguiculata (L.) Walp. subsp.tenuis *	у	Y	native
	<i>V. unguiculata</i> (Harms) Verdc. subsp. <i>dekindtiana</i> **	у	Y	native
	V. vexillata (L.) A.Rich. subsp.angustifolia	n	Y	native
	V. vexillata (L.) A. Rich. var.vexillata	n	Y	native
	V. kirkii (Baker) J.B.Gillett	n	Y	native
	V. platyloba Welw. ex Hiern	n	Y	native
	V. pygmaea R.E.Fr.	n	Y	native
	V. reticulata Hook.f.	n	Y	native
	V. schimperi Baker	n	Y	native
	V. juncea Milne-Redh.	n	Y	native
	V. nyangensis R.Mithen & H.Kibblewhite	n	Y	unknown
	V. radicans Baker	n	Y	native
	V. frutescens subsp. frutescens A.Rich. var. buchneri (Harms) Verdc.	n	Y	native
	V. macrorhyncha (Harms) Milne-Redh.	n	Y	native
	V. oblongifolia A. Rich. var.parviflora (Baker) Verdc.	n	Y	native
Cucumber	Coccinia mildbraedii Harms	n	Ν	native
	Cucumis anguria L. anguria	n	Ν	native
	C. hirsutus Sond	n	Ν	native
	Oreosyce africana Hook. f.	n	Ν	unknown
	Mukia maderaspatana (L.) M. Roem.	n	Ν	native
	Oreosyce africana Hook. f.	n	Ν	unknown

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
Date palm	Phoenix reclinata Jacq. **	у	N	native
Desmodium	Desmodium ospriostreblum Chiov. **	n	Ν	introduced
Eggplant	Solanum anguivi Lam.	n	Y	native
	S. tettense Klotzsch	n	Y	native
	S. aethiopicum L.	n	Y	native
	S. dasyphyllum Schumach.	n	Y	native
	S. goetzei Dammer	n	Y	native
	S. incanum L. *	n	Y	native
	S. lichtensteinii Willd.*	у	Y	native
	S. richardii Dunal var.richardii	n	Y	native
	S. richardii Dunal var.burtt-davyi	n	Y	native
	S. torvum Sw.	n	Y	native
	S. aculeatissimum Jacq.	n	Y	native
	S. aculeatissimum Dunal var.aculeatissimum	n	Y	native
	S. aureitomentosum Bitter *	у	Y	native
	S. campylacanthum Hochst. ex A.Rich.*	у	Y	native
	S. chrysotrichum Schltdl.	n	Y	introduced
	S. macrocarpon L.	n	Y	native
	S. nigrum/retroflexum L.	n	Y	introduced
	S. aculeastrum Dunal subsp. aculeastrum	n	Y	native
	S. delagoense Dunal	n	Y	native
	S. hispidum Pers.	n	Y	native
	S. schumannianum Dammer	n	Y	native
	S. seaforthianum Andrews var.disjunctum O.E.Schulz	n	Y	native
	S. terminale Forssk. subsp.terminale	n	Y	native

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
	S. mammosum L.	n	Y	native
	S. panduriforme E.Mey.	n	Y	native
	S. giganteum Jacq.	n	Y	native
	S. memphiticum J.F.Gmel.	n	Y	native
	S. pseudospinosum C.H.Wright	n	Y	native
	S.grossidentatum A. Rich.	n	Y	native
Faba beans	Vicia paucifolia Barker	n	Y	unknown
	V. paucifolia Baker subsp. malosana (Baker) Verdc. *	n	Y	native
Finger millet	Eleusine indica (L)Gaenth*	У	Y	unknown
	E. coracana (L.) Gaertn. subsp.africana**	У	Y	unknown
Foxtail millet	Setaria italica (L.) P.Beauv. **	n	Ν	introduced
	S. atrata Hackel *	n	Ν	native
	S. grandis Stapf	n	Ν	native
	S. nigrirostris (Nees) Dur. & Schinz	n	Ν	native
	S. pumila (Poir.) Roem. & Schult.	n	Ν	native
Ginger	Siphonochilus aethiopicus (Schweinf.) B.L.Burtt	n	Ν	native
	S. parvus Lock	n	Ν	native
	S. rhodesicus (T.C.E.Fr.) Lock	n	Ν	native
	S. carsonii (Baker) Lock	n	Ν	native
	S. kirkii (Hook.) B.L. Burtt	n	Ν	native
Gourds	Lagenaria sphaerica (Sond.) Naudin	n	Ν	native
Grapes	Vitis rotundifolia (Forssk.) Vahl**	n	Ν	native
	V. cornifolia (Baker) Planch*	n	Ν	native
	V. gracilis (Guill. & Perr.) Suess.	n	Ν	native
	V. integrifolia (Baker) Planch.	n	Ν	unknown

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
	V. petiolata Hook. f.	n	N	unknown
	V. quadrangularis L.	n	Ν	native
Hyacinth beans	Lablab purpureus (L.) Sweet subsp.uncinatus var.uncinatus*	n	Ν	native
Millet	Echinochloa frumentacea Link * *	n	Ν	introduced
Kaki/persimmon	<i>Diospyros abyssinica</i> (Hiern) F. White subsp. <i>attenuata</i>	n	Ν	native
	D. loureiriana G. Don. subsp. loureiriana	n	Ν	native
	D. quiloensis (Hiern) F. White	n	Ν	native
	D. truncatifolia A.N. Caveney	n	Ν	native
Lettuce	Lactuca attenuate Stebbins	n	Ν	native
	L. glandulifera Hook. f.	n	Ν	native
	L. paradoxa Sch. Bip. ex A. Rich.	n	Ν	native
Lima bean	<i>Macroptilium atropurpureum</i> (Moç. & Sessé ex DC.) Urb.*	n	Y	unknown
Livingstone potato	Plectranthus mandalensis Baker	n	Ν	native
Lupine	Lupinus mexicanus Cerv.*	n	Y	native
Millet	Echinochloa haploclada (Stapf) Stapf *	n	Ν	native
	E. jubata Stapf	n	Ν	native
	E. pyramidalis (Lam.) Hitchc. & Chase	n	Ν	native
Millet	E. colona (L.) Link**	n	Ν	native
Millet	E. crus-galli (L.) P.Beauv.	n	Ν	introduced
	E. stagnina (Retz.) P.Beauv.(L).P. Beauv.	n	Ν	native
Olives	Olea capensis L.	n	Ν	unknown
	O. capensis L. subsp.macrocarpa	n	Ν	unknown
	<i>O. europaea</i> L. subsp. <i>cuspidata</i> **	у	Ν	native

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
	O. welwitschii (Knobl.)	n	N	native
Panicum	Panicum adenophorum K.Schum.	n	Ν	native
	P. nymphoides Renvoize*	n	Ν	native
	P. lukwangulense Pilg.	n	Ν	native
	P. miliaceum L.	n	Ν	unknown
	P. repens L.	У	Ν	native
Pearl millet	Cenchrus purpureus (Schumach.)Morrone **	У	Y	native
	C. clandestinum Hochst. ex Chiov	n	Y	introduced
	C. geniculatus Thunb	n	Y	native
	C. polystachios L. subsp.polystachios	n	Y	native
	C. polystachios L.Morrone. subsp. atrichus	n	Y	native
	C. sphacelatum (Nees) T.Durand & Schinz	n	Y	native
	C. ciliaris (L.) Link	n	Y	unknown
	C. atrichum Stapf & C.E.Hubb	n	Y	native
	C. kirkii Stapf	n	Y	native
	C. macrourum Trin	n	Y	native
	C. mildraedii Mez	n	Y	native
	C. setosum (Sw.) Rich.	n	Y	native
	C. thunbergii Kunth	n	Y	native
	C. unisetus (Nees) Morrone	n	Y	native
Pigeon pea	Pearsonia cajanifolia (Baker) Polhill. subsp. cryptantha	n	Y	native
Plum	Prunus africana (Hook.f.) Kalkman	n	Ν	native
Potato	Solanum tuberosum L. (wild types)	У	Y	native
	S. wendlandii Hook.f.	n	Y	native
	S. wrightii Benth.	n	Y	native

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
Pumpkin	Gunnera perpensa L.	n	N	native
Quinoa	Chenopodium procerum Hochst. ex Moq.	n	Ν	native
	C. ambrosioides L.	n	Ν	introduced
Raspberry	Rubus iringanus Gust.	n	Ν	native
	R. scheffleri Engl.	n	Ν	unknown
	R. niveus Thunb*	n	Ν	introduced
	R. ellipticus Sm. **	n	Ν	introduced
	R. rosifolius Sm.*	n	Ν	unknown
Rhodes grass	Chloris roxburghiana Schult.	n	Ν	unknown
	Chloris pilosa Schumach.	n	Ν	introduced
Rice	Oryza punctata Kotschy ex Steud.*	у	Y	unknown
	O. barthii A.Chev. **	у	Y	native
	O. longistaminata A.Chev.&Roehr. **	у	Y	native
Sesame	Sesamum angolense Welw.	n	Ν	native
	S. angustfolium (Oliver) Engl.	у	Ν	native
	S. calycinum Welw. subsp.calycinum	n	Ν	unknown
Sauchum	S. calycinum Seidenst. ex HD.Ihlenfeldt subsp.pseudoangolense Souchum alwum (J. Doordi	n	N Y	unknown
Sorghum	Sorghum almum (L) Parodi	n		native
	S. bicolor (L.) Moench subsp. arundinaceum**	у	Y	native
	S. bicolor (L.) Moench subsp. bicolor **	n	Y	native
	S. bicolor (L.) Moench subsp.drummondii**	n	Y	native
	S. bicolor (L.) Moench subsp.verticilliflorum**	n	Y	native
	S. halepense (L.) Pers.*	n	Y	native
	S. rigidifolium Stapf	n	Y	native
	S. sudanense (Piper) Stapf	n	Y	native

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
	S. versicolor Andersson	у	Y	native
Soybean	Neonotonia wightii subsp.wightii (Wight & Arn.) J.A. Lackey var.longicauda (Schweinf.) J.A. Lackey	n	Ν	unknown
	Ophrestia unifoliolata (Baker f.) Verdc.	n	Ν	native
	<i>Rhynchosia sublobata</i> (Schumach. & Thonn.) Meikle	n	N	native
Spiny Cucumber	Cucumis metuliferus E.Mey. ex Naudin	n	Ν	native
Sugar cane	Eriochrysis pallida Munro	n	Ν	native
	Imperata cylindrica (L.) Raeusch.	n	Ν	unknown
	Saccharum officinarum L**	n	Ν	native
	S. spontaneum L.subsp.aegyptiacum **	у	Ν	unknown
	S. spontaneum L. *	n	Ν	unknown
Sweet potato	<i>Ipomoea. coptica (L.)</i> Roth ex Roem. & Schult. var. <i>acuta</i>	n	Y	native
	I.turbinata Lag.	n	Y	introduced
	I. sinensis (Desr.) Choisy subsp. blepharosepala	n	Y	native
	I. blepharophylla Hallier f.	n	Y	native
	I. kituiensis Vatke	n	Y	native
	I. marginata (Desr.) Verdc.	n	Y	native
	I. mauritiana Jacq.	n	Y	unknown
	I. oenotherae (Vatke) Hallier f.	n	Y	native
	I. aquatica Forssk	n	Y	native
	I. barteri Baker var.barteri	n	Y	native
	I. cairica (L.) Sweet var. cairica	n	Y	unknown
	<i>I. coptica</i> (L.) Roth ex Roem. & Schult. var. <i>coptica</i>	n	Y	native
	I. plebeia R. Br. subsp.africana A. Meeuse	n	Y	native

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
	I. involucrata P.Beauv var.involucrata	n	Y	native
	I. muricata (L.) Jacq.	n	Y	unknown
	I. obscura (L.) Ker Gawl. var. sagittifolia Verdc.	n	Y	native
	I. obscura (L.) Ker Gawl. var.obscura	n	Y	native
	I. pes-tigridis L. var. africana Hallier f.	n	Y	native
	Itigridis L. var .pes-tigridis	n	Y	native
	I. tenuirostris Steud. ex Choisy subsp.tenuirostris	n	Y	native
	I. crassipes Hook.var. crassipes	n	Y	native
	I. involucrata P. Beauv. var. operosa (C.H. Wright) Hallier f.	n	Y	native
	I. pileata Roxb.	n	Y	native
	I. dichroa Hochst. ex Choisy	n	Y	native
	<i>I. fulvicaulis</i> (Hochst. ex Choisy) Boiss. ex Hallier f. var. <i>asperifolia</i> (Hallier f.) Verdc.	n	Y	native
	I. hederifolia L	n	Y	introduced
	<i>I. fulvicaulis</i> (Hochst. ex Choisy) Boiss. ex Hallier f. var. <i>heterocalyx</i> (Schulze-Menz) Verdc.	n	Y	native
	I. linosepala Hallier f.subsp. alpina (Rendle) Lejoly & Lisowski	n	Y	native
	I. rubens Choisy	n	Y	native
	<i>I. pes-caprae (L.)</i> R. Br. subsp. <i>brasiliensis</i> (L.) van Oststr.	n	Y	native
	I. eriocarpa R. Br.	n	Y	native
	I. trinervia Schulze-Menz	n	Y	native
	I. verbascoidea Choisy	n	Y	native
	I. welwitschii Vatke ex Hallier f.	n	Y	native

Сгор	Priority crop wild relatives for Malawi	Included in the SADC inventory (Yes/No)	Related to crop of global importance (Yes/No)	Status
	I. wightii (Wall.) Choisy var.wightii	у	Y	native
Sword/Jack bean	Canavalia Africana Dunn*	n	Y	unknown
Teff	Eragrostis tef (Zuccagni) Trotter **	n	Ν	native
	<i>E. aethiopica</i> Chiov.	n	Ν	native
	E. heterolomera StapF. **	n	Ν	native
	E. fastigiata Cope	n	Ν	native
	E. sylviae Cope.	n	Ν	native
	E. pilosa (L).P.Beauv. *	n	Ν	native
Tobacco	Nicotiana rustica L. *	n	Ν	introduced
Tomato	Solanum tarderemotum Bitter*	n	Ν	native
Yam bean	Sphenostylis briartii (De Wild.) Baker f.	n	Ν	unknown
	<i>S. erecta</i> (Baker f.) Hutch. ex Baker f. subsp.e <i>recta</i>	n	Ν	native
	S. erecta (Baker f.) Hutch. ex Baker subsp.obtusifolia (Harms) Potter & Doyle	n	Ν	unknown
	S. stenocarpa (Hochst. ex A. Rich.) Harms	n	Ν	unknown
Yam	Dioscorea praehensilis Benth. * *	У	Y	native
	D. hirtiflora Benth. subsp.orientalis*	n	Y	native
	D. asteriscus Burkill	n	Y	native
	D. bulbifera (L.) L**	n	Y	native

(no=wild relative not priority for the SADC region and is not related to crop of global importance; yes=wild relative of priority to SADC region and is related to crop of global importance; *= wild relatives with documented use in crop improvement)

Related crop	Crop Wild Relative taxa	Genepool concept	IUNC 2018 global red listing	South Africa Plants red listing
Cassava	Manihot glaziovii Müll. Arg*.	GP2	NA	NA
Chinese/Indian mustard, Rape seed	Brassica juncea (L.)Czern.	GP1b	NA	NA
Coffee Arabica	Coffea arabica (wild types) L.*	GP1b	EN	NA
Coffee Arabica	C. eugenioides S.Moore	GP2	LC	NA
Coffee Arabica	C. ligustroides S.Moore	GP2	VU	NA
Coffee Arabica	C. mufindiensis Hutch.ex Bridson	GP2	LC	NA
Coffee Arabica	C. salvatrix Swynnerton & Phillipson.	GP2	EN	NA
Coffee Arabica	<i>C. mufindiensis</i> Hutch ex Bridson <i>subsp.australis</i> Bridson	GP2	NA	NA
Coffee Arabica	C. mufindiensis Hutch ex Bridson subsp.lundaziensis Bridson	GP2	NA	NA
Coffee Arabica	C. mufindiensis Hutch ex Bridson subsp.pawekiana Bridson	GP2	NA	NA
Cotton	Gossypium barbadense L*.	GP1b	NA	NA
Cowpeas	<i>Vigna unguiculata</i> (L.)Walp. <i>var.spontanea</i> (Schweinf.) Pasquet	GP1b	NA	NA
Cowpeas	V. unguiculata (L.)Walp. subsp.pawekiae Pasquet	GP2	NA	NA
Cowpeas	<i>V</i> . <i>unguiculata</i> (L.)Walp. <i>subsp.pubescens</i> (R.Wilczek) Pasquet	GP2	NA	NA
Cowpeas	<i>V. unguiculata</i> (L.)Walp. <i>subsp.stenophylla</i> (Harv.) Marechal et al.	GP1b	NA	LC

Table 2.3: High priority taxa closely related to some cultivated crops and with highest potential use in crop improvement

Related crop	Crop Wild Relative taxa	Genepool concept	IUNC 2018 global red listing	South Africa Plants red listing
Cowpeas	V. unguiculata (L.)Walp. subsp.tenuis (E.Mey.) Marechal et al.	GP1b	NA	LC
Cowpeas	<i>V. unguiculata</i> (L.)Walp. <i>subsp.dekindtiana</i> (Harms) Verdc.	GP1b	NA	LC
Date palm	Phoenix reclinata Jacq.	GP1b	NA	LC
Eggplant	Solanum incanum L.	GP2	NA	NA
Eggplant	S. lichtensteinii Willd.	GP2	NA	LC
Eggplant	S. aureitomentosum Bitter	GP2	NA	NA
Eggplant	S. campylacanthum Hochst.ex A.Rich.	GP2	NA	LC
Finger millet	Eleusine.indica (L.) Gaertn	GP2	NA	NA
Finger millet	<i>E. coracana</i> (L.) Gaertn. <i>subsp.africana</i> (Keen O'Byrne) Hilu & de Wet	GP1b	LC	LC
Foxtail millet	Setaria italica (L.) P.Beauv.	GP1b	NA	NA
Indian barnyard millet	<i>E. frumentacea</i> Link	GP1b	LC	NA
Jack bean	Canavalia africana Dunn.	GP2	NA	NA
Millet/Indian Barnyard	Echinochloa colona (L.) Link	GP1b	LC	LC
Millet/Japanese Barnyard	E. stagnina (Retz.) P.Beauv./ (L).P.Beauv.	GP1b	LC	LC
Olives	<i>Olea europaea</i> L.subsp. <i>cuspidata</i> (Wall. ex G.Don) Cif.	GP1b	NA	NA
Pearl millet	Pennisetum purpureus (Schumach.) Morrone*	GP2	LC	NA
Raspberry(black)	Rubus niveus Thunb	GP2	NA	NA
Raspberry(red)	R. ellipticus Sm.	GP1b	NA	NA
Raspberry(red)	R. rosifolius Sm.	GP2	NA	NA

Related crop	Crop Wild Relative taxa	Genepool concept	IUNC 2018 global red listing	South Africa Plants red listing
Rice	Oryza punctata Kotschy ex Steud.*	GP2	LC	LC
Rice	O. barthii A.Chev*.	GP1b	LC	NA
Rice	O. longistaminata A.Chev.& Roehr.*	GP1b	LC	VU
Sorghum	Sorghumbicolor(L.)Moenchsubsp.arundinaceum(Desv.) de Wet and Harlan*	GP1b	NA	LC
Sorghum	S. bicolor (L.)Moench subsp. Drummondii (Steud.) de Wet and Harlan	GP1b	NA	LC
Sorghum	S. bicolor (L.)Moench subsp.verticilliflorum (Steud.) de Wet and Harlan	GP1b	NA	NA
Sorghum	S. halepense (L.) Pers.	GP2	NA	NA
Sorghum	S. bicolor (L.) Moench subsp.bicolor*	GP1b	NA	NA
Sugar cane	Saccharum.spontaneum L. subsp. aegyptiacum (Willd.) Hack*	GP1b	NA	NA
Sugar cane	S. spontaneum L*.	GP2	NA	NA
Teff	Eragrostis tef (Zaccagni) trotter	GP2	LC	NA
Teff	E. heterolomera StapF.	GP1b	NA	NA
Teff	E. pilosa (L).P.Beauv.	GP2	NA	LC
White guinea yam	Dioscorea praehensilis Beth.	GP1b	NA	LC

NA means taxa whose threat status is unknown (not assessed yet by the time of data collation), LC= Least concern, VU=vulnerable, EN=endangered taxa; GP1b=taxa in the genepool as cultivated crops, GP2=taxa in the secondary genepool, (*represents taxa with verified use in crop improvement).

2.4. DISCUSSION

2.4.1. The general checklist

The results of this study indicate, for the first time, existence of a relatively great diversity of CWR taxa (at species, subspecies and variety levels) in Malawi occurring across its regions. This provides an opportunity for establishing genetic reserves for *in situ* conservation of priority CWR across all agro-ecological zones of Malawi, capturing unique adaptive zones that possibly represent unique and/or rare genes useful for improvement of specific traits in crops. Collection and conservation of such taxa under *ex situ*, could provide a broad range of unique alleles specific for each agroecology.

The general checklist had 446 taxa and 74.7% of which were native to Malawi, although they had the centre of diversity of their related crops elsewhere (Vincent *et al.*, 2013). It is important to note that more than 50% of these taxa had unknown threat status at both global and SADC regional levels. This was expected as most conservation institutions have different mandates, inadequate expertise in redlisting, as well as lacking adequate resources to do the redlisting exercise (Hunter and Heywood, 2011).

The general checklist captured taxa of national, regional (SADC) and of global important crops, and of crops not cultivated in the country, but with wild relatives in Malawi. Related studies in Zambia, Mauritius and South Africa reported similar results of existence of CWR of regional and global priority (Ng'uni *et al.*, 2017; Bissessur *et al.*, 2019; Holness *et al.*, 2019) an indication that the SADC region share the CWR diversity providing an opportunity for germplasm exchange. Zambia for example, took a step further by collecting such diversity to facilitate its utilization in pre-breeding, crop improvement programmes (Ng'uni *et al.*, *al.*, *al*

2017), and Malawi has similar plans. Regarding the wild relatives of fodder and forage crops, it should be noted that only major fodder crops were considered, given their complex botanic classification, as noted by Vincent *et al.* (2013), and the inadequate information about the exact species regarded as crops in Malawi, as most fodder species occur in the wild. With such status, it is practically impossible to put under active conservation of such fodder unless well defined. Therefore, the total number of CWR occurring in Malawi could be slightly higher than 446, hence the checklist should be regarded as a working list and it should be updated whenever new information is available.

2.4.2. The National inventory

Malawi's CWR inventory includes 277 taxa, of which 26.4% has potential for crop improvement, 33.6% were a priority to the SADC region (Allen *et al.*, 2017; 2019), and 59.2% taxa were related to crops of global importance (FAO, 2009;Vincent *et al.*, 2013). With inter dependency on food and raw materials among nations, (Khoury *et al.*, 2010; Kell *et al.*, 2015) and harmonised access to plant genetic resources at all levels (FAO, 2009; Dempewolf *et al.*, 2014; Allen *et al.*, 2019); presence of such taxa allows for continued germplasm exchange, and places Malawi in an important role as providing a broad genetic diversity relevant to the improvement of crops that are important at global and regional levels.

In terms of conservation, this study provided fundamental information such as the amount of priority diversity for conservation and this will guide the formulation of specific conservation action plans for the priority taxa. However, to address the conservation needs of all priority taxa, the next step should be field mapping of such taxa to assess their current conservation status and have the real picture on the ground. The criteria and methods used to prioritize

CWR were tailored to the conservation of plant genetic resources context in Malawi. Due to differences in conservational needs, other countries and/or regions have used other criteria, or the same criteria but different prioritization methods (Vincent *et al.*, 2013; Allen *et al.*, 2017; Allen *et al.*, 2019); and this only shows that CWR prioritization varies according to the different contexts.

About 12.6% of the priority taxa occurring in Malawi confirmed uses in crop improvement (Table 2.3) for traits such as pests and diseases resistance, drought tolerance and increase yield (Hajjar and Hodgkin, 2007). These were used to improve crops like cotton (Jafar *et al.*, 2018), sorghum (Wilson *et al.*, 2000; Jordan *et al.*, 2004), rice (Khush *et al.*, 2004; Brar, 2005), pearl millet (Hanna, 1989), cowpeas (Hajjar and Hodgkin, 2007) and sugarcane (Ramdoyal and Badaloo, 2002; Edmé *et al.*, 2005). Availability of drought tolerance genes in taxa such as *O. Barthii* A. Chev. and *O. Longistaminata* A.Chev. & Roehr. provides an opportunity for rice improvement whose cultivation in Malawi is confined to lakeshore areas with reliable water sources.

The occurrence of taxa with genes controlling traits of economic importance has potential to improve agricultural productivity and diversified production considering that (i) the present food security in Malawi relies on a few crops such as maize, rice, cowpeas and a few minor crops whose genetic diversity has been significantly explored due to agricultural intensification and continuous selection for high yielding traits to meet high food demands; (ii) breeding for drought tolerance and pests and disease resistance is complex and resource demanding (Witcombe *et al.*, 2007); and (iii) use of populations with known resistance and

tolerance could potentially save on time, hence the need to take advantage of the available taxa with such genes to save on time and resources.

About 73 of the priority taxa in the inventory have potential use for crop improvement, but only 3 taxa have *ex situ* collections at the national genebank; and these may need to be evaluated to benefit national breeding programme. Malawi has vibrant breeding programmes in crops like, rice, coffee, cowpeas, soya bean, millets, sorghum and in horticultural crops, which include leaf, and fruit vegetables whose wild relatives occur in the country. These crops would benefit from the collections and further exploration of their wild relatives. However, it is important to note that information about taxa potential and confirmed use for crop improvement was not available for some taxa. With the use of modern breeding methods, distantly related taxa in the general checklist could potentially be useful in breeding programmes, and these taxa were not reflected in the inventory, implying that the number of priority taxa would increase with availability of such information.

The importance of the developed National Inventory cannot be over emphasised; its use has already been demonstrated through development of proposed national conservation strategy for CWR in Malawi, as part of the Darwin Initiative funded project "Bridging agriculture and environment: Southern African cropwildrelative regional network" that was initiated in April 2019. This immediate application shows its significance to conservation efforts in Malawi, and its availability will facilitate active and sustainable conservation of priority CWR, as noted by Maxted *et al.* (2015) and Magos Brehm *et al.* (2017). It could also facilitate utilisation of such taxa by breeders (Dempewolf *et al.*, 2014; Zhang *et al.*, 2017).

However, an inventory alone may not be sufficient for effective conservation of the identified taxa; other complementary analyses such as distribution and diversity analyses need to be considered in order to identify hot spots potential for active *in situ* conservation and designation of genetic reserves that capture broad range of diversity (Maxted, 2003). Such additional analyses can be useful in the identification of populations for *in situ* conservation that represent the genetic diversity in the wild (Maxted *et al.*, 2012). More importantly, these analyses will assist in the identification of both *in situ* and *ex situ* conservation gaps of the priority CWR. As formulation of a national CWR conservation strategy is in progress, the inventory can provide as background information such taxa distribution, native and threat status, taxon endemicity, rarity and potential use in crop improvement, to guide initial stages in conservation planning. As a temporary measure, we recommend that priority taxa with potential use for crop improvement, taxa that are endemic and threatened, should be priority for *ex situ* collections because they are vulnerable to localised natural and anthropogenic factors.

The study also noted the insufficiency of information about the threat status of the priority taxa at national level, and with only few taxa considered for red listing at global level, relying on such information might be misleading in that taxa threatened at global level may not be threatened at national level. With such information gaps, it is recommended that threat assessments be conducted at national level to have a true reflection that adequately guide the formulation of strategic conservation actions of such taxa.

2.5. CONCLUSION

The development of the CWR inventory is a first step towards a comprehensive system that will systematically guide the conservation and sustainable utilization of CWR in Malawi. The tool is timely, especially now when Malawi is facing challenges of loss of biodiversity and increased demand for food as the population continues to grow. However, more information on taxa *ex situ* and *in situ* conservation status is required to facilitate an effective conservation planning. The study recommends conducting ecogeographic surveys, diversity analyses, and modelling of climate change impact on their future distributions as next step towards effective conservation planning of CWR. The proposed studies will help verify status of CWR considering that there has been changes in land use in some sites where the taxa were observed. Threat assessment results will ensure formulation of conservation actions that address the needs of threatened taxa. Although the inventory adequately covers taxa of important crops for Malawi, it should be updated whenever more information is available in

order to make it relevant to the prevailing conservation needs.

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APPENDIX 1

SUPLIMENTARY MATERIALS

Supplementary Table 4: National Stakeholder Workshop on Crop and Crop Wild Relatives Species Prioritization and validation of the priority checklist for conservation in Malawi, held at the Silver Sand Resort, Salima, Malawi, 19th October 2017.

Participant	Designation	Institution
Dr. Wilkson I. Makumba	Director of Agricultural Research Services	Department of Agricultural Research Services (DARS) P.O. Box 30779, Lilongwe 3.
Dr. Tenyson Mzengeza	Plant breeder (Rice) and Senior Deputy Director of Agricultural research Services	Chitedze Research Station, P.O. Box 158, Lilongwe, Malawi
Dr. Obedi Mwenye	Plant Breeder (Horticultural crops)	Bvubwe Research Station, P.O. Box 5748, Limbe, Blantyre, Malawi.
Prof. James Bokosi	Professor of Plant Breeding (Legumes)	Lilongwe University of
Prof. Moses F.A. Maliro	Professor of Plant Breeding and Biotechnology	Agriculture and Natural Resources, Bunda College Campus, P.O. Box 219,
Mr. H.D. Musiska (ex official)	Lecture in Pasture agronomy	Lilongwe, Malawi.
Dr. Zacharia L. K. Magombo	Senior Scientist	National Herbarium and Botanic Gardens of Malawi, head office, Livingstone Old Naisi Roads Junction PO Box 528, Zomba Malawi.
Dr. Tembo Chanyenga	Forest research	Forestry Research Institute of Malawi, P.O. Box 270, Zomba, Malawi.

Dr. Lawrent Pungulani	Curator (National Genebank Manager)	Malawi Plant Genetic Resources
Nolipher Khaki Mponya	In situ /on farm conservation and Germplasm collection Officer and Lead Researcher for crop wild relatives	Centre, Chitedze Research Station, P.O. Box 158, Lilongwe.
Comfort Kamwendo Mphangamu	Technical officer	
Mrs. Hilda Kabuli	Chief Statistician and National Coordinator for Technical and Adversory Services	Chitedze Research Station, P.O. Box 158, Lilongwe, Malawi.
Mrs. Judith Chikoti	Livestock Scientist (Pasture)	
Mr. Yohane Chimbalanga	Research Officer (Agriculture and Natural resources)	National Commission for Science and Technology, 1st Floor Lingadzi House, Robert Mugabe Crescent, Private Bag B303, Lilongwe 3, Malawi.
Mr. Chris Manda	Environmental Officer (Biodiversity)	Environmental Affairs Department, P/Bag 394, Lilongwe3
Mr. Matthias. Nkhoma	Chief Crops officer (Field crops)	Department of Crop Production P/Bag 30145, Lilongwe 3.

Сгор	Priority crop wild relatives (CWR) for Malawi	Criteria for selecting the crop	Criteria for selecting CWR
Amaranth	Amaranthus dubius Mart. ex Thell.*	Nutritional value (NE)	PCI, IRC
	A. graecizans L. subsp. Silvestris (Vill.) Brenan*		IRC, PCI
	A. hybridus L		IRC
Asparagus	Asparagus laricinus Burch.	Economic importance	IRC
	A. asparagoides (L.) Druce	importance	IRC
	A. buchananii Baker		IRC
	A. migeodii Sebsebe		IRC
	A. migeoun Sebsebe A. pendulus (Oberm.) Fellingham & N.L. Mey.		IRC
	A. psilurus Welw. ex Baker		IRC
	A. suaveolens Burch.		IRC
	A. virgatus Baker		IRC
	A. africanus Lam. Var. africanus		IRC
	A. africanus (Baker) Sebsebe var. puberulus		IRC
	A. racemosus Willd.		IRC
	A. saundersiae Baker		IRC
	A. schroederi Engl.		IRC
	A. setaceus (Kunth) Jessop		IRC
Bambara groundnut	Vigna hosei (Craib) Backer var. pubescens	Food security, potential for value addition	IRC
	V. luteola (Jacq.) Benth.		IRC
	V. oblongifolia A.Rich.		IRC
	V. fischeri Harms		IRC
	V. heterophylla A.Rich. subsp.ambacensis		IRC
D	V. racemosa (G.Don) Hutch. & Dalziel	Es e d'accorditor	IRC
Banana	Ensete ventricosum (Welw.) Cheesman	Food security, Economic importance	IRC
	Musa livingstonianum (J.Kirk) Cheesman	1	IRC
Bitter melon	Momordica foetida Schumach.*	NA	PCI
	Coccinia adoensis (A. Rich.) Cogn.*		PCI
	Momordica boivinii Baill.		TD
	<i>M. friesiorum</i> (Harms) C. Jeffrey		TD
Black pepper	Peperomia exigua (Blume) Miq.	NA	NS
ыаск реррег	<i>P. retusa</i> (L.f.) A. Dietr.	NA	NS
	Piper capense L. fil. subsp. capense **		PCI
	P. capense L. fil. var.brachyrhachis *		PCI
	P. umbellatum L.		NS
Blue berry	Vaccinium exul Bolus **	NA	PCI
Cardamom	Aframomum alboviolaceum (Ridl.) K. Schum.	NA	NS
	A. albiflorum Lock		NS
	A. alboviolaceum (Ridl.) K. Schum.		NS
	A. angustifolium (Sonn.) K. Schum.		NS
	A. zambesiacum (Baker) K. Schum. subsp. Zambesiacum		NS
Cassava	Manihot glaziovii Müll. Arg.**	Food security, climate change adaptation	IRC, PCI
Chinese/Indian mastard,	Brassica juncea (L.) Czern. **	Food security	IRC, PCI
Rape Clover	Trifolium polystachyum Fresen. var. psoraleoides	NA	NS
	Welw. ex Hiern		
	T. pseudostriatum Baker f.		NS
	T. rueppellianum Fresen. var. rueppellianum		NS
	T. semipilosum Fresen		NS
	T. simense Fresen.		NS
	T. usambarense Raub.		NS

Supplementary Table 5: Priority crop wild relatives for conservation in Malawi and their prioritization criteria

Coffee	Coffea arabica L. wild types **	Economic importance of the	IRC, PCI, Taxon threat status, TD (national
		crop	endemic)
	C. eugenioides S.Moore *		IRC, PCI
	C. ligustroides S.Moore*		IRC, PCI, Taxon threat
			status
	C. mufindiensis Hutch. ex Bridson subsp.		IRC, PCI
	Mufindiensis*		
	C. mufindiensis Hutch ex Bridson subsp. australis *		IRC, PCI
	C. mufindiensis Hutch ex Bridson subsp.		IRC, PCI, TD (near
	lundaziensis *		endemic)
	C. mufindiensis Hutch ex Bridson subsp.		IRC,PCI, TD (endemic)
	pawekiana * C. aglustrin Summartan & Phillipson *		IDC DCI Toyon throat
	C. salvatrix Swynnerton & Phillipson.*		IRC, PCI, Taxon threat
C	Comming hashadayaa I **	Economia	status
Cotton	Gossypium barbadense L **	Economic	IRC, PCI
		importance of the	
Compose	Viena comora Daleas	crop Food accurity	ШC
Cowpeas	Vigna comosa Baker	Food security,	IRC
		climate change	
	V nhoonin Promitte	adaptation	ШC
	V. phoenix Brummitt V. scabra (L.f.) Sond subsp scabra		IRC IRC
	V. scabra (L.f.) Sond subsp.scabra V. schimpari Bakar		IRC
	V. schimperi Baker V. unguiculata (E.Mey.) Marechal & al.		IRC
	0 (),		INU
	subsp.tenuis V. adapartha (C. May) Marcahal & al		IRC
	V. adenantha (G.Mey.) Marechal & al V. antunesii Harms		IRC
	V. frutescens A.Rich. subsp.frutescens		IRC
	V. gazensis Baker f.		IRC
	V. gazensis baker J. V. nuda N.E.Br.		IRC
	V. nudu N.E.Br. V. unguiculata (L.) Walp. subsp. unguiculata var.		IRC, PCI
	spontanea **		IKC, I CI
	V. unguiculata (L.) Walp. subsp. pawekiae*		IRC, PCI
	V. unguiculata (L.) Walp. subsp. pawekiae V. unguiculata (L.) Walp.subsp. pubescens*		IRC, PCI
	V. unguiculata (L.) Walp.subsp. pubescens V. unguiculata (L.) Walp.subsp.stenophylla **		IRC, PCI
	V. unguiculata (L.) Walp.subsp.stenophytia ** V. unguiculata (L.) Walp. subsp.tenuis *		IRC, PCI
	V. unguiculata (Harms) Verdc. subsp.		IRC, PCI
	dekindtiana **		IKC, I CI
	V. vexillata (L.) A.Rich. subsp.angustifolia		IRC
	V. vexillata (L.) A. Rich. var.vexillata		IRC
	V. kirkii (Baker) J.B.Gillett		IRC
	V. platyloba Welw. ex Hiern		IRC
	V. pigmaea R.E.Fr.		IRC
	V. reticulata Hook.f.		IRC
	V. renculata Hook.j. V. schimperi Baker		IRC
	V. schimperi Baker V. juncea Milne-Redh.		IRC
	V. juncea Mune-Rean. V. nyangensis R.Mithen & H.Kibblewhite		IRC
	V. nyangensis K.Minen & H.Kibblewhite V. radicans Baker		IRC
	V. frutescens subsp. frutescens A.Rich. var.		IRC
	v. jrulescens subsp. jrulescens A.Rich. var. buchneri (Harms) Verdc.		inc
	V. macrorhyncha (Harms) Milne-Redh.		IRC
	V. oblongifolia A. Rich. var.parviflora (Baker)		IRC
	V. obiologijona A. Kich. var.parvijiora (Baker) Verdc.		ше
Cucumber	Coccinia mildbraedii Harms	NA	TD, NS
Cucumoti	Cucumis anguria L. anguria	- 14 -	TD, NS
	C. hirsutus Sond		TD, NS
	Oreosyce africana Hook. f.		TD, NS
	Mukia maderaspatana (L.) M. Roem.		TD, NS
	Oreosyce africana Hook. f.		TD, NS
Date palm	Phoenix reclinata Jacq. **	NA	PCI
Desmodium	Desmodium ospriostreblum Chiov. **	NA	PCI
Eggplant	Solanum anguivi Lam.**	NA	PCI
~55P	S. tettense Klotzsch	- 12 -	NS
	S. aethiopicum L**.		PCI

	S. dasyphyllum Schumach. **		PCI
	S. goetzei Dammer		NS
	S. incanum L. *		PCI
	S. lichtensteinii Willd.**		PCI
	S. richardii Dunal var.richardii		NS
	S. richardii Dunal var.burtt-davyi		NS
	S. torvum Sw*.		PCI
	S. aculeatissimum Jacq.		NS
	S. aculeatissimum Dunal var.aculeatissimum		NS
	S. aureitomentosum Bitter *		PCI
	S. campylacanthum Hochst. ex A.Rich.*		PCI
	S. chrysotrichum Schltdl.		NS
	S. macrocarpon L.		NS
	S. nigrum/retroflexum L.		NS
	S. aculeastrum Dunal subsp. aculeastrum		NS
	S. delagoense Dunal		NS
	S. hispidum Pers.		NS
	S. schumannianum Dammer		NS
	S. seaforthianum Andrews var.disjunctum O.E.Schulz		NS
	S. terminale Forssk. subsp.terminale		NS
	S. mammosum L.		NS
	S. panduriforme E.Mey.		NS
	S. giganteum Jacq.		NS
	S. memphiticum J.F.Gmel.		NS
	S. pseudospinosum C.H.Wright		NS
	S.grossidentatum A. Rich.	-	NS
Faba beans	Vicia paucifolia Barker	Food security,	IRC
		economic importance of the	
	V and it. Data and an Malazana (Data)	crop	
	V. paucifolia Baker subsp. Malosana (Baker) Verdc.*		IRC, PCI
Finger millet	Eleusine indica (L)Gaenth*	Nutritional value	IRC, PCI
	E. coracana (L.) Gaertn. subsp.africana**		IRC, PCI
Foxtail millet	Setaria italica (L.) P.Beauv.**	NA	NS, PCI
	S. atrata Hackel *		NS, PCI
	S. grandis Stapf		TD (near endemic)
	S. nigrirostris (Nees) Dur. & Schinz		NS
	S. pumila (Poir.) Roem. & Schult.		NS
Ginger	Siphonochilus aethiopicus (Schweinf.) B.L.Burtt	Economic importance of the crop, medicinal value, potential for value addition	IRC
	S. parvus Lock		IRC
	S. rhodesicus (T.C.E.Fr.) Lock		IRC
	S. carsonii (Baker) Lock		IRC
	S. kirkii (Hook.) B.L. Burtt		IRC
Gourds	Lagenaria sphaerica (Sond.) Naudin	NA	TD
Grapes	Vitis rotundifolia (Forssk.) Vahl**	NA	PCI
-	V. cornifolia (Baker) Planch*		PCI
	V. gracilis (Guill. & Perr.) Suess.		TD
	V. integrifolia (Baker) Planch.		TD
	V. petiolate Hook. f.		TD
	V. quadrangularis L.		TD
Hyacinth beans	Lablab purpureus (L.) Sweet subsp.uncinatus	NA	PCI
Kaki/persimmon	var.uncinatus* Diospyros abyssinica (Hiern) F. White	NA	NS
	subsp.attenuata		NG
	D. loureiriana G. Don. subsp. Loureiriana		NS
	D. quiloensis (Hiern) F. White		NS
_	D. truncatifolia A.N. Caveney		NS
Lettuce	Lactuca attenuate Stebbins	Food security	NS
	L. glandulifera Hook. f.		NS
	L. paradoxa Sch. Bip. ex A. Rich.		NS

Lima bean	Macroptilium atropurpureum (Moç. & Sessé ex DC.) Urb.*	NA	PCI
Livingstone potato	Plectranthus mandalensis Baker	NA	TD (endemic)
Lupine	Lupinus mexicanus Cerv.*	NA	PCI
Millet	Echinochloa haploclada (Stapf) Stapf *	NA	PCI
	E. jubata Stapf		TD
	E. pyramidalis (Lam.) Hitchc. & Chase		TD
	E. colona (L.) Link**		PCI
	E. colona (E.) Link ** Echinochloa frumentacea Link * *		PCI
	E. crus-galli (L.) P.Beauv.		TD
0.11	E. stagnina (Retz.) P.Beauv.(L).P. Beauv.		TD
Olives	Olea capensis L.	NA	NS
	O. capensis L. subsp.macrocarpa		NS
	O. europaea L. subsp.cuspidata**		NS, PCI
	O. welwitschii (Knobl.)		NS
Panicum	Panicum adenophorum K.Schum.	NA	NS
	P. nymphoides Renvoize*		NS,PCI
	P. lukwangulense Pilg.		NS
	P. miliaceum L.		NS
	P. repens L.		NS
Pearl millet	Cenchrus purpureus (Schumach.)Morrone **	Food/feed security	IRC, PCI
I carr minet	C. clandestinum Hochst. ex Chiov	1000/leeu security	IRC, I CI
	C. geniculatus Thunb		IRC
	C. polystachios L. subsp.polystachios		IRC
	C. polystachios L.Morrone. subsp. atrichus		IRC
	C. sphacelatum (Nees) T.Durand & Schinz		IRC
	C. ciliaris (L.) Link		IRC
	C. atrichum Stapf & C.E.Hubb		IRC
	C. kirkii Stapf		IRC
	C. macrourum Trin		IRC
	C. mildraedii Mez		IRC
	C. setosum (Sw.) Rich.		IRC
	C. thunbergii Kunth		IRC
	÷		IRC, NS
D '	C. unisetus (Nees) Morrone	Es a d as annitas	
Pigeon pea	Pearsonia cajanifolia (Baker) Polhill. subsp.	Food security	IRC, NS
	cryptantha		
Plum	Prunus africana (Hook.f.) Kalkman	NA	NS, Threat status
Potato	Solanum tuberosum L.	Food security,	IRC, NS
		economic	
		importance of the	
		crop	
	S. wendlandii Hook.f.	•	IRC, NS
	S. wrightii Benth.		IRC, NS
Pumpkin	Gunnera perpensa L.	NA	NS
Quinoa	Chenopodium procerum Hochst. ex Moq.	NA	NS
Zuilloa		11/1	
D	C. ambrosioides L.	NT A	NS
Raspberry	Rubus iringanus Gust.	NA	NS
	R. scheffleri Engl.		NS
	R. niveus Thunb*		NS, PCI
	R. ellipticus Sm. **		NS, PCI
	R. rosifolius Sm.*		NS, PCI
Rhodes grass	Chloris roxburghiana Schult.	Food/feed security	IRC
Rice	Oryza punctata Kotschy ex Steud.*	Food security,	IRC, PCI
	,	economic	- , -
		importance of the	
		1	
	O handhii A Chan **	crop	IDC DCI
	O. barthii A.Chev. **		IRC, PCI
	O. longistaminata A.Chev.&Roehr. **		IRC, PCI, Taxon thre
			status
Sesame	Sesamum angolense Welw.	Potential for value	IRC
		addition	
	S. angustfolium (Oliver) Engl.		IRC
	S. calycinum Welw. subsp.calycinum		IRC
	2. cm, cm, norm, subspectively children		
	S. calycinum Seidenst. ex HD.Ihlenfeldt		IRC

Sorghum	Sorghum almum (L) Parodi	Food security, climate change adaption	IRC	
	S. bicolor (L.) Moench subsp. arundinaceum**	adaption	IRC, PCI	
	S. bicolor (L.) Moench subsp. bicolor **		IRC, PCI	
	S. bicolor (L.) Moench subsp. drummondii**		IRC, PCI	
	S. bicolor (L.) Moench subsp.verticilliflorum**		IRC, PCI	
	S. halepense (L.) Pers.*		IRC, PCI	
	S. rigidifolium Stapf		IRC	
	S. sudanense (Piper) Stapf		IRC	
	S. versicolor Andersson		IRC	
Soybean	Neonotonia wightii subsp.wightii (Wight & Arn.)	Economic	IRC	
•	J.A. Lackey var.longicauda (Schweinf.) J.A.	importance of the		
	Lackey	crop		
	Ophrestia unifoliolata (Baker f.) Verdc.	*	IRC	
	Rhynchosia sublobata (Schumach. & Thonn.)		IRC	
	Meikle			
Cucumber	Cucumis metuliferus E.Mey. ex Naudin	NA	TD	
Sugar cane	Eriochrysis pallida Munro	Economic	IRC	
		importance of the		
		crop		
	Imperata cylindrica (L.) Raeusch.		IRC	
	Saccharum officinarum L**		IRC, PCI	
	S. spontaneum L.subsp.aegyptiacum **		IRC, PCI	
	S. spontaneum L. *		IRC, PCI	
Sweet potato	Ipomoea. coptica (L.) Roth ex Roem. & Schult.	Food security,	IRC	
	var.acuta	climate change adaptation		
	I. turbinata Lag.	1	IRC	
	I. sinensis (Desr.) Choisy subsp. blepharosepala		IRC	
	I. blepharophylla Hallier f.		IRC	
	I. kituiensis Vatke		IRC	
	I. marginata (Desr.) Verdc.		IRC	
	I. mauritiana Jacq.		IRC	
	I. oenotherae (Vatke) Hallier f.		IRC	
	I. aquatica Forssk		IRC	
	I. barteri Baker var.barteri		IRC	
	I. cairica (L.) Sweet var. cairica		IRC	
	I. coptica (L.) Roth ex Roem. & Schult. var.		IRC	
	coptica			
	I. plebeia R. Br. subsp.africana A. Meeuse		IRC	
	I. involucrata P.Beauv var.involucrata		IRC	
	I. muricata (L.) Jacq.		IRC	
	I. obscura (L.) Ker Gawl. var.sagittifolia Verdc.		IRC	
	I. obscura (L.) Ker Gawl. var.obscura		IRC	
	I. pes-tigridis L. var. africana Hallier f.		IRC	
	Itigridis L. var .pes-tigridis		IRC	
	I. tenuirostris Steud. ex Choisy subsp.tenuirostris		IRC	
	I. crassipes Hook.var. crassipes		IRC	
	I. involucrata P. Beauv. var. operosa (C.H.		IRC	
	Wright) Hallier f.		ma	
	I. pileata Roxb.		IRC	
	I. dichroa Hochst. ex Choisy		IRC	
	I. fulvicaulis (Hochst. ex Choisy) Boiss. ex Hallier		IRC	
	f. var.asperifolia (Hallier f.) Verdc.		mc	
	I. hederifolia L		IRC	
	5		IRC	
	I. fulvicaulis (Hochst. ex Choisy) Boiss. ex Hallier		inc	
	I. fulvicaulis (Hochst. ex Choisy) Boiss. ex Hallier f. var.heterocalyx (Schulze-Menz) Verdc.			
	I. fulvicaulis (Hochst. ex Choisy) Boiss. ex Hallier f. var.heterocalyx (Schulze-Menz) Verdc. I. linosepala Hallier f.subsp. alpina (Rendle)		IRC	
	I. fulvicaulis (Hochst. ex Choisy) Boiss. ex Hallier f. var.heterocalyx (Schulze-Menz) Verdc. I. linosepala Hallier f.subsp. alpina (Rendle) Lejoly & Lisowski		IRC	
	I. fulvicaulis (Hochst. ex Choisy) Boiss. ex Hallier f. var.heterocalyx (Schulze-Menz) Verdc. I. linosepala Hallier f.subsp. alpina (Rendle) Lejoly & Lisowski I. rubens Choisy		IRC IRC	
	I. fulvicaulis (Hochst. ex Choisy) Boiss. ex Hallier f. var.heterocalyx (Schulze-Menz) Verdc. I. linosepala Hallier f.subsp. alpina (Rendle) Lejoly & Lisowski I. rubens Choisy I. pes-caprae (L.) R. Br. subsp. brasiliensis (L.)		IRC	
	I. fulvicaulis (Hochst. ex Choisy) Boiss. ex Hallier f. var.heterocalyx (Schulze-Menz) Verdc. I. linosepala Hallier f.subsp. alpina (Rendle) Lejoly & Lisowski I. rubens Choisy I. pes-caprae (L.) R. Br. subsp. brasiliensis (L.) van Oststr.		IRC IRC IRC	
	I. fulvicaulis (Hochst. ex Choisy) Boiss. ex Hallier f. var.heterocalyx (Schulze-Menz) Verdc. I. linosepala Hallier f.subsp. alpina (Rendle) Lejoly & Lisowski I. rubens Choisy I. pes-caprae (L.) R. Br. subsp. brasiliensis (L.) van Oststr. I. eriocarpa R. Br.		IRC IRC IRC IRC	
	I. fulvicaulis (Hochst. ex Choisy) Boiss. ex Hallier f. var.heterocalyx (Schulze-Menz) Verdc. I. linosepala Hallier f.subsp. alpina (Rendle) Lejoly & Lisowski I. rubens Choisy I. pes-caprae (L.) R. Br. subsp. brasiliensis (L.) van Oststr.		IRC IRC IRC	

	I. welwitschii Vatke ex Hallier f.		IRC
	I. wightii (Wall.) Choisy var.wightii		IRC
Sword/Jack bean	Canavalia Africana Dunn*	NA	PCI
Teff	Eragrostis tef (Zuccagni) Trotter **	NA	NS, PCI
	E. aethiopica Chiov.		NS
	E. heterolomera StapF. **		NS, PCI
	E. fastigiata Cope.		NS, TD (endemic)
	E. sylviae Cope.		NS, TD (endemic)
	E. pilosa (L).P.Beauv. *		NS, PCI
Tobacco	Nicotiana rustica L. *	Economic importance of the crop	PCI
Tomato	Solanum tarderemotum Bitter*	NA	PCI
Yam bean	Sphenostylis briartii (De Wild.) Baker f.	NA	NS
	S. erecta (Baker f.) Hutch. ex Baker f. subsp.erecta	1474	IRC
	S. erecta (Baker f.) Hutch. ex Baker subsp.obtusifolia (Harms) Potter & Doyle		IRC
	S. stenocarpa (Hochst. ex A. Rich.) Harms		IRC
Yam	Dioscorea praehensilis Benth.**	Food security	IRC, PCI
	D. hirtiflora Benth. subsp.orientalis*		IRC, PCI
	D. asteriscus Burkill		IRC
	D. bulbifera (L.) L**		IRC, PCI
Rhodes grass	Chloris pilosa Schumach.	Important fodder crop/Feed security	IRC

((*=potential use for crop improvement) (**=potential use for crop improvement and in GP1b),

PCI=potential use for crop improvement, IRC= importance of related crop, TD=taxon distribution, NS=taxon native status, NA=crop of low priority to Malawi)

CHAPTER 3

IN SITU AND *EX SITU* CONSERVATION GAP ANALYSES OF CROP WILD RELATIVES FROM MALAWI

ABSTRACT

The study analysed the conservation gaps of the priority crop wild relatives (CWR) taxa for Malawi in order to contribute to the development of a harmonized conservation strategy that helps secure the priority CWR under *in situ* and *ex situ*.

Taxa distribution modelling, complementarity analysis and ecogeographic land characterization map were used to analyse spatial diversity and distribution of 123 priority taxa across different adaptive scenarios. Areas of observed and predicted richness, the minimum number of protected areas (PAs) that conserve the broadest ecogeographic diversity *in situ* and the minimum number of grid cells that capture highest diversity outside PAs were identified to recommend the establishment of genetic reserves. The representativeness of the conserved ecogeographic diversity of target taxa in *ex situ* collections was analysed to identify *ex situ* conservation gaps and advise for priority areas for *ex situ* collections.

For the 123 taxa, 70.7 % of the total diversity occurs in 36 PAs with 66.8 % of the diversity captured in only 10 complementary PAs. Outside PAs, the broadest diversity was conserved in three grid cells of size 5 x 5 km. Fifty- three of 123 taxa have *ex situ* collections with only three taxa having *ex situ* collections at the Malawi Plant Genetic Resources Centre.

The findings of this study will guide formulation of conservation actions for the priority taxa as well as lobbying for active conservation of the same under *in situ* and *ex situ*.

Key words: Crop wild relative, conservation gaps, genetic reserves, *in situ*, *ex situ*, protected areas.

3.1. INTRODUCTION

The global community is currently challenged with feeding an expanding human population (FAO 2018; UN 2017; UN 2019). This puts more pressure on already limited resources amidst increased climatic shocks, which have destroyed crops, associated biodiversity and rendered some agricultural land unproductive. The calls for building up of resilient production systems have been echoed in the Sustainable Development Goals (SDGs) 2 and 15 that target reducing hunger, environmental degradation and loss of biodiversity (FAO, 2015; UN, 2015; UNDP 2019). Contribution of plant diversity to food security and its sustainable conservation has received much recognition by many other international bodies such as the Food and Agricultural Organization of the United Nations (FAO, 2012), the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, 2009) and Convention on Biological Diversity (CBD, 2019).

Crop wild relatives (CWR) have potential use in crop improvement (Hajjar and Hodgkin 2007; Vincent *et al.*, 2013). Many crops cultivated in the Southern African Development Community (SADC) region and of global importance such as cotton, wheat, maize, coffee and rice have benefited from adaptive traits originating from CWR (Brar, 2005; Edmé *et al.*, 2005; Hajjar and Hodgkin, 2007; Vincent *et al.*, 2013; Allen *et al.*, 2017; Allen *et al.*, 2019). However, their conservation has been grossly passive with very low representation in many gene banks (Castañeda-Álvarez *et al.*, 2016; Phillips *et al.*, 2016; Contreras-Toledo *et al.*, 2019).

Recognizing the need for improved crop production to meet with increasing food demand in the face of unprecedented livelihood damage and biodiversity loss, the SADC region developed a regional inventory of CWR potential for crop improvement (Allen *et al.*, 2019).

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Currently, the region is developing a CWR conservation strategy in an effort to link conservation and utilization of CWR (Magos Brehm et al. in prep). Further, the 16 SADC member states have a significant contribution in the implementation of the regional strategy by developing their own national strategies that resonate with regional conservation priorities. At present, only Zambia, Mauritius and South Africa have such strategies in place (Ministry of Agriculture 2016; Ng'uni et al., 2017; Bissessur et al., 2019; Holness et al., 2019). Malawi has about 6000 plant taxa excluding the bryophytes and 446 CWR out of which 277 are priorities for conservation based on various criteria including the economic importance of the related crop, their potential use in crop improvement, threat status, native status, taxon national and global distribution (Mponya et al., 2020). National stakeholders in nature conservation and agrobiodiversity community agreed upon the prioritization criteria and methodology. In order to sustainably conserve these resources, Malawi plan to develop a national conservation strategy for the conservation of priority CWR. Having a stand alone CWR strategy has been found to act as a catalyst to a more systematic *in situ* and *ex situ* conservation helping reduce loss of these valuable resources (Magos Brehm et al., 2017a). Complementary conservation helps conserve the broadest range of CWR taxa (Maxted et al. , 1997; Maxted et al., 2015), and with 126 terrestial protected areas in Malawi, there is potential to update management plans in order to accommodate CWR active conservation. However, the current status is that the country lacks knowledge of the distribution of CWR diversity and of the precise locations where conservation that could capture maximum diversity could be designated. In addition, ex situ conservation gaps for the priority taxa are not yet known. The lack of such fundamental information deterred conservation efforts of CWR in Malawi as such guide conservation planning and development of the national conservation strategy (Magos Brehm *et al.*, 2017a).

Thus the aim of this study was to undertake gap analyses of the CWR of Malawi, through (a) analysing the spatial distribution of priority CWR diversity for Malawi; (b) modelling the potential distribution of priority taxa; (c) identifying the minimum number of complementary potential *in situ* sites within protected areas (PA) that could conserve the broadest range of ecogeographic diversity *in situ*; (d) identifying locations outside the PAs with high CWR presence where non-PA *in situ* conservation or novel PAs could be established; and (e) identify locations where priority CWR for *ex situ* collections could be sampled.

3.2. METHODS

3.2.1. Occurrence data collation, verification and quality check

To achieve the above aims, methods adapted from Magos Brehm *et al.* (2017a) and widely used at national and global level CWR conservation were applied (e.g. Hunter and Heywood (2011); Fielder, 2015; Castañeda-Álvarez *et al.* (2016); Phillips *et al.* (2016); Taylor *et al.* (2017); Contreras-Toledo (2018)).

The inventory contains 277 CWR taxa but only 123 taxa were included for this study because of lack of coordinates and specific site occurrence data. The standard template for collation of CWR distributional data was used (Magos *et al.*, 2017b) for the 123 priority CWR taxa (Mponya *et al.*, 2020). Not all priority CWR taxa are included in the current eFlora of Malawi (Hyde *et al.*, 2018) and therefore additional occurrence data was obtained from institutions holding Malawi plant herbarium specimen, accessions and other plant information. Such include Royal Botanical Gardens–Kew herbarium and Kew.org/herbcat/navigator.do (2017); Bioversity Collecting Missions Database (2016); Global Biodiversity Information Facility

(GBIF 2017); and Genesys Global Portal on Plant Genetic Resources (2016), National Herbarium and Botanic Gardens of Malawi and the Malawi Plant Genetic Resources Centre (MPGRC). Since 67% of the records did not have associated coordinates, the initial stage was georeferencing of these records using Google Maps (http://maps.google.com/) and a national gazetteer (https://www.diva-gis.org/gdata). Records with coordinates of the records using Canadensys (http://data.canadensys.net/tools/coordinates). Geographic outliers were filtered in DIVA-GIS version 7.5.0 (http://www.diva-gis.org) (Hijmans *et al.*, 2012). Occurrence data was then organised in FAO-Bioversity passport descriptors format (FAO-Bioversity, 2012). Quality of georeferencing of the collection sites was assessed using the GEOQUAL tool of the CAPFITOGEN Version 2.0. package that enables to assess the quality of the geographic coordinates (COORDQUAL), suitability of the indicated sites (SUITQUAL) and quality of the location information (LOCALQUAL) (Parra-Quijano *et al.*, 2016). Occurrence records with total quality (TOTALQUAL) above 50% were used for this study.

3.2.2. Distribution and ecogeographic diversity analyses

Observed taxa distribution and sampling bias were mapped in DIVA-GIS 7.5.0 (Hijmans *et al.*, 2012) for 1621 records with TOTALQUAL >50% for a grid cell size of approximately 10x10 km. The potential taxa distribution and ecogeographic diversity were obtained using the Maximum Entropy (MaxEnt) algorithm (Phillips *et al.*, 2006) and circular buffer (CA₅₀) in ArcMap 10.4.1 (ESRI 2015; Hijmans and Spooner, 2001; Contreras Toledo *et al.*, 2019).

3.2.3. Taxa distribution modelling

Potential taxa distribution was estimated by individual distribution models generated for taxa (Supplementary Table 2) with ≥ 10 occurrence records in MaxEnt (Phillips *et al.*, 2006; Elith *et al.*, 2006; Hernandez *et al.*, 2006) based on individual sets of ecogeographic variables

(Supplemementary Table1) from Worldclim (https://www.worldclim.org/bioclim) and by circular buffer (CA₅₀) for taxa with < 10 records. Random forest was used to select variables for each of three categories (bioclimatic, edaphic and geophysical with a cell size of 5 x 5 km (\approx 2.5 arc minutes at the Equator)) for each taxon in SelecVar (Parra-Quijano et al. 2016). To reduce dimensionality, Bivariate correlation analysis was run in SelecVar and only variables with weak correlation (Pearson value of \leq 0.3) or not correlated (Pearson value =0) were used to generate species distribution models for each taxon (Supplementary Table 2).

Cross validation test and maximum training sensitivity plus specificity threshold were applied. Taxa with \geq 50 occurrence records used 10 replications and 5 replications for taxa with \geq 10 records. Models that had; 1) average area under the Test ROC Curve (ATAUC) > 0.7; 2) standard deviation of ATAUC (STAUC) below 0.15; and 3) proportion of potential distribution area with standard deviation above 0.15 (ASD15) is below 10%, were considered stable and used for estimating potential taxa distribution (Ramírez-Villegas *et al.*, 2010; Liu and Matt, 2016; Contreras Toledo *et al.*, 2019). For the taxa that did not pass the MaxEnt models validation criteria above and for taxa with <10 occurrence records a circular buffer method was applied adapting a 19 km buffer diameter for Malawi based on country size. For studies targeting larger areas, a 50 km circular buffer (CA₅₀) was considered (e.g. Hijmans and Spooner, 2001; Contreras Toledo *et al.*, 2019).

3.2.4. Complementarity analysis

Complementarity analysis was run in CAPFITOGEN with the Complementa tool at a resolution of 5 x 5 km (approximately 30 arc segment at the Equator). PAs network data for Malawi (UNEP-WCMC 2019) was used in Complementa to identify PAs containing highest taxa diversity and those with large number of unique taxa to propose for genetic reserves. For PAs with similar number of unique taxa, random selection was applied. The complementary

analyses maps were visualised in DIVA-GIS 7.5.0 (Hijmans *et al.*, 2012) and ArcMap 10.4.1 (ESRI 2015). A grid cell analysis was also run in Complementa and identified hotspot grid cells outside PAs that would optimize *ex situ* collections as well as conserve CWR diversity outside PAs.

3.2.5. Ecogeographic land characterization map

Finally, a generalist Ecogeographic Land Characterization (ELC) map that defines general land characteristics where taxa could occur was produced with the ELCmapas tool in CAPFITOGEN 2.0, using the elbow method, with a cell size of 5 x 5 km (approximately 2.5 arc minutes at the Equator), as described by Parra-Quijano *et al.* (2016). Eleven variables (Supplementary Table 1) were used to produce the ELC map. Variables were selected in SelecVar as described in taxa distribution modelling.

3.2.6. Conservation gaps

Average Maxent models for each taxon and potential distribution map produced by a circular buffer (CA_{50}) method were combined in DIVA GIS.7.5.0 to create potential taxa distribution map for 123 priority CWR taxa. The observed taxa distribution map was subtracted from the potential distribution map. *In situ* conservation gaps were estimated by 1) comparing the coverage of the observed richness already passively conserved *in situ* in the PAs and that which is outside of PAs; 2) comparing number of ELC zones captured in PAs against those outside PAs (this helps understand the distribution of taxa diversity in different environments) and 3) by comparing populations of taxa conserved in PAs versus that outside of PAs.

The taxa and ecogeographic diversity represented in genebank accessions held by MPGRC and international genebanks (Table 3.5b) were analysed using Representa tool in CAPFITOGEN tools (Parra-Quijano *et al.*, 2016). Adaptive scenarios (ELC zones) from ELC map developed earlier were used to divide the ELC map into four classes (Low, medium,

medium to high and high) based on their frequency on ELC map as well as based on collections. *Ex situ* conservation gaps were identified by comparing representation of the ELC classes in the *ex situ* collections held at the MPGRC and international genebanks and by comparing the diversity conserved *ex situ* against that present *in situ*.

3.3. RESULTS

3.3.1. Observed and potential taxa diversity and distribution

Analyses were done on 123 priority taxa out of the 277 priority taxa included in the national inventory, as there were no data for the remaining priority CWR. Hotspots of CWR taxa were observed in the district of Zomba (42 taxa) in the Southern Region with part of the diversity occurring in Zomba Forest Reserve and extends outside the protected area, Dedza (22) in the Centre and Mzimba (25) District in the Northern Region bordering Kaning'ina Forest Reserve on Nkhata Bay District side (Figure 3.1a). These hotspots correspond to the same areas where observational bias was noted (Figure S1).

An average potential distribution map created from the 15 taxa models that passed the validation criteria (Supplementary Tables1 and 2) and that from circular buffer (CA50) for the taxa that did not pass the validation criteria and those with < 10 records indicates wider coverage of diversity of priority CWR in Malawi (Figure 3.1b). Most of the diversity was predicted outside of PAs and possibly in cultivated land and settlement areas. In the Northern Region, much of the diversity is predicted in Nyika National Park, Kaning'ina Forest Reserve in the vicinity of Mzuzu city and Mughese and Wilindi Forest Reserves (Figure 3.1a). Diversity in Blantyre, Thyolo and Dedza Districts was predicted within the towns raising more concern on the availability of such taxa as the demand for settlement is on increase.

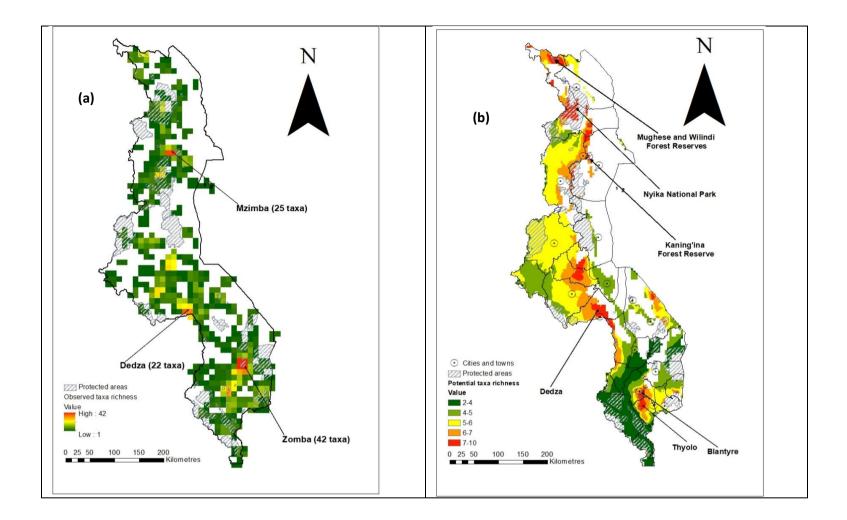


Figure 3. 1: Observed (a) and Potential richness (b) and distribution of priority CWR taxa in Malawi, grid square size of 0.1 degree (10 x 10 km at the Equator).

3.3.2. Complementarity analyses

Coverage analysis identified 36 PAs with at least one priority CWR taxon (Figure S2) and these in total conserve 70.7% diversity of 123 priority CWR taxa and diversity is defined by number of taxa. The 36 PAs represent 63.2% of the total PAs area in Malawi. However, 32 PAs were identified as complementary (Figure S3). Within the complementary PAs, 66.83% of the diversity is conserved in ten (10) PAs. (Figure 3.2).

Higher numbers of CWR taxa were conserved in South Viphya Forest Reserve (38 taxa), Nyika National Park (36), Mulanje (24) and Zomba (19) Forest Reserves; Lengwe (11) and Kasungu National Parks (seven) (Figure 3.2 and Table 3.1) and the other four PAs had less than seven taxa. The six PAs with highest diversity had also higher overall taxa occurrences (range of 10–86) (Figure S2, Table 3.1). However, 61 taxa present in these PAs have low (\leq 3) known occurrences (Table 3.1 and Supplementary Table 3). Taxa with low population size had on average 75% of their population located outside PAs (Supplementary Tables 3 and Table 4). Ten (10) taxa recorded a single population (Supplementary Tables 3) which means that they only occur at one site within a PA. Unfortunately, none of these has *ex situ* collections at MPGRC (Supplementary Table 5a) and *ex situ* collections of 11 taxa held at international genebanks (Supplementary Table 5b) had no duplicates at the MPGRC (Supplementary Table 5b) had no duplicates at the MPGRC (Supplementary Table 5a).

Those with accessions in the international genebanks include *Vigna unguiculata* (L.)Walp subsp. *dekindtiana* (Harms) Verdc., *V. unguiculata* (L.)Walp subsp. *pawekiae* Pasquet, *V. unguiculata* (L.)Walp subsp. *pubescens* (R.Wilczek) Pasquet, *V. unguiculata* (L.)Walp subsp. *stenophylla* Harms (Mponya *et al.*, 2020). These have potential use in crop improvement and require immediate field exploration for their conservation. These populations are then priority

for collection and conservation in *ex situ* genebanks. Grid cell analyses identified hotspots in Dedza District (Point a), Lilongwe District (point b) and the boundary between Dowa and Ntchisi Districts (point c) in Figure 3.2.

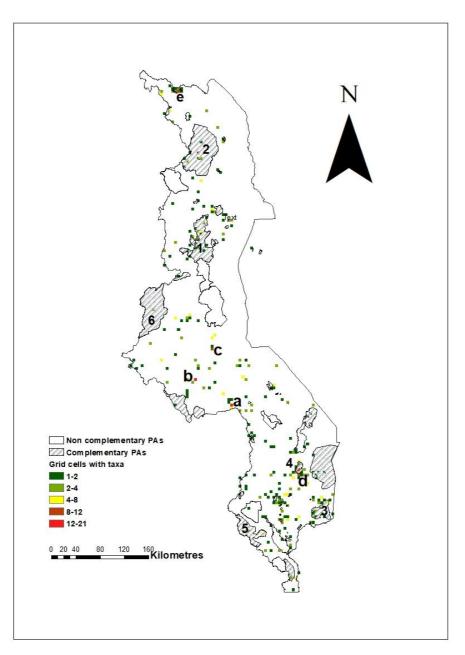


Figure 3.2: Complementary network of protected areas identified to passively conserve *in situ* priority CWR taxa in Malawi and number of grid cells (size 5 x 5 km at the equator) with taxa. Letters refer to grid cells outside protected areas with high number of taxa and numbers refer to 6 ranked complementary protected areas with high number of taxa and potential for genetic reserves.

3.3.3. Ecogeographic land characterization map

Eleven environmental variables (4 bioclimatic, 4 edaphic and 3 geophysical) were used to generate 27 ELC zones (Fig.3.3) which represented potential adaptive scenarios for 123 priority taxa (Parra-Quijano *et al.*, 2012, Parra-Quijano *et al.*, 2011). The ecogeographic diversity of 19 ELC zones is passively conserved in complementary PAs (Supplementary Table 6) and 12 out of the 19 ELC are located in the 6 complementary PAs with highest diversity (Table 3.1, Figure 3.3 and Figure 3. 4b) and with potential for genetic reserve designation.

Rank	Complementary PA	No. occurrences	No. CWR	No. unique CWR	ELC zones	PA coverage (Km ²)
1	South Viphya	49	38	38	0*,18*,19,21*,2 4,25	1147.8
2	Nyika National Park	65	37	36	0*,18,19*,24	3092.32
3	Mulanje Forest reserve	59	26	24	0*,9*,15*	552.09
4	Zomba Forest reserve	86	33	19	0*	59.57
5	Lengwe National Park	15	12	11	0*,5*,7*,8*	928.19
6	Kasungu National Park	10	7	7	18,19*,21,22,24 ,25*	2358.62

Table 3.1: Occurrences and diversity coverage of priority crop wild relatives taxa (CWR) represented by ELC zones across six priority complementary PAs.

* means ELC zones where taxa were observed

Protected areas coverage data source: Protected planet (<u>https://protectedplanet.net/</u>, (UNEP-WCMC (2019)).

3.3.4. Ecogeographic diversity representativeness in *ex situ* collections and taxa *in situ* By percentage, reprensentativeness results indicate that only 25.9 % of the diversity of the nalaysed priority CWR is conserved *ex situ* and the rest remains in the wild and passively conserved. This diversity represents 53 taxa whose collections are held at MPGRC (102) with 555 accessions held by international genebanks (Supplementary Tables 5a and S5b). The 53 taxa represent ecogeographic diversity of 20 ELC zones (Fig.3.4a) and the diversity of 7 ELC zones is not represented. Twelve of the 20 ELC zones are conserved by both MPGRC and international genebanks (Table 3.2) with ELC zones 0, 8 and 19 being relatively represented in both genebanks collections. Coincidently, these seem to be ELC zones with high frequency on the ELC map (Table 3.2). In terms of population size, only ELC zones 0 and 8 had sufficient (\geq 10 %) representation at MPGRC and the rest had less than 5% representation to zero (no *ex situ* collections). The trend was similar to international genebanks but in either case ELC zone 0 had high representation in both genebanks collections and much of its diversity was also passively conserved *in situ* (Figure 3.4a).

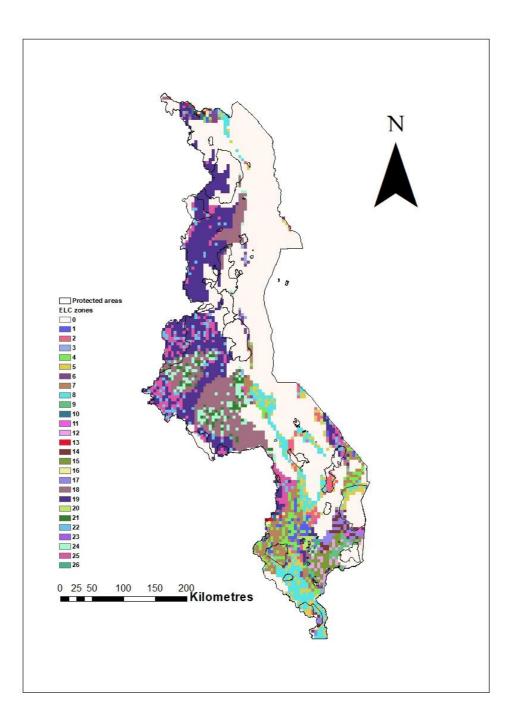


Figure 3.3: ELC map for Malawi composed of 27 ELC zones. Each zone represents a combination of environmental variables. Zone 0 represents areas to which information on some environmental variables is missing.

By numbers, the MPGRC conserved three of the 53 taxa with *ex situ* collections and these included *Oryza longistaminata*, *O. barthii and O. punctata* (Supplementary Table 5a). With exception of *O. longistaminata* which was categorized as threatened (Vulnerable) by South African plants red listing (2009), taxa threatened at global level such as *Coffea ligustroides* S.Moore, *C. salvatrix* Swynnerton & Phillipson, and *C. arabica* L. (wild types) and *Prunus africana* (Hook.f.) Kalkman, (IUNC, 2018) had no *ex situ* collections held by external gene banks either, had restricted geographic distribution with small population size (Supplementary Tables 3 and 4).

Oryza and *Vigna* have more collections than other taxa. ELC zones 19 and 20 (Figure 3.4a) had extensive collections by external partners. Coincidently, the most collected ELC zones happened to be represented *in situ* especially in PAs with the highest diversity (Table 3.1).

Table 3.2: ELC categories classification based on taxa collections and the frequency of ELC zones on the ELC map for future taxa exploration

	ELC category	Classification by frequency of taxa occurrence (based on National gene bank collections) ^a	ELC category	Classification by frequency of the category on ELC map ^b
-	1,3,4,9,10,11,12,13,14,16,17, 20,23,24,26	Not collected	Not applicable	Not applicable
	2,6,15,18,21,22,25	Low**	3,10,11,13,16,23,26	Low
	Not applicable ^c	Medium-Low	1,6,9,12,14,19,20	Medium-Low
	5,7	Medium-High	2,4,15,21,22,24	Medium-High
	0,8,19	High	0,5,7,8,18,19,25	High

**Low refers to classification where <5 samples were collected.

^a Refers to the frequency of ELC classes as observed in the *ex situ* collections.

^b Refers to the frequency of ELC category on ELC map.

"ELC Zones categories not represented in the ex situ collections of the national gene bank.

3.3.5. *In situ* conservation gaps

About 19 different envornments with occurrence of CWR were noted in 36 PAs. These represent 19 ELC zones being passively conserved in 36 PAs, however only three ELC zones have relatively high (>20) number of taxa (Figure 3. 4b). This agrees with Supplementary Tables 3 and 4 that indicated highest number of taxa having greater proportion of their population outside PAs. Figure 3.5 indicated similar outputs of having most of the predicted hotspots outside PAs with exception of potential richness captured in Nyika National Park, South Viphya and Mughese Forest Reserves in the Northern Region.

In Central Region, hotspots were in Dedza (a), Dowa (b), Ntchisi (c) and Ntcheu (d), Districts (Figure 3.5). In the Southern Region, hots pots were in Mangochi, Blantyre, and Thyolo Districts (Figure 3.5). The diversity conserved *in situ* covers 87 taxa out of 123. Ecogeographic diversity that does not occur in PA include that falling in ELC zones of 1, 3, 4, 6, 11, 12, 16 and 26 (Supplementary Table 6 and Figure 3.4b).

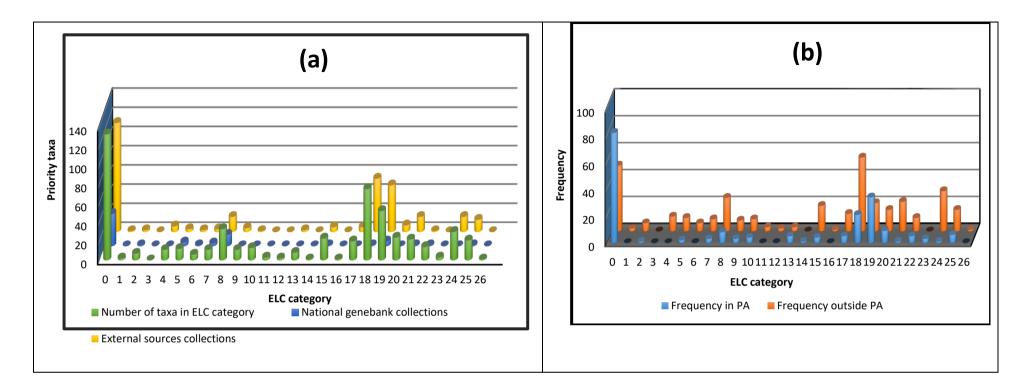


Figure 3.4: *Ex situ* (a) and *in situ* (b) conservation gaps of priority CWR taxa based on taxa representation at national and international genebanks and taxa passively conserved *in situ* in PAs and outside PAs across the 27 ELC categories.

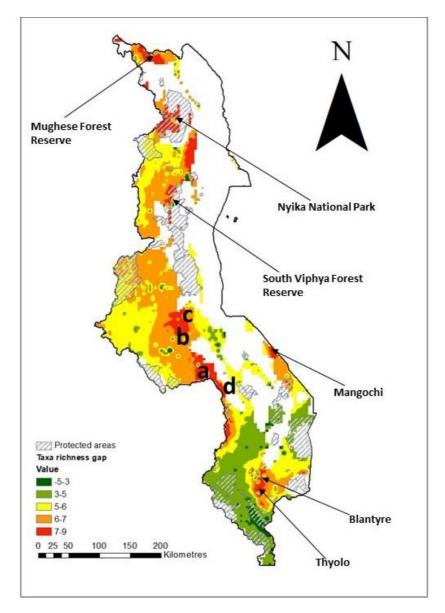


Figure 3.5: Taxa richness gaps for further exploration in Malawi. Grid square size of 0.1 degrees ($10 \times 10 \text{ km}$ at the equator). Labelled sites have the highest number of predicted taxa than observed.

3.4. DISCUSSIONS

In Malawi, the diversity of priority CWR has a wide geographic coverage and no CWR populations are actively conserved in existing PAs. For the analysed taxa, a relative high amount of diversity (70.7%) is passively conserved in 36 PAs, and the remainder occurs outside PAs. Outside PAs, hotspots were observed in grid cells located in Dedza and Lilongwe Districts and the boundary between Ntchisi and Dowa Districts. These sites are documented as forest reserves in the Protected Planet database although their polygons were not yet available when the study was conducted. It is therefore likely that the percentage of diversity passively conserved *in situ* for 123 taxa is >70.7%.

Having a huge amount of diversity being already passively conserved in PAs provides for a great opportunity to advocate for an active *in situ* conservation given that the establishment of genetic reserves will require minimal negotiations as PAs would only have to adapt their management plans to accommodate CWR conservation (Maxted and Kell 2009).

For an active *in situ* conservation of priority taxa in Malawi, 10 PAs could be potential for active *in situ* conservation as they passively conserve more that 60% of the priority CWR taxa for which data are available ie they capture 73 out of 123 priority taxa. However, only 6 PAs (South Viphya, Mulanje and Zomba Forest Reserves; Nyika, Kasungu and Lengwe National Parks) have; (1) stable taxa populations with high numbers of occurrences (range of 10–86), (2) high number of unique taxa not found in any of the other complementary PAs; (3) they represent unique adaptations for the taxa (being located in different agro ecological zones), (4) harbour rare taxa. For efficiency and cost effectiveness, these complementary PAs should be considered for genetic reserves.

Zomba Forest Reserve is of particular interest because it has a small coverage area (59.57 km²) and yet has the highest (86) population of taxa and taxa richness (33) with 19 unique taxa that only occur in this complementary PA and taxa diversity pattern extends outside its borders as well. This was not surprising because Rapid Botanical Surveys conducted in the Shire River Basin in 2016 reported that Zomba-Malosa Forest Reserve harbours taxa of significant global uniqueness and has the fourth-highest level of globally-rare, restricted range taxa (Shire River Management Programme, 2017).

The overflow taxa richness was also predicted in Mughese, Wilindi and Kaning'ina Forest Reserves and Nyika National Park where diversity extends beyond borders of these PAs. In order to ensure there is minimal disturbance to the taxa and its biosphere, the reserve should consider including all areas with unique taxa that is not captured within the sites considered as hotspots. (Hunter and Heywood 2011 Chapter 9). Genetic reserves for the other five PAs may be considered in localized grids of 10 x 10 km considering that they have large area coverage (500–3092 km²) which make them practically impossible to effectively monitor the species population (Maxted and Kell 2009) and difficult to manage the associated threats (Hunter and Heywood 2011 Chapter 9).

It is important to note that populations of 72% of individual taxon occurring across PAs were less than 5 with 32% having population range of 1–2. It is particularly concerning that these taxa may only occur once or twice in a PA and may be prone to localized threats. It was also noted that a large proportion (>75%) of the population of these taxa was outside PAs. Efforts to survey their occurrence in other sites predicted by species modelling can help establish the present population and this helps in designing an effective monitoring mechanism (Iriondo *et al.*, 2008).

Of particular interest are taxa with no population in PAs (Supplementary Table 3). Probably the indicated ELC zones captured in PAs in which they are present should be used for surveying as these represent environments into which they are potentially adapted. Diversity outside PAs was observed in Thyolo, Dedza, Ntchisi, Dowa and Chitipa Districts (Figures 3.1 and 3.5). Some of these sites are near and or at the centre of the towns (Figure 3.1) and having such diversity not conserved elsewhere is a risk.

When compared with the observed diversity, it was also noted that more sites in Malawi were predicted to have CWR and much of the diversity was predicted outside PAs. This could mean that Malawi is under surveyed. In order to secure a broad range of the diversity of taxa outside PAs, these sites should be explored for genetic reserves. In that case, it would require three grid cells of 10 x 10 km to conserve this diversity of priority CWR outside PA given that other grids with highest taxa are within and or close to PAs. Although these sites occur within or close to towns, efforts to assess their suitability for establishing genetic reserves should not be undermined as some of these towns have gardens with natural vegetation, forest reserves that could be potential for active *in situ* conservation of the priority CWR taxa. However, for effective conservation, priority must be given for *ex situ* collections because taxa present in these sites are likely to be more threatened than those in PAs due to changes in land use that may result to habitat loss. There is more ecogeographic diversity of priority CWR outside PA than within existing PA. Therefore conserving this diversity ensures capturing of both low represented ELC zones in PAs and ELC zones that are rare.

The results of taxa representativeness in *ex situ* conservation were a true reflection of global gap analysis outcomes (Castañeda-Álvarez *et al.*, 2016). Priority taxa are poorly represented

at MPGRC. Currently, only three taxa (*Oryza barthii, O. longistaminata and O. punctata*) out the 277 priority taxa have *ex situ* collections at the MPGRC and this calls for urgent action. When possible, the existing 555 *ex situ* accessions of the 50 CWR taxa held at international genebanks should be retrieved to have their duplicates conserved at MPGRC. Retrieval and conservation of these accessions at MPGRC will provide breeders with an opportunity for pre breeding and further exploration as all the accessions have potential for crop improvement. *O. punctata* is under-represented with three records at MPGRC and zero collections reported for external genebanks. Even within Malawi, this taxon is rare with only two occurrence sites hence the need to put effective conservation measures before it disappears.

Although the large amount of priority CWR diversity seems to be passively conserved *in situ*, under representation in the MPGRC defeats the very purpose of utilization, which is the focus of CWR conservation. The need to conserve such taxa *ex situ* is paramount if we are to facilitate access and utilization in crop improvement as well as further exploration by other users (Hunter and Heywood 2011 Chapter 1).

Ex situ collections are needed for 121 taxa without collections at MPGRC and the same applies to 154 priority taxa not included in this study. Predicted hotspots outside PAs should be targeted in order to capture broad range of diversity with minimal expeditions to safeguard priority CWR taxa before they go extinct due to climate change and anthropogenic related threats.

Ecogeographic diversity of ELC zone 0 had more than 100 accessions from 86 taxa under *ex situ* conservation and with high frequency of occurrence in PAs compared to other ELC zones (Table 3.2, Figure 3.3, Figure 3.4a and Figure 3.4b). Rare ELC zones represent unique potential adaptive scenarios and taxa from such environments might represent unique genes

(Parra-Quijano *et al.*, 2016) and should be priority for *ex situ* collections. However, for wider ecogeographic diversity representativeness, Figure 3.5 and Table 3.2 should guide the *ex situ* collection missions as conserving the full range of diversity ensures unique genes are also captured (Parra-Quijano *et al.*, 2016).

Although species seed dispersal mechanisms and geographic barriers may influence potential species distribution, potential richness based on MaxEnt models and circular buffer (CA₅₀) closely resembled the pattern of observed richness and therefore gave a true reflection of the diversity distribution in Malawi. Taxa richness was predicted in sites previously observed through grid cell analysis signifying that the richness in these sites could be one aspect of the observational bias noted in this study. The reason for this could be that most collectors and botanists tend to concentrate their collections in areas where diversity is high (Hunter and Heywood 2011). However, more work should be done to establish the status of the remaining 154 priority taxa excluded from this study as some of these taxa have potential use in crop improvement and such include *Brassica juncea* (L.) Czern., *Gossypium barbadense* L., *Olea europaea* L. subsp. *cuspidata* (Wall. ex G.Don) Cif (Mponya *et al.*, 2020). Predicting occurrence and distribution of the priority taxa was the first step. As a follow up to this study, the following conservation actions are recomended:

- Conduct field surveys to establish the current distribution of 123 priority taxa targeting potential hotspots as predicted by the SDMs and circular buffer (CA₅₀) and for the distribution of 154 taxa not included in this study.
- Assess the status of taxa with fewer populations (1–4) in both PAs and outside the PAs in order to establish their current status and design sustainable measures for their conservation.

- 3. Assess the status and suitability of the six complementary PAs with highest diversity of priority CWR taxa for establishing genetic reserves based on the recommendations of Dulloo *et al.* (2008) and the quality standards described in Iriondo *et al.* (2012).
- 4. Initiate negotiations for border expansion for the suitable complementary PAs whose diversity spans beyond the set boundaries and this should only be considered if the diversity of CWR in question is not conserved within the borders of the PAs.
- 5. Initiate urgent *ex situ* collection expeditions for the 121 taxa not represented in the MPGRC and for *O.punctata* that is underrepresented targeting:
 - a. Hotspots outside protected areas first and then rare adaptive environments (ELC zones) see Figure S2 and Table 3.2.
 - b. Taxa whose largest (>60%) population is outside PAs (Supplementary Table 4).
- 6. Plan for retrieval of the *ex situ* collections of the taxa held at international genebanks but have no duplicates at MPGRC and duplicate these with the SADC gene bank.
- **7.** Update findings in this chapter and recommendations periodically based on available data and or information.

3.5. CONCLUSION

The outcomes of this study provide a foundation for conservation planning for CWR in Malawi. Although only based on analysis of 123 priority CWR taxa, they act as a reference point for the other taxa not included in this study, as the methods used are applicable to both. Understanding that conservation needs for CWR and that of users may change overtime, the recommendations provided on these findings should be regarded as guidance and where more information is made available, they can be modified. Considering that this nature of work is holistic, the views of stakeholders during reserve evaluation should not be undermined and the implementation of the recommended conservation actions should be a shared

responsibility. Any support to ensure that these resources are safeguarded brings a difference. Lastly, the results provide an opportunity for other SADC member states to draw lessons from having a number of member states without knowledge of current conservation gaps of CWR.

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APPENDIX 2

SUPLEMENTARY MATERIALS

Supplementary Table 1: Climatic, edaphic and geophysical variables used in taxa distribution modelling in MaxEnt based on SelecVar analysis

TAXA	ENVIRO	NMENTAL V.	ARIABLES US	ED FOR SPE	CIES DISTRI	BUTION MOI	DELLING		
CHENOPODIUM AMBROSIOIDES	bio_2	bio_14	t_sand	t_oc	s_ph_h2o	eastness	northeness	slope	
COFFEA LIGUSTROIDES	bio_10	bio_14	t_bs	t_cec_soil	s_gravel	slope	northeness	aspect	
COFFEA MUFINDIENSIS SUBSP.	bio_2	bio_4	bio_15	t_caco3	s_gravel	s_cec_soil	eastness	aspect	
AUSTRALIS									
CUCUMIS ANGURIA VAR. ANGURIA	bio_4	bio_15	t_sand	t_caco3	northess	slope			
ELEUSINE INDICA	bio_1	bio_2	bio_15	t_caco3	s_cec_soil	t_ce_soil	aspect	slope	eastness
ELEUSINE CORACANA SUBSP.	bio_2	bio_10	bio_15	s_cec_soil	t_oc	aspect	slope		
AFRICANA									
IPOMOEA PILEATA	bio_1	bio_4	bio_15	s_gravel	t_oc	s_sec_soil	aspect	slope	eastness
IPOMOEA OBSCURA SUBSP.	bio_2	bio_4	bio_10	t_caco3	t_bs	t_cec_soil	aspect	alt	slope
OBSCURA									
IPOMOEA TENUIROSTRIS	bio_4	bio_10	s_gravel	s_cec_soil	slope	eastness			
ORYZA BARTHII	bio_2	bio_10	bio_14	t_caco3	t_oc	northeness	aspect		
ORYZA LONGISTAMINATA	bio_1	bio_2	t_oc	s_cec_soil	aspect	slope			
PRUNUS AFRICANA	bio_10	bio_15	t_oc	s_cec_soil	aspect	slope			
SOLANUM ACULEATISSIMUM	bio_2	bio_7	t_caco3	t_ph_h2o	aspect	alt	eastness		
SOLANUM ANGUIVI	bio_1	bio_3	bio_7	t_gravel	s_cec_clay	slope	aspect	alt	
SOLANUM CAMPYLACANTHUM	bio_1	bio_4	bio_15	t_caco3	s_cec_clay	slope	northeness	alt	
SOLANUM HISPIDUM	bio_1	bio_2	t_ph_h2o	t_gravel	northess	eastness			
SOLANUM INCANUM	bio_1	bio_3	t_ph_h2o	t_sand	aspect	northness			
SOLANUM NIGRUM	bio_1	bio_4	bio_19	t_sand	t_ph_h2o	aspect	northeness		
SOLANUM PANDURIFORME	bio_1	bio_3	t_gravel	s_gravel	t_caco3	aspect	slope		
SOLANUM RICHARDII SUBSP.	bio_4	bio_15	s_gravel	t_cec_clay	slope	northness			
RICHARDII									
SOLANUM RICHARDII	bio_1	bio_7	bio_19	s_ph_h2o	s_gravel	slope	eastness		
SOLANUM SCHUMANNIANUM	bio_2	bio_4	t_sand	s_cec_clay	slope	eastness			
SOLANUM TARDEREMOTUM	bio_1	bio_3	bio_7	t_gravel	t_ph_h2o	alt	northeness	eastness	
SOLANUM TERMINALE	bio_2	bio_3	bio_15	t_gravel	t_cec_clay	slope	eastness		

TAXA	ENVIRONMENTAL VARIABLES USED FOR SPECIES DISTRIBUTION MODELLING						
SOLANUM TERMINALE SUBSP.	bio_4	bio_15	t_sand	s_cec_clay	alt	northness	
TERMINALE		_	_	į			
SOLANUM TORVUM	bio_4	bio_12	t_sand	s_cec_clay	alt	northness	
SORGHUM BICOLOR SUBSP.	bio_1	bio_4	t_gravel	t_ph_h2o	aspect	slope	
ARUNDINACEUM			U	•			
SORGHUM VERSICOLOR	bio_1	bio_19	t_ph_h2o	t_oc	slope	alt	
VIGNA FRUTESCENS	bio_1	bio_15	t_ece	s_gravel	slope	alt	
VIGNA UNGUICULATA VAR.	bio_1	bio_2	t_sand	t_caco3	alt	eastness	
UNGUICULATA							
VIGNA PYGMAEA	bio_4	bio_15	t_sand	t_ph_h2o	slope	northness	
VIGNA PLATYLOBA	bio_1	bio_15	t_oc	t_caco3	slope	alt	
VIGNA OBLONGIFOLIA	bio_2	bio_19	s_gravel	s_ph_h2o	aspect	nortness	
VIGNA PHOENIX	bio_2	bio_10	t_cec_clay	slope	aspect		
VIGNA HETEROPHYLLA SUBSP	bio_14	bio_16	t_caco3	t_ref_bulk	alt	aspect	
.AMBACENSIS							
VIGNA UNGUICULATA SUBSP.	bio_4	bio_15	t_ph_h2o	t_oc	aspect	slope	
DEKINDTIANA							
VIGNA UNGUICULATA SUBSP.	bio_2	bio_3	bio_15	t_oc	t_bs	eastness	aspect
SPONTANEA							
VIGNA GAZENSIS	bio_7	bio_15	t_ece	s_ph_h2o	aspect	slope	
VIGNA VEXILLATA	bio_1	bio_7	t_oc	t_cec_clay	eastness	alt	
SUBSP.ANGUSTIFOLIA							
VIGNA VEXILLATA	bio_1	bio_3	bio_7	t_oc	t_bs	alt	eastness
VAR.VEXILLATA							
VIGNA COMOSA	bio_7	bio_15	t_ph_h2o	s_gravel	slope	northness	
VIGNA LUTEOLA	bio_1	bio_15	t_ece	s_ph_h2o	eastness	northness	alt
VIGNA RACEMOSA	bio_3	bio_12	t_cec_clay	t_oc	slope	alt	
VIGNA RETICULATA	bio_7	bio_15	t_ph_h2o	t_caco3	slope	aspect	

Sources 1.Bioclimatic data https://www.worldclim.org/bioclim

___2. Edaphic data and geophysical data (Harmonised soil data base): http://www.fao.org/soils-portal/soil-survey (Fischer et al., 2008).

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ТАХА	TEST AUC	AUC STANDARD	MAXIMUM TRAINING	PREDICTED AREA BEING	MODEL
		DEVIATION	SENSITIVITY PLUS SPECIFICITY	<10% OF THE AUC	VALID(YES/NO)
			CLOGLOG THRESHOLD	STANDARD DEVIATION>0.15	
CUCUMIS ANGURIA VAR. ANGURIA	0.4805	-0.5496	0.634	N/a	No
COFFEA LIGUSTROIDES	0.8811	0.0643	0.3914	6.72	Yes
COFFEA MUFINDIENSIS SUBSP.	0.9191	0.0576	0.3982	0	Yes
AUSTRALIS					
CHENOPODIUM AMBROSIOIDES	0.5172	0.1048	0.6269	N/a	No
ELEUSINE CORACANA SUBSP.	0.6616	-0.5622	0.6152	N/a	No
AFRICANA					
ELEUSINE INDICA	0.758	0.1074	0.5293	N/a	No
IPOMOEA OBSCURA SUBSP. OBSCURA	0.5352	-1	0.758	N/a	No
IPOMOEA PILEATA	0.8477	0.0816	0.4869	2.737	Yes
IPOMOEA TENUIROSTRIS	0.819	0.1146	0.4193	28	No
ORYZA BARTHII	0.7213	0.1303	0.5186	1558	No
ORYZA LONGISTAMINATA	0.7137	0.0839	0.6495	4.11	Yes
PRUNUS AFRICANA	0.8034	-0.7991	0.5607	134	No
SOLANUM ACULEATISSIMUM	0.6242	-0.5298	0.6692	N/a	No
SOLANUM ANGUIVI	0.9125	0.0387	0.3155	2.84	Yes
SORGHUM ARUNDINACEUM	0.8151	0.06	0.4505	2.07	Yes
SOLANUM CAMPYLACANTHUM	0.6299	-0.2018	0.6939	N/a	No
SOLANUM HISPIDUM	0.8204	-1	0.616	12.7	Yes
SOLANUM INCANUM	0.389	-0.5633	0.6592	N/a	No
SOLANUM NIGRUM	0.6741	0.1144	0.6575	N/a	No
SOLANUM PANDURIFORME	0.4987	0.1149	0.6498	N/a	No
SOLANUM RICHARDII	0.6999	0.134	0.6794	N/a	No
SOLANUM RICHARDII SUBSP.	0.5965	-0.1009	0.6644	N/a	No
RICHARDII					
SOLANUM SCHUMANNIANUM	0.7943	0.0739	0.5937	6.84	Yes

Supplementary Table 2: Taxa used for MaxEnt modelling and the validation criteria ((N/a) means criteria not applied).

ТАХА	TEST AUC	AUC STANDARD	MAXIMUM TRAINING	PREDICTED AREA BEING	MODEL
		DEVIATION	SENSITIVITY PLUS SPECIFICITY	<10% OF THE AUC	VALID(YES/NO)
			CLOGLOG THRESHOLD	STANDARD DEVIATION>0.15	
SOLANUM TARDEREMOTUM	0.7252	0.1536	0.5992	N/a	No
SOLANUM TERMINALE	0.733	-0.3586	0.6657	0	Yes
SOLANUM TERMINALE SUBSP.	0.4231	-1	0.4428	N/a	No
TERMINALE					
SOLANUM TORVUM	0.5	-1	0.6321	N/a	No
SORGHUM VERSICOLOR	0.688	0.1531	0.5884	N/a	No
VIGNA COMOSA	0.9677	-1	0.7167	2.4	Yes
VIGNA UNGUICULATA SUBSP.	0.5546	0.0944	0.611	N/a	No
DEKINDTIANA					
VIGNA FRUTESCENS	0.7215	0.1253	0.6744	0	Yes
VIGNA GAZENSIS	0.4895	0.1302	0.6514	N/a	No
VIGNA HETEROPHYLLA SUBSP.	0.2065	-1	0.6697	N/a	No
AMBACENSIS					
VIGNA LUTEOLA	0.5047	0.1747	0.6414	N/a	No
VIGNA OBLONGIFOLIA	0.7817	0.07	0.586	0	Yes
VIGNA PHOENIX	0.9944	-1	0.429	71.87	No
VIGNA PLATYLOBA	0.6903	0.1506	0.69	N/a	No
VIGNA PYGMAEA	0.3628	0.0804	0.6455	N/a	No
VIGNA RACEMOSA	0.7662	-0.1518	0.576	0	Yes
VIGNA RETICULATA	0.6747	0.0814	0.5623	N/a	No
VIGNA UNGUICULATA SUBSP.	0.3952	0.1238	0.6392	N/a	No
SPONTANEA					
VIGNA UNGUICULATA	0.8643	0.0293	0.5984	0	Yes
VIGNA VEXILLATA VAR.VEXILLATA	0.6418	0.1121	0.646	N/a	No
VIGNA VEXILLATA	0.8436	-0.1441	0.6306	0	Yes
SUBSP.ANGUSTIFOLIA					

*AUC is area under the curve

Taxa	South Viphya Forest	Nyika National Park	Mulanje Forest	Zomba Forest Reserve	Lengwe National Park	Kasungu National
	Reserve		Reserve			Park
Coffea mufindiensis	0	0	3	0	0	0
Coffea mufindiensis subsp. lundaziensis	0	2	0	0	0	0
Cucumis anguria var.anguria	0	0	0	1	0	0
Eleusine coracana subsp.africana	0	0	0	1	0	0
Eleusine indica	0	0	1	0	0	0
Glycine wightii	1	0	0	0	0	0
Ipomoea obscura subsp. fragilis	1	0	0	0	0	0
Ipomoea obscura subsp. obscura	1	0	0	0	0	0
Olea capensis	0	0	2	0	0	0
Olea europaea	0	1	0	0	0	0
Oryza barthii	0	0	1	0	2	0
Oryzapunctata	0	0	0	0	1	0
Pennisetum macrourum	0	0	1	0	0	0
Prunus africana	0	0	4	0	0	0
Setaria sphacelata	0	0	0	1	0	0
Solanum aureitomentosum	0	0	0	1	0	0
Solanum campylacanthum	0	2	0	0	0	0
Solanum mammosum	0	0	0	2	0	0
Solanum pseudospinosum	0	2	0	0	0	0
Solanum retroflexum	0	0	1	0	0	0
Solanum seaforthianum subsp. disjunctum	0	0	1	0	0	0
Solanum wendlandii	0	0	0	2	0	0
Solanum torvum	0	0	1	0	0	0
Sorghum bicolor subsp. arundinaceum	0	0	1	0	0	0
Sorghum versicolor	0	1	0	0	0	0
Sorghum drumondii	0	0	0	0	3	0
Vigna comosa	0	0	4	0	0	0
Vigna hosei subsp. pubescens	0	0	1	0	0	0
Vigna juncea	3	0	0	0	0	0
Vigna juncea subsp. corbyi	1	0	0	0	0	0
Vigna kirkii	1	0	0	0	0	0
Vigna luteola subsp. fischeri	1	0	0	0	0	0
Vigna phoenix	0	8	0	0	0	0
Vigna platyloba	0	2	0	0	0	0
Vigna pygmaea	0	4	0	0	0	0
Vigna unguiculata subsp. tenuis	0	0	0	1	0	0
Vigna adenantha	1	0	0	0	0	0
Vigna fischeri	2	0	0	0	0	0
Vigna heterophylla subsp. ambacensis	0	1	0	0	0	0

Supplementary Table 3: Taxa with low population numbers within 6 complementary PAs and that require immediate surveying and ex situ collections

Taxa	South Viphya Forest	Nyika National Park	Mulanje Forest	Zomba Forest Reserve	Lengwe National Park	Kasungu National
	Reserve		Reserve			Park
Vigna schimperi	0	3	0	0	0	0
Coffea mufindiensis subsp. australis	0	0	8	2	0	0
Ipomoea pileata	3	0	0	5	0	0
Ipomoea tenuirostris	1	0	0	1	0	0
Phoenix reclinata	0	0	1	2	0	0
Solanum panduriforme	1	0	0	1	0	0
Solanum tarderemotum	1	1	0	0	0	0
Solanum terminale subsp. sanaganum	0	1	0	1	0	0
Vigna luteola	3	0	0	1	0	0
Vigna nyangensis	2	1	0	0	0	0
Vigna frutescens	2	0	2	0	0	3
Vigna unguiculata subsp. pawekiae	1	1	0	0	0	0
Solanum aculeatissimum	1	4	0	3	0	0
Solanum nigrum	0	1	2	3	0	0
Solanum richardii	2	1	0	1	0	0
Solanum schumannianum	1	2	0	4	0	0
Solanum terminale subsp. terminale	1	0	2	1	0	0
Vigna gazensis	1	0	4	1	0	0
Vigna unguiculata subsp. dekindtiana	1	2	0	1	1	0
Vigna vexillata var. vexillata	1	1	0	2	0	0
Vigna vexillata subsp.angustifolia	1	1	0	1	0	0
Coffea ligustroides	2	1	1	2	0	0
Vigna unguiculata var. unguiculata	1	2	1	3	0	0
Solanum goetzei	0	0	0	0	2	0
Coccinia senensis	0	0	0	0	2	0
Solanum dasyphyllum	0	0	0	0	1	0
Vigna oblongifolia	0	0	0	0	0	3
Vigna racemosa	0	0	0	0	0	2
Vigna antunesii	0	0	0	0	0	2

Taxa	Total taxa occurrence	Taxa population in PA	% of taxa population in PA
Vigna reticulata	61	1	1.6
Oryza barthii	58	5	8.6
Solanum anguivi	58	20	34.5
Vigna vexillata	53	7	13.2
Sorghum arundinaceum	43	3	7.0
Vigna unguiculata subsp. dekindtiana	43	5	11.6
Solanum panduriforme	39	8	20.5
Oryza longistaminata	38	2	5.3
Solanum nigrum	36	11	30.6
Vigna luteola	29	9	31.0
Vigna platyloba	27	3	11.1
Coffea ligustroides	24	15	62.5
Ipomoea tenuirostris	24	6	25.0
Ipomoea pileata	21	10	47.6
Eleusine indica	20	2	10.0
Solanum campylacanthum	20	5	25.0
Vigna gazensis	20	6	30.0
Solanum richardii	19	5	26.3
Cucumis anguria var.anguria	18	2	11.1
Vigna pygmaea	17	5	29.4
Vigna unguiculata	16	8	50.0
Coffea mufindiensis subsp. australis	15	12	80.0
Solanum aculeatissimum	15	8	53.3
Solanum schumannianum	15	10	66.7
Sorghum versicolor	15	1	6.7
Vigna frutescens	15	6	40.0
Eleusine coracana subsp.africana	14	1	7.1
Solanum tarderemotum	14	3	21.4
Ipomoea obscura subsp.obscura	12	5	41.7
Vigna oblongifolia	12	2	16.7
Vigna racemosa	12	1	8.3
Solanum richardii subsp.richardii	11	2	18.2
Vigna unguiculata subsp.spontanea	11	1	9.1
Vigna unguiculata subsp.spontanea	11	1	9.1
Chenopodium ambrosioides	10	1	10.0
Vigna vexillata subsp.angustifolia	10	5	50.0
Prunus africana	9	5	55.6
Solanum incanum	9	1	11.1
Solanum terminale	8	4	50.0

Supplementary Table 4: Population of priority taxa observed across complementary network of protected areas (PAs) and outside PAs.

Taxa	Total taxa occurrence	Taxa population in PA	% of taxa population in PA
Vigna antunesii	8	3	37.5
Vigna phoenix	8	8	100.0
Coffea mufindiensis	7	6	0.9
Solanum retroflexum	7	4	57.1
Solanum seaforthianum subsp. disjunctum	7	1	14.3
Solanum terminale subsp. terminale	7	2	28.6
Solanum torvum	7	1	14.3
Vigna heterophylla subsp.ambacensis	7	1	14.3
Coffea mufindiensis subsp.lundaziensis	6	2	33.3
Cucumis metuliferus	6	1	16.7
Solanum aculeastrum	6	1	16.7
Solanum mammosum	6	2	33.3
Solanum tettense	6	0	0.0
Vigna comosa	6	4	66.7
Vigna juncea	6	3	50.0
Vigna kirkii	6	1	16.7
Vigna unguiculata subsp.pawekiae	6	2	33.3
Coffea mufindiensis subsp. pawekiana	5	2	50.0
Solanum giganteum	5	0	0.0
Solanum goetzei	5	2	40.0
Solanum hispidum	5	1	20.0
Vigna schimperi	5	3	60.0
Vigna fischeri	5	2	40.0
Ipomoea coptica	4	1	25.0
Ipomoea pes-tigridis subsp.pes-tigridis	4	0	0.0
Oryza punctata	4	2	50.0
Phoenix reclinata	4	4	100.0
Solanum dasyphyllum	4	3	75.0
Vigna nyangensis	4	3	75.0
Vigna unguiculata subsp.tenuis	4	0	0.0
Glycine wightii	3	1	33.3
Ipomoea obscura subsp.sagittifolia	3	0	0.0
Solanum lichtensteinii	3	0	0.0
Solanum seaforthianum	3	0	0.0
Solanum terminale subsp.sanaganum	3	2	57.0
Solanum wendlandii	3	2	75.0
Vigna juncea subsp.corbyi	3	1	33.3
Vigna luteola subsp. fischeri	3	1	33.3
Chenopodium procerum	2	0	0.0
Coccinia senensis	2	1	50.0
Coffea arabica	2	0	0.0

Taxa	Total taxa occurrence	Taxa population in PA	% of taxa population in PA
Coffea eugenioides	2	2	100.0
Cucumis anguria	2	0	0.0
Musa livingstonianum	2	0	0.0
Olea capensis	2	2	100.0
Pennisetum polystachion	2	0	0.0
Piper capense	2	0	0.0
Solanum aculeastrum subsp.aculeastrum	2	0	0.0
Solanum chrysotrichum	2	0	0.0
Solanum delagoense	2	1	50.0
Solanum pseudospinosum	2	2	100.0
Sorghum drummondii	2	2	100.0
Sorghum rigidifolium	2	0	0.0
Vigna radicans	2	0	0.0
Vigna adenantha	2	1	50.0
Vigna macrorhyncha	2	0	0.0
Cenchrus ciliaris	1	0	0.0
Coffea salvatrix	1	0	0.0
Dioscorea praehensilis	1	1	100.0
Echinochloa colona	1	0	0.0
Ipomoea obscura subsp.fragilis	1	1	100.0
Ipomoea pes-tigridis var. africana	1	0	0.0
Ipomoea turbinata	1	0	0.0
Olea europaea	1	1	100.0
Pennisetum macrourum	1	1	100.0
Rubus rigidus	1	1	100.0
Setaria longiseta	1	0	0.0
Setaria orthosticha	1	0	0.0
Setaria sphacelata	1	1	100.0
Siphonochilus kirkii	1	0	0.0
Solanum aureitomentosum	1	1	100.0
Solanum memphiticum	1	0	0.0
Solanum richardii subsp. burtt-davyi Solanum schumannianum susb schumannianum	1 p. 1	0	0.0 100.0
Solanum wrightii	1	0	0.0
Solanum hispidum	1	0	0.0
Sorghum bicolor subsp. verticilliflorum	1	0	0.0
Sorghum halepense	1	0	0.0
Vigna hosei pubescens	1	1	100.0
Vigna unguiculata susbp. tenuis	1	1	100.0
Vigna antunesii subsp. nuda	1	0	0.0
Vigna unguiculata subsp. naaa	1	0	0.0

Taxa	Total taxa occurrence	Taxa population in PA	% of taxa population in PA
Vigna unguiculata subsp. pubescens	1	0	0.0
Vigna unguiculata subsp. stenophylla	1	0	0.0

ТАХА	NUMBER OF EX SITU	NATIONAL GENEBANK	INTERNATIONAL GENEBANKS
	COLLECTIONS		
COFFEA MUFINDIENSIS	1	0	1
COFFEA PAWEKIANA	1	0	1
DIOSCOREA PRAEHENSILIS	2	0	2
ECHINOCHLOA COLONA	2	0	2
ELEUSINE CORACANA	10	0	10
SUBSP.AFRICANA			
GLYCINE WIGHTII	3	0	3
IPOMEA PES-TIGRIDIS	1	0	1
IPOMEA COPTICA	1	0	1
OLEA CAPENSIS	2	0	2
ORYZA BARTHII	74	74	0
ORYZA LONGISTAMINATA	27	25	21
ORYZA PUNCTATA	3	3	0
PENNISETUM MACROURUM	1	0	1
PENNISETUM POLYSTACHION	2	0	2
PHOENIX RECLINATA	1	0	1
PIPER CAPENSE	2	0	2
PRUNUS AFRICANA	13	0	13
SETARIA LONGISETA	1	0	1
SETARIA ORTHOSTICHA	1	0	1
SOLANUM LICHTENSTEINII	1	0	1
SOLANUM ANGUIVI	16	0	16
SOLANUM RICHARDII	9	0	9
SOLANUM KICHARDH SOLANUM TERMINALE	3	0	3
SORGHUM BICOLOR	1	0	1
SUBSP.VERTICILLIFLORUM	1	0	1
VIGNA RADICANS	1	0	1
VIGNA ADENANTHA	2	0	2
			2 7
VIGNA ANTUNESH SUBSD NUDA		0 0	1
VIGNA ANTUNESII SUBSP. NUDA	1	•	
VIGNA COMOSA	8	0	8
VIGNA FISCHERI	2	0	2
VIGNA FRUTESCENS	15	0	15
VIGNA GAZENSIS	19	0	19
VIGNA HETEROPHYLLA SUBSP.	6	0	6
AMBACENSIS		0	
VIGNA JUNCEA	4	0	4
VIGNA KIRKII	6	0	6
VIGNA LUTEOLA	28	0	28
VIGNA MACRORHYNCHA	4	0	4
VIGNA NYANGENSIS	3	0	3
VIGNA OBLONGIFOLIA	17	0	17
VIGNA PHOENIX	6	0	6
VIGNA PLATYLOBA	27	0	27
VIGNA PYGMAEA	13	0	13
VIGNA RACEMOSA	25	0	25
VIGNA RETICULATA	84	0	84
VIGNA SCHIMPERI	5	0	5
VIGNA UNGUICULATA	19	0	19
VIGNA UNGUICULATA SUBSP.	31	0	31
DEKINDTIANA			

Supplementary Table 5a: *Ex situ* collections of priority, CWR taxa sourced from national and international genebanks.

GRAND TOTAL	657	102	555
VIGNA VEXILLATA	99	0	99
TENUIS			
VIGNA UNGUICULATA SUBSP.	3	0	3
SPONTANEA			
VIGNA UNGUICULATA SUBSP.	11	0	11
SPONTANEA			
VIGNA UNGUICULATA SUBSP.	11	0	11
PUBESCENS			
VIGNA UNGUICULATA SUBSP.	1	0	1
PAWEKIAE			
VIGNA UNGUICULATA SUBSP.	2	0	2

Supplementary Table 5b. International genebanks holding crop wild relatives accessions from Malawi

External genebank	Institution code according to FAO Wiews (<u>http://www.fao.org/wiews</u> -archive/institute.htm	Passport data source
National Botanic Garden of Belgium)	BEL014	Genesys (<u>https://www.genesys-</u> pgr.org/a/v2WdgrK7KKR)
International Institute of Tropical Agriculture	NGA039	Genesys (<u>https://www.genesys-</u> pgr.org/a/v2WdgrK7KKR
International Crop Research Institute for the Semi-Arid Tropics	IND002	Genesys (<u>https://www.genesys-</u> pgr.org/a/v2WdgrK7KKR
Centro Internacional de Agricultura Tropical	COL003	Genesys (<u>https://www.genesys-</u> pgr.org/a/v2WdgrK7KKR)
Colombia		
International Livestock Research Institute	ETH013	Genesys (https://www.genesys- pgr.org/a/v2WdgrK7KKR
Ethiopia		
Plant Genetic Resources Conservation Unit, Southern Regional Plant Introduction Station, University of Georgia, USDA-ARS	USA016	Genesys (<u>https://www.genesys-</u> pgr.org/a/v2WdgrK7KKR)
United States of America		
Australian Grains Genebank, Department of Environment and Primary Industries Australia	AUS165	Genesys (<u>https://www.genesys-</u> pgr.org/a/v2WdgrK7KKR)
Bioversity International		Genesys (<u>https://www.genesys-</u> pgr.org/a/v2WdgrK7KKR)

ELC ZONE	OCCUR IN PA (YES (Y)/NO(N))	NO(N)) PA(YES/NO) /Y/N		FREQUENCY	
0	у	82	у	49	
1	n	0	y	2	
2	у	1	y	6	
3	n	0	n	0	
4	n	0	у	11	
5	у	2	y	10	
6	n	0	y	6	
7	у	3	y	9	
8	y	8	y	25	
9	y	3	y	8	
10	y	4	y	9	
11	n	0	y	3	
12	n	0	y	2	
13	у	5	y	3	
14	y	1	n	0	
15	y	4	у	19	
16	n	0	y	1	
17	у	5	y	13	
18	y	21	y	55	
19	y	34	y	21	
20	y	9	y	16	
21	y	1	y	22	
22	ÿ	5	y	10	
23	v	3	n	0	
24	ÿ	1	y	30	
25	y V	6	y V	16	
26	n	0	ÿ	1	

Supplementary Table 6: Frequency of occurrence of ELC zones in Protected Areas (PAs) and outside PAs

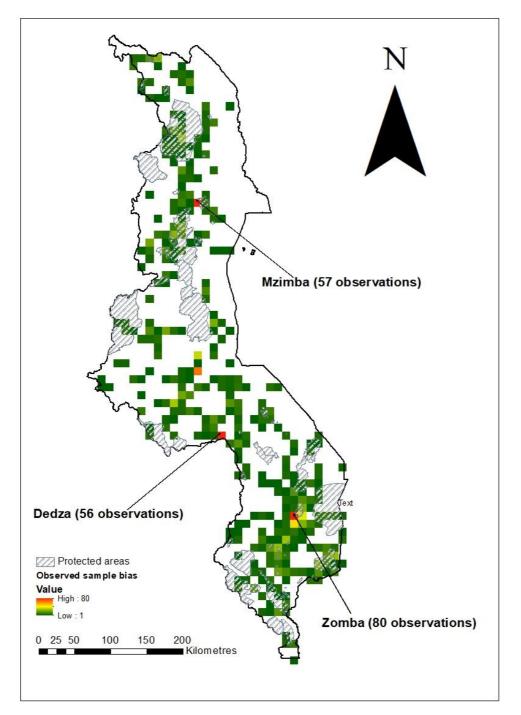


Figure S1: Observed number of records of 123 priority CWR, at a resolution of approximately 10 x 10 km.

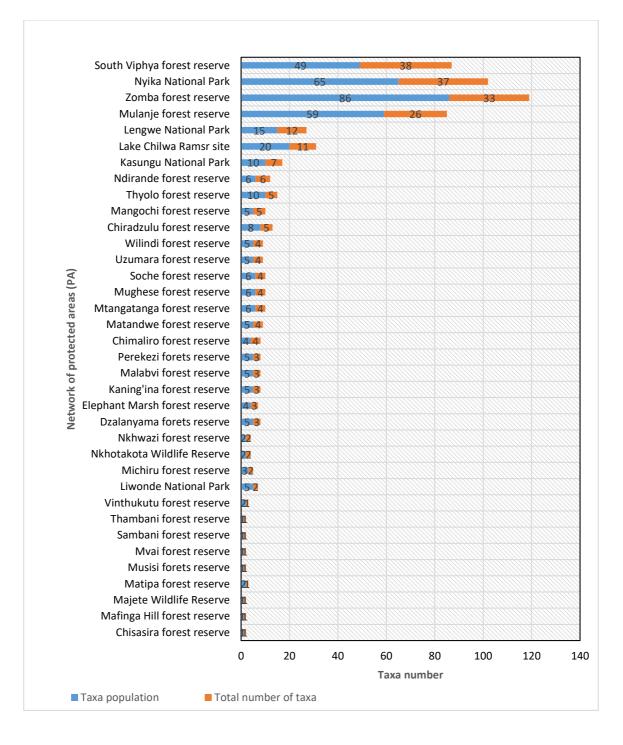


Figure S2: Protected areas in which priority taxa is present based on species coverage analysis

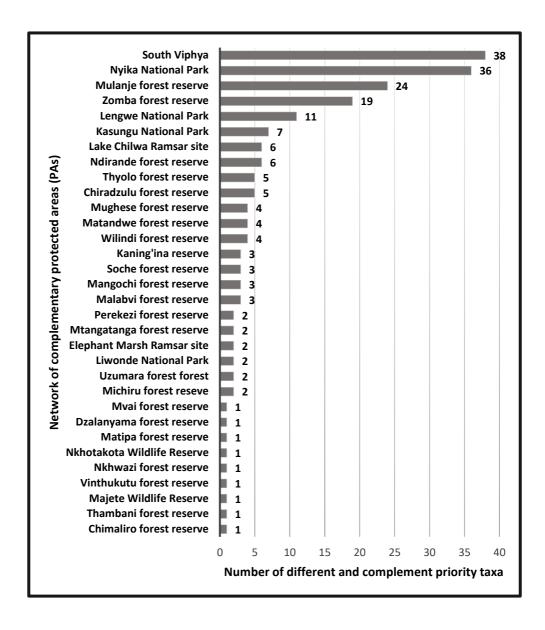


Figure S3: Complementary network of protected areas that registered occurrence of priority CWR taxa.

CHAPTER 4

CLIMATE CHANGE IMPACT AND CONSERVATION OF CROP WILD RELATIVES IN MALAWI.

ABSTRACT

Climate change is predicted to have negative impact on Malawi's agriculture and biodiversity, though previous research has been generalised and not species specific. To assess the specific impact of climate change on future distribution and diversity of crop wild relatives (CWR) in Malawi, we studied 14 national priority CWR taxa. The impact was projected for 2030, 2050 and 2070 under the Representative Concentration Pathways (RCPs) 4.5 and 8.5 with the General Circulation Models bcc csm1 1 m, csiro access1 0 and gfdl esm2m. We used MaxEnt to produce distribution models for individual taxon and calculated taxa area of occupancy (AOO) and taxa richness in ArcMap and DIVA GIS. Change in taxa richness under present and future climatic conditions was noted. The results indicated that under both RCPs, climate change is expected to negatively impact 9 (64%) of the 14 priority taxa. These taxa are predicted to shift their distribution by 2050 and lose 50-98% of their AOO by 2070. Taxa found outside-protected areas (PAs) are expected to be impacted more compared to those occurring within existing PAs. The taxa most negatively impacted include Coffea ligustroides S.Moore, Vigna unguiculata (L) Walp var. unguiculata (wild species) and V. vexillata (L.) A.Rich subsp. anguistifolia with 82–95% loss in AOO. Based on A3(c) criteria of IUCN, these taxa will become critically endangered. Ipomoae pileata Roxb, Solanum anguivi Lam, V. comosa Baker and V. frutescens subsp. frutescens A.Rich. were categorized as Endangered (EN) having lost distribution area of about 75%. Oryza longistaminata, S. schumannianum Dammer, V. oblongifolia A.Rich. var. parviflora, V. racemosa (G. Don) Hutch. & Dalziel and Sorghum bicolor subsp. arundinaceum were categorized as Least Concern (LC). These had lost ≤30% of their AOO by 2070 with O.longistaminata and S. bicolor subsp.arundinaceum projected to double their (AOO) by 2070. CWR taxa in Nyika National Park, South Viphya, Kaning'ina, Zomba and Mulanje Forest Reserves are predicted to be the least impacted by climate change. The formulation of a conservation strategy on priority CWR should therefore incorporate actions to address the projected impact of climate change and consider the above PAs as potential genetic reserves for long-term in situ conservation of national priority CWR. Taxa outside PAs are likely to disappear in the future hence urgent collecting for ex situ conservation is required.

Keywords: conservation strategies, climate change impact, crop wild relatives.

4.1. INTRODUCTION

Global warming, loss and fragmentation of species habitat increase species vulnerability and negatively affect their ability to survive (Bellard, Bertelsmeier, Leadley, Thuiller, and Courchamp, 2012; Hoegh-Guldberg *et al.*, 2018). Negative impacts of climate change tender agricultural land unproductive so affecting food security (Arndt *et al.*, 2019; FAO, 2019; Loboguerrero *et al.*, 2018). The Intergovernmental Panel on Climate Change (IPCC, 2019) report illustrates how the four pillars of food security, i.e. availability, access, utilization and stability, may be negatively affected by climate change. Having already over 821 million people suffering malnourishment or starvation globally (FAO, 2019), increases the urgency to develop resilient and more productive crop varieties that reduce yield gaps. However, global human population relies on a few crops (e.g. wheat, maize, rice, barley, etc. (ITPGRFA, 2009) and to feed over 7.7 billion people and to improve these crops require wider genetic diversity from their progenitors and wild relatives (Harlan and de Wet, 1971; Loboguerrero *et al.*, 2018).

Crop wild relatives (CWR) of maize saved the world from corn blight, which infected Mexican corn in the early 1970's, and wheat was rescued from powdery mildew with resistant genes from its wild relative, *Triticum boeoticum* Bois (Maxted and Kell, 2009). However, globally 90% of CWR are reportedly underrepresented in *ex situ* genebanks (Castañeda-Álvarez *et al.*, 2016) and lack active *in situ* conservation (Maxted *et al.*, 2011), and this situation is mirrored in Malawi. There is a growing number of threats on biodiversity in Malawi ranging from changes in land use to climate change impact (Chavula *et al.*, 2017; Tananga and Undi, 2016). For example, an increase in the number of warm days (with >30 °C) and a temperature rise of between 2.3–6.3 °C throughout the country are expected by 2090 (England *et al.*, 2018; Mittal *et al.*, 2017), leading to drying up of the soils and water resources. An estimated rise in temperature between 1°C and 3 °C during 2040–2070 was highlighted by Chibwana Gama *et al.* (2014) and slightly lower rise temperatures (0.5 °C–1.5 °C) by 2040 was projected by Mittal

et al. (2017) under RCP 8.5. In both cases, these predictions are directly associated with negative impacts on crop productivity as noted by (England *et al.*, 2018; Verhage, Cramer, Thornton, and Campbell, 2018). Although the focus of these studies was on crop production, the findings help us understand the magnitude of vulnerability of Malawi's biodiversity including its CWR diversity.

Previous studies on CWR in Malawi revealed that 274 of priority CWR taxa are not protected, even though these have potential for crop improvement of important crops in Malawi (Mponya *et al.*, 2020). Some taxa (29.7%) were predicted to occur outside protected areas (PAs) but even those within PAs are not actively conserved (Khaki Mponya *et al.*, 2020). Assessing climate change impact on the future diversity and distribution of priority CWR taxa is therefore a priority for Malawi where already one million people suffer malnourishment or starvation (FAO, 2019) and where natural disasters attributed to climate change are an annual occurrence. The information generated will contribute to the development of a national CWR conservation strategy that is climate smart and able to respond to future food security challenges. The results of this study will also inform on potential sites that will be least impacted by climate change in the future to advise for *in situ* genetic reserve designation. This paper therefore discusses the projected impact of climate change on priority CWR taxa and the implication of this on their future richness, distribution and habitat suitability. Recommendations are provided on conservation actions that respond to forthcoming challenges.

4.2. METHODS

4.2.1 Priority CWR and occurrence data

Data was collated for all 277 priority CWR taxa included in the national CWR inventory developed for Malawi (Mponya *et al.*, 2020). Occurrence data was collated from the Malawi Plant Genetic Resources Centre and National Herbarium and Botanical Gardens of Malawi, the Global Biodiversity Information Facility (GBIF, 2017), the Royal Botanical Gardens, Kew,

eFlora of Malawi(<u>https://www.malawiflora.com/</u>) and Genesys (<u>https://www.genesys-pgr.org/</u>). The National gazetteer from the Geographic Names Database (http://maps.google.com/) was used to georeference sites with unknown coordinates. The tool GEOQUAL of CAPFITOGEN version 2.0. was used to check for the quality of coordinates of the taxa collection sites and included for the analysis only those with TOTALQUAL (Parra-Quijano, Torres, Iriondo, López and Molina, 2016) of above 50% as reported by Khaki Mponya *et al.* (2020).

4.2.2. Species distribution models

Due to the lack of sufficient occurrence data for 233 priority CWR taxa, we modelled the distribution of the 44 CWR taxa that had >10 occurrence records (Contreras-Toledo et al., 2019; Elith et al., 2006). The Maximum Entropy algorithm (MaxEnt) (Phillips and Dudik, 2008) was used to model the distribution of each of 44 taxa using important and non-correlated environmental variables. MaxEnt was chosen due to its performance in ecological modelling of species distribution relative to other programs (Elith et al., 2006; Ramírez-Villegas, Khoury, Jarvis, Debouck and Guarino, 2010) and its wide use allows for replication of our study and comparison of our results with similar studies. The bioclimatic, edaphic and geophysical variables (Supplementary Table1) used were initially selected from a list of 63 by Random Forest and bivariate correlations (Khaki Mponya et al., 2020). Bioclimatic variables (averages for 1970-2000) at 2.5 min spatial resolution (Fick and Hijmans, 2017) were obtained from Worldclim Version 2.0 (https://www.worldclim.org/bioclim). Edaphic and geophysical variables were sourced from the Harmonised World Soil Database version 1.2 (http://www.fao.org/soils-portal/soil) (Fischer et al., 2008). Cross validation with 5 and 10 folds was implemented and the modelled potential distributions were restricted to the maximum training sensitivity plus specificity threshold as recommended by Liu et al. (2005) and Young et al. (2011). The distribution models were considered stable if they met three model validation criteria: i) average area under the Test ROC Curve (ATAUC) >0.7; ii) standard deviation of ATAUC <0.15; and iii) proportion of potential distribution area with standard deviation above 0.15 (ASD15) <10 % (Ramírez-Villegas *et al.*, 2010).

4.2.3. Impact of climate change on richness and distribution of priority CWR taxa

Three General Circulation Models (GCMs) were used: bcc csm1 1 m, csiro access1 0 and gfdl_esm2m, with future bioclimatic variables obtained from CCAFS (www.ccafsclimate.org/data). The projections were done for three climate change scenarios 2030, 2050 and 2070 under the 4.5 and 8.5 Representative Concentration Pathways (RCPs) (Meinshausen et al., 2011; Riahi et al., 2011). The GCMs were selected for their performance in modelling climate change impact in Southern Africa (Huntingford et al., 2003) and these have already been used specifically for climate change modelling studies in Malawi (Chibwana Gama et al., 2014; England et al., 2018; Mittal et al., 2017). RCP 4.5 assumes stabilisation of total radiative forcing before 2100 due to use of technologies and employment of strategies that reduce greenhouse gas emissions (Meinshausen et al., 2011). Malawi is initiating the implementation of such strategies, e.g. re-afforestation and use of alternatives energy sources (Coulibaly et al., 2015; Mauambeta, David, and Reginald, 2010). RCP 8.5 assumes an increase in greenhouse gas emission over time representative for scenarios leading to high greenhouse gas concentration levels (Riahi et al., 2011). Such factors as increased in deforestation, human population and continued use of fossils and electricity as energy sources coupled with slow technological advancement are associated with this scenario. Some of the drivers associated with this scenario are a characteristic of the current status of Malawi (Coulibaly et al., 2015; Mauambeta et al., 2010) although efforts to mitigate such drivers are underway.

An ensemble of the individual taxon models that passed the validation criteria under both climate change scenarios were generated in ArcMap10.4 (ESRI, 2015). Each model was run for each taxon for both scenarios and comparisons were made on how climate change will

impact that particular taxon across all projected years. PAs maps from the World Database on Protected Areas (UNEP-WCMC, 2019) were overlaid on the stacked scenarios maps. Change in taxa richness and shift in its distribution was compared under current and future climatic conditions. Changes in taxa temporal and spatial distribution were analysed from the potential species distribution models under present and future climatic conditions in ArcMap10.4 (ESRI, 2015) on a grid size of 5x5 km (approximately 2.5 arc minutes at the equator) for both RCP scenarios. Collectively, proportions of taxa distribution that were negatively affected by climate change were noted.

4.2.4. Climate change impact and projected taxa threats

Changes in taxa coverage under both present and future climate change scenarios were mapped. Gain and loss in area by each taxon across the projected years was noted by comparing the area occupied by taxa under present climatic conditions with the area under projected future climatic conditions. Change in area of occupancy (AOO) were noted for both RCP4.5 and 8.5. Taxa threat levels were assigned based on change in area as guided by IUCN threat assessment criteria A3 (c). The criterion uses decline in area of occupancy (AOO), extent of occurrence and or habitat quality as a proxy to projected threats on taxa. In this study, we used a decline in AOO to assign threat levels to the priority taxa since change in area is assumed to have a direct impact on species population size (IUCN, 2019). In this criteria, taxa projected to lose 100% of its area is categorized as Extinct (EX), loss of >80% area as Critically Endangered (CR), a loss in area of >50% as Endangered (EN) and taxa with loss in area of >30% as Vulnerable (V) (IUCN, 2019). Species with less than 30% reduction in area were considered of Least Concern (LC).

Taxa turnover (T) for each projected year was calculated under limited and unlimited migration and across the projected periods using the formula $T = 100 X \frac{(L+G)}{(SR+G)}$, where L is loss in taxa per grid cell, G is gain in taxa per grid cell and SR is current taxa richness (Thuiller, Lavorel, Araújo, Sykes and Prentice, 2005). Under unlimited migration, changes in distribution and taxa richness were calculated per grid cell for both present and future climate scenarios. Gain or loss of grid cells on all taxa was expressed as a percentage of the total number of grids where 0% represents no change (assuming there is no migration) and 100% means a total change in taxa composition or total loss of the taxa in a particular habitat. This implies total migration of the taxa (if considered under migration) and or total loss of the taxa under limited and no migration scenarios. The analyses were done in DIVA GIS 7.5 (Hijmans, Guarino, and Mathur, 2012) and in ArcMap10.4 (ESRI, 2015) on grid size of 5x5 km (approximately 2.5 arc min at the Equator).

4.3. RESULTS

4.3.1. Current predicted CWR distribution

Although 44 CWR taxa had sufficient records for modelling the potential distribution, only 14 had stable models based on the validation criteria and were therefore used for further analyses. Table 4.1 lists the 14 priority CWR with valid distribution models and these belong to the genera of *Coffea*, *Oryza*, *Solanum*, *Sorghum* and *Vigna*. Together, these taxa were predicted to occur in PAs with small patches outside PAs (Figure 4.1). PAs predicted as CWR hotspots include Nyika National Park, Mughese, Wilindi, Musisi, Mafinga hills, South Viphya and Kaning'ina Forest Reserves in the Northern Region of Malawi. In Southern Region, hotspots were predicted in Mulanje Mountain and Zomba Forest Reserves and all Forest Reserves in Thyolo and Blantyre Districts. Outside PAs, most CWR richness was predicted in Ntcheu and Dedza Districts, in the areas connecting PAs in the Northern Region and in Blantyre, Thyolo and Chiradzulu Districts (Figure 4.1).

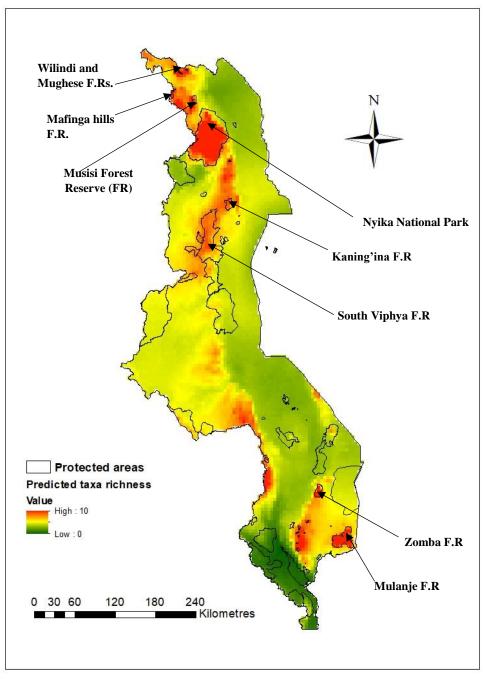


Figure 4. 1: Predicted richness distribution of 14 priority CWR under present climatic conditions in Malawi. Map resolution at 5x5 km (2.5 arc minutes at Equator).

Таха	ATAUC	STAUC	ASD15 (%)	Maximum training sensitivity plus specificity cloglog threshold
Coffea ligustroides S.Moore	0.88	0.06	6.72	0.39
Coffea mufindiensis Hutch ex Bridson subsp. australis	0.92	0.06	0.00	0.40
Ipomoea pileata Roxb	0.85	0.08	2.74	0.49
Oryza longistaminata A.Chev.& Roehr	0.71	0.08	4.11	0.65
Solanum anguivi Lam	0.91	0.04	2.84	0.32
Solanum schumannianum Dammer	0.79	0.07	6.84	0.59
Solanum terminale Forssk. subsp. terminale	0.73	-0.36	0.00	0.67
Sorghum bicolor (l.) Moench subsp. arundinaceum	0.82	0.06	2.07	0.45
Vigna comosa Baker	0.97	-1.00	2.40	0.72
Vigna frutescens A.Rich. subsp. frutescens	0.72	0.13	0.00	0.67
<i>Vigna oblongifolia</i> A. Rich. var. <i>parviflora</i> (Baker) verdc.	0.78	0.07	0.00	0.59
Vigna racemosa (G. Don) Hutch. & DALZIEL	0.77	-0.15	0.00	0.58
Vigna unguiculata (L.) Walp subsp. unguiculata (wild species)	0.86	0.03	0.00	0.60
Vigna vexillata (L.) A.rich. subsp. angustifolia	0.84	-0.14	0.00	0.63

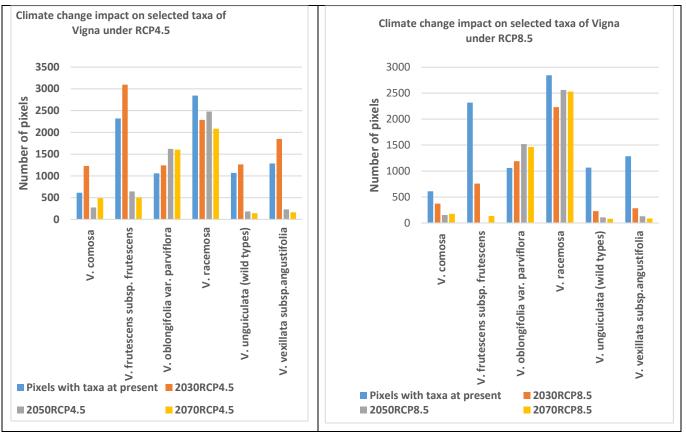
Table 4.1: CWR with stable distribution models and model validation criteria.

ATAUC is the test area under operating curve, STAUC is the standard deviation of the test area under the operating curve, ASD15 is the proportion of potential distribution area with standard deviation above 0.15

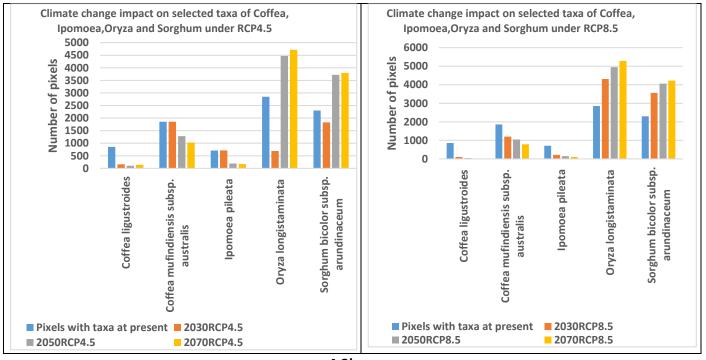
4.3.2. Impact of climate change on distribution and richness of priority CWR

Climate change negatively affected the priority taxa across all projected years with the exception of three taxa (*Vigna oblongifolria* A.Rich. subsp. *parviflora* (Baker) Verdc, *Oryza longistaminata* A.Chev. & Roehr and *Sorghum bicolor* (L.) Moench subsp. *arundinaceum*) which were only impacted in 2030 under RPC4.5. By area coverage, the most impacted taxa included *Ipomoea pileata* Robx, *Coffea ligustroides* S.Moore, *Solanum anguivi* Lam, *Vigna comosa* Baker, *Vigna frutescens* A.Rich. subsp. *frutescens*, *Vigna unguiculata* (L.) Walp subsp.*unguiculata* (wild species) and *Vigna vexillata* (L.) A.Rich. subsp. *angustifolia*. These will have lost almost 90% of their range with taxa by 2070 under both scenarios while the least impacted taxa will have increased area coverage by more than 50% of the current predicted coverage (Figures 4.2a, 4.2b and 4.2c). Eight taxa will have reduced distribution range by 2030

and will also be impacted by 2050 and 2070. In terms of taxa richness, no changes will be observed between 2030 and 2050 under RCP4.5 (Figures 4.3a and 4.3b). Increase by 10% taxa richness was noted in 2070 (Figure 4.3c) in areas where most diversity was predicted. The situation was slightly different for scenario RCP8.5 where a reduction in taxa richness will be observed in 2050 and 2070. Most diversity was predicted to be found in PAs, similar to those noted under present conditions (Figures 4.1, 4.3a and 4.4a). Diversity outside PA was predicted in Blantyre, Dedza, Ntcheu and part of Thyolo Districts and the strip connecting South Viphya and Nyika National Park (Figures 4.1, 4.3a and 4.4a). However, this diversity was visibly lost between 2050 and 2070 under RCP 8.5 in Dedza (Y), Ntcheu (Z) and area connecting Nyika National Park and South Viphya Forest Reserve (Figure 4.4c).









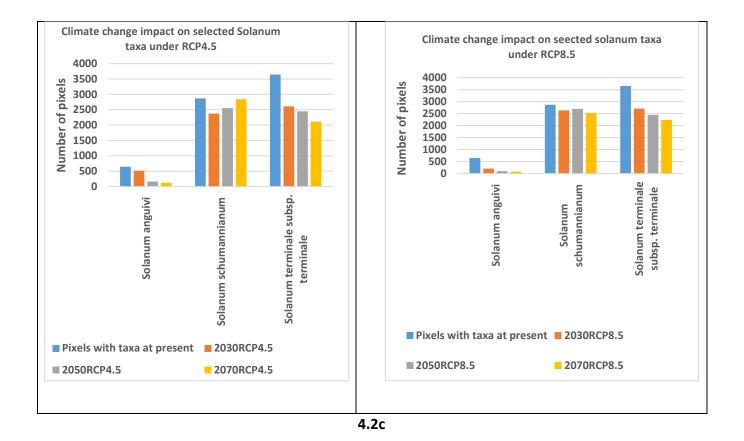


Figure 4.2: (**a**, **b** and **c**).Climate change impact on 14 priority crop wild relative taxa in 2030, 2050 and 2070 under climate change scenarios RCP4.5 and RCP8.5.

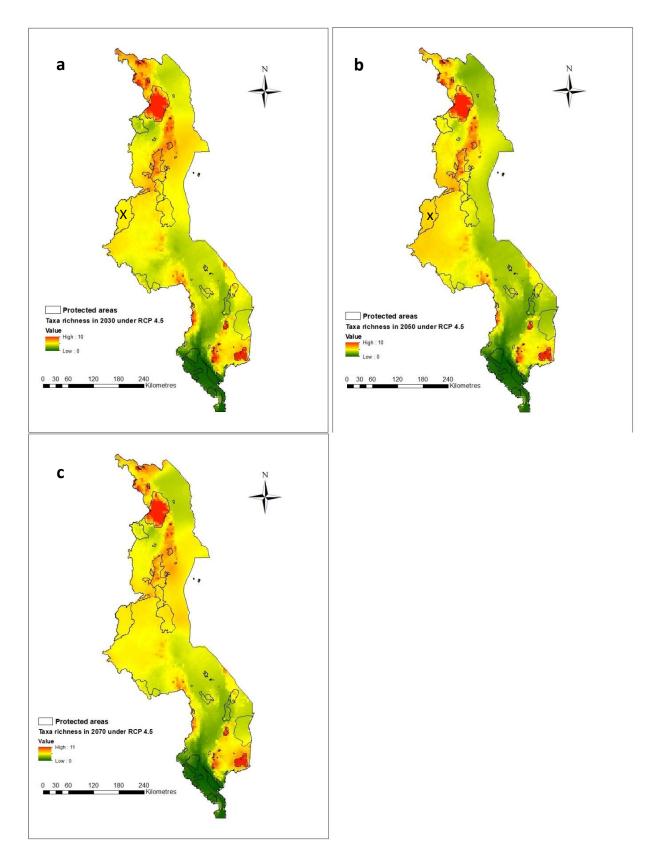


Figure 4.3: (**a**, **b** and **c**). Future predicted richness of 14 priority CWR taxa in Malawi under RCP 4.5 for 2030 (a), 2050 (b) and 2070 (c) projection periods. Map resolution at 5x5 km (approx. 2.5 arc minutes at the Equator).

Dedza (Y), Ntcheu (Z)

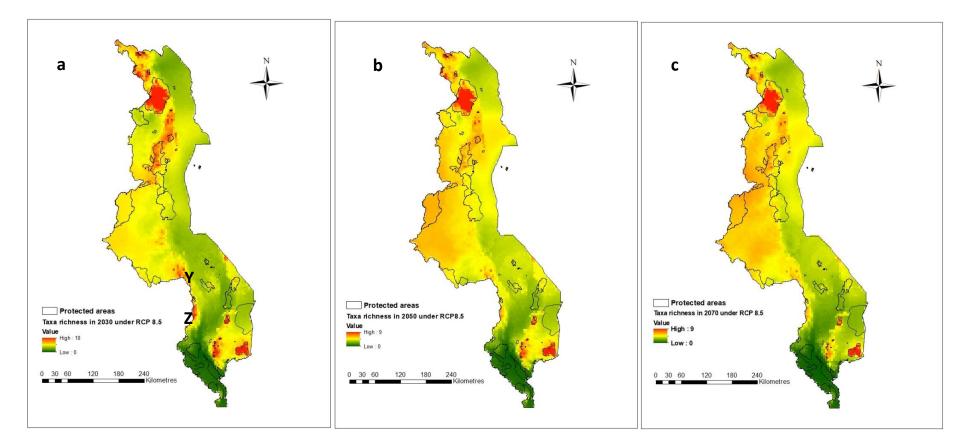


Figure 4.4: (**a**, **b** and **c**). Predicted richness and distribution of 14 priority CWR taxa under RCP 8.5 for 2030 (a), 2050 (b) and 2070 (c) projection periods. Map resolution at 5x5 km (approx. 2.5 arc minutes at the Equator).

A shift in taxa distribution was more visible in Central and Northern Regions between 2050 and 2070. Taxa tend to migrate northwards occupying areas where no taxa were previously observed and predicted. A good example is Kasungu National Park (\mathbf{X}, \mathbf{x}) and parts of Mzimba District in Figures 4.3a and 4.3b. In the Southern Region, not much migration of taxa was observed and taxa tend to concentrate in the Ndirande, Chiradzulu, Mulanje, Zomba and Thyolo Forest Reserves. Areas between these PA were also predicted for occurrence of priority taxa in 2070 under RCP8.5. But for both RCP scenarios, PAs previously predicted with the highest number of taxa e.g. Zomba and Mulanje Forest Reserves were consistently projected as hotspots from now to 2070 under both climate change scenarios considered in this study.

4.3.3. Taxa turnover and projected threat levels

Taxa turnover will slightly be lower in 2030 RCP4.5 under unlimited migration compared to the limited migration scenario. High (>60 %) taxa turnover was consistently projected from 2050 to 2070 for both scenarios for unlimited migration (Figure 4.6a). However, high turnover noted in 2050 under RCP4.5 dropped by 10% in 2070. Within the same period, there will be increased loss in taxa coverage with 78.6% of the taxa losing more than half their geographic coverage (Figures 4.5a and 4.5b). Under RCP8.5, high taxa turnover (>80%) will be observed in 2070. During this period, six taxa were predicted to lose >80% of their AOO, 2 taxa had gained over 90% of its AOO, one priority taxa had gained 45% AOO and taxa richness increased by 9% in hotspots.

Under limited migration, >50% of CWR turnover will be observed in 2030 under RCP4.5 and the rest of the projected years registered <30% taxa turnover with 2070 being the year with lowest taxa turnover under RCP 8.5 (Figure 4.6a and 4.6b). Under unlimited migration, *Coffea ligustroides, Vigna unguiculata* (wild species) and *V. vexillata.* subsp. *anguistifolia* will lose 82–95% of their area on average from 2050-2070 under both RCPs. *Coffea ligustroides* will be notably the most affected taxa with loss of >80% of the pixels with

taxa observed as early as 2030 for both RCPs. These three taxa will be categorized as critically endangered (CR) in relation to IUCN Red list criterion A3(c). On average, *I. pileata*, *S. anguivi*, *V. comosa* and *V. frutescens*. subsp. *frutescens* will lose about 75% of the taxa coverage across the projected period and fall in the Endangered category (EN). Four taxa will be categorized as Least Concern (LC) with aprojected lost of <30% to 0% of their current occupancy. Taxa in this category include *O. longistaminata*, S. *schumannianum*, *V. oblongifolia* var. *parviflora* and *V. racemosa* and *S. bicolor* subsp. *arundinaceum*.

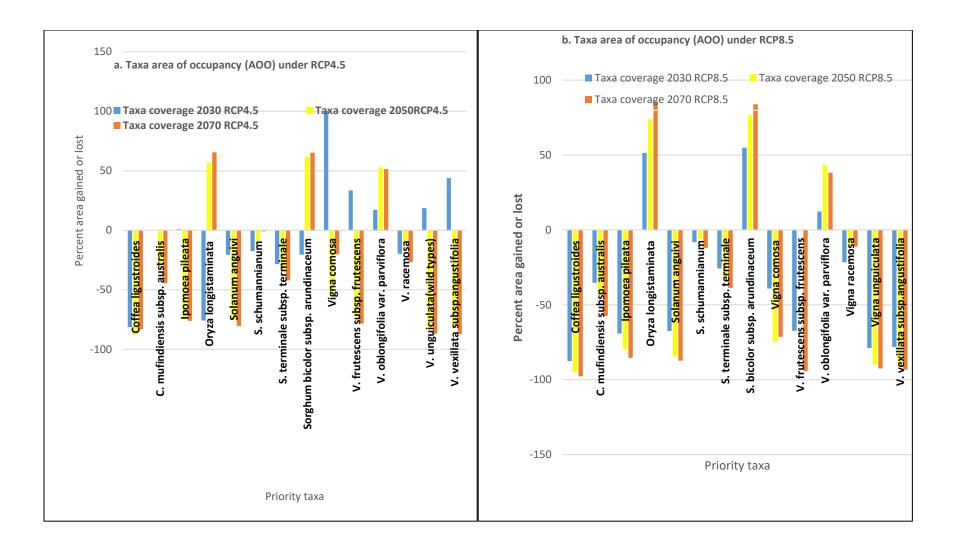


Figure 4.5: Change in priority taxa distribution under climate change scenarios RCP4.5 (a) and RCP8.5 (b).

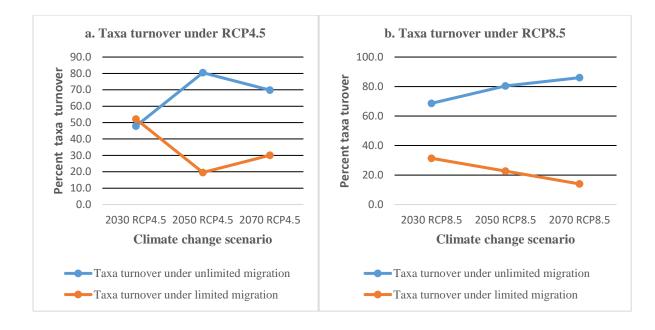


Figure 4.6: (a) Taxa turnover as predicted from 2030 through 2070 and (b) change in taxa coverage for 14 priority taxa under RCPs 4.5 and 8.5.

4.4. DISCUSSIONS

Climate change is expected to impact the distribution and richness of 14 priority CWR taxa in Malawi with the worst impact to be felt by nine taxa (*Ipomoea pileata, Coffea ligustroides, Coffea mufindiensis* subsp. *australis, Solanum anguivi, Solanum terminale, Vigna comosa, Vigna frutescens* subsp. *frutescens, Vigna unguiculata* subsp.*unguiculata* (wild types) and *Vigna vexillata* subsp. *angustifolia*) across all projected years under both climate change scenarios. Taxa that will have least impact include *V. oblongifolia* var. *parviflora, Oryza longistaminata, Sorghum bicolor* subsp.*arundinaceum and Solanum schumannianum*. These taxa were observed across Malawi (from Sotuhern to Northern Region), have high numbers of occurences and are in abundance compared to other taxa. The probability of survival for these taxa is therefore relatively high than those taxa expected to be negatively impacted by climate change in the future. As expected, the impact varied across projected period but overall, the impact will be felt more in 2070 under both RCP4.5 and RCP8.5.

The impacted taxa will be characterized by reduction in area of occupancy of between 50–98%. Three priority taxa (*V. unguiculata* (wild types), *V. vexillata* subsp. *anguistifolia* and *C. ligustroides*) were projected to loose almost 93% of their current areas of occupancy and these taxa will categorically fall in the Critically Endangered IUCN threat level. *C. ligustroides* was also reported as Vulnerable (VU) at global level (IUCN, 2019). Unfortunately, these taxa were noted to occur in only 1 and or 2 sites in Malawi (Khaki Mponya *et al.*, 2020) and so will need special conservation attention in order to make sure they persist in the wild. Additionally, four taxa (*I. pileata*, *S. anguivi*, *V. comosa* and *V. frutescens*. subsp. *frutescens*) will be categorized as Endangered (EN), three taxa as Vulnerable (VU) and four taxa; *V. oblongifolia* var. *parviflora, Oryza longistaminata, Sorghum bicolor* subsp.*arundinaceum and Solanum schumannianum* will be of Least Concern (LC).

V. oblongifolia var. *parviflora* and *Oryza longistaminata* were projected to increase their areas of occupancy consistently for the projected period with *O. longistmana* increasing AOO by 85.7%, while the former increased area by 38% (Supplementary Tables 2a and 2b). The shift in geographic climates will likely have a positive impact on the three taxa and will potentially make them to expand their area of occupancy. However, increase in AOO of these taxa cannot be entirely explained by rising temperatures but a combination of factors such as taxa migration rates and survival rates of each taxon (Lawler *et al.*, 2006).

It was also noted that the same rising temperatures will reduce habitat suitability and cause migration and or disappearing of nine taxa. The degree of impact varied from taxon to taxon within each projected year under both climate scenarios. For example, some taxa lost more AOO compared to others although this did not affect taxa richness except for RCP8.5 in 2070. Taxa turnover was higher under unlimited migration scenario for all project years compared to limited migration scenario. High taxa turnover could mean high taxa survival rate since taxa will migrate freely to favourable environments (Jarvis *et al.*, 2008). High species turnover for this study could also mean that there was low-shared number of species between two areas and that the number of gained species equalled the lost species (Koleff and Gaston 2002).

Future taxa hot spots were consistently projected in PAs and these results showed similar taxa distribution pattern to the potential taxa distribution under present climatic conditions (Khaki Mponya *et a l.*, 2020). In this study, these PAs were projected to be the least impacted with climate change in the next 30 to 50 years meaning that the environmental conditions will potentially be favourable for the survival of the species in the future. Further, this could also mean that most of the evaluated taxa were niche specific meaning that they are adapted to specific conditions and these may be more vulnerable once their habitat is disturbed. Such taxa may require active *in situ* and *ex situ* conservation if they are to be secured for the future.

The lowest species turnover was projected in 2050 under RCP4.5 and under RCP 8.5; the lowest species turnover is expected in 2070. This could be attributed to species migration experienced during the same period with some taxa losing more than 50% of their distribution area (Supplementary Tables 2a and 2b). Hotpots occurring outside PAs, those in the peripheral of the PAs and those occurring in high altitude areas are expected to be hit significantly due to reduction in habitat suitability. These hotspots were also projected to disappear by 2050 under RCP 8.5. This was more visible in Ntcheu and Dedza Districts where hotspots had almost disappeared and some taxa hotspots area being lost around the peripherals of Nyika National Park, Kaning'ina and South Viphya Forest Reserves in the Northern Region of Malawi in the years 2050 and 2070 under RCP 8.5.

However, some areas in the Central and Northern Malawi were predicted to become more suitable for the occurrence of the taxa in the future. Taxa tend to migrate to the Central and Northern parts of Malawi. Taxa migration was likely influenced by the change in habitat suitability which could have resulted due to modification of some useful ecological interactions (Lane and Jarvis, 2007). In the Central Region, we noted Kasungu National Park and in the Northern Region, a change was observed in some parts of Mzimba District becoming potential habitats for the priority taxa from 2050 to 2070 and these could be additional sites for *in situ* conservation in the future. On the other hand, minimal taxa migration was noted in the Southern Region compared to Central and Northern Regions.

It was also noted that most of the diversity of priority taxa was predicted to be present in PAs and some hotspots were predicted not to lose taxa under both climate change scenarios for the projected period. In the Southern Region, most taxa diversity was predicted in the protected areas of Ndirande, Chiradzulu, Mulanje, Zomba and Thyolo Forest Reserves. In the Northern Region, hotspots were predicted in PAs of Nyika National Park, Mughese, Wilindi, Musisi, Mafinga hills, South Viphya and Kaning'ina Forest Reserves. With exception of 2030 under RCP 4.5, all the PAs previously predicted as hotspots will have maintained their status in the next 30–50 years. The consistency in the results is an indicator that these PAs may relatively be less affected by the future impact of climate change hence providing an option for long-term active *in situ* conservation.

These results will inform the conservation strategies of the 14 priority taxa. With revelation that, some CWR are expected to be least impacted by climate change in the future in some selected PAs and that some CWR diversity hotspots will be maintained, gives Malawi an opportunity for advocating for active *in situ* conservation of the 14 priority taxa. PAs such as South Viphya, Zomba and Mulanje Mountain Forest Reserves and Nyika National Park were already predicted with high diversity of priority CWR (Khaki Mponya *et al.*, 2020) and are therefore potential future sites for the active conservation of the 14 priority CWR taxa.

Additional PA conservation should be considered in Wilindi, Mughese and Kaning'ina Forest Reserves. These PAs have rare taxa and taxa in these PAs are predicted to be less impacted by climate change in the next 50 years. Additionally, the results also advise for *ex situ* collections of taxa occurring in high impact areas of climate change. Such areas include Dedza and Ntcheu Districts and in the peripherals of Nyika National Park, Kaning'ina and South Viphya Forest Reserves. For active and sustainable *in situ* conservation of the priority taxa in the proposed PAs, we recommend review of the management plans and border expansion to capture the priority taxa along the PAs perimeter. Where possible, measures to control genetic erosion such as control of logging and periodical monitoring of taxa population be considered in the PAs with relatively high diversity of CWR.

4.5. CONCLUSIONS

Climate change will negatively affect the distribution of priority CWR and is expected to affect habitat suitability causing a significant number of taxa to loose distribution area. For instance, the study indicates that 9 out of 14 priority CWR taxa will be threatened by 2070 having lost

an average AOO of 50–90%. However, this will not have much negative impact on taxa richness in hotspots occurring within PAs such as South Viphya, Zomba and Mulanje Mountain Forest Reserves and Nyika National Park compared to those outside PAs hence making these PAs potential future sites for priority taxa. Most PAs will only be impacted around their borders and they may require border expansion depending on their management plans. Border expansion will be more applicable to PAs like Mafinga Hills, Musisi and Kaning'ina Forest Reserves that are at a distance from other PAs. But for those in a network of PAs, creating the species corridors might increase the survival of the taxa. Finally, for active *in situ* conservation, authors recommend establishing genetic reserves in PAs of South Viphya, Zomba and Mulanje Mountain Forest Reserves and Nyika National Park and recommend for *ex situ* collection of taxa occurring in Ntcheu and Dedza Districts.

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4.7. SUPPLEMENTARY MATERIAL

Supplementary Table 1: Climatic, edaphic and geophysical variables used in taxa distribution modelling in MaxEnt based on SelecVar analysis

Taxa	Environme	ental variables	used for speci	es distribution	modelling				
Chenopodium ambrosioides	bio_2	bio_14	t_sand	t_oc	s_ph_h2o	eastness	northeness	slope	
Coffea ligustroides	bio_10	bio_14	t_bs	t_cec_soil	s_gravel	slope	northeness	aspect	
Coffea mufindiensis subsp. australis	bio_2	bio_4	bio_15	t_caco3	s_gravel	s_cec_soil	eastness	aspect	
Cucumis anguria var. anguria	bio_4	bio_15	t_sand	t_caco3	northess	slope			
Eleusine indica	bio_1	bio_2	bio_15	t_caco3	s_cec_soil	t_ce_soil	aspect	slope	eastness
Eleusine coracana subsp. africana	bio_2	bio_10	bio_15	s_cec_soil	t_oc	aspect	slope		
Ipomoea pileata	bio_1	bio_4	bio_15	s_gravel	t_oc	s_sec_soil	aspect	slope	eastness
Ipomoea obscura subsp. obscura	bio_2	bio_4	bio_10	t_caco3	t_bs	t_cec_soil	aspect	alt	slope
Ipomoea tenuirostris	bio_4	bio_10	s_gravel	s_cec_soil	slope	eastness			
Oryza barthii	bio_2	bio_10	bio_14	t_caco3	t_oc	northeness	aspect		
Oryza longistaminata	bio_1	bio_2	t_oc	s_cec_soil	aspect	slope			
Prunus africana	bio_10	bio_15	t_oc	s_cec_soil	aspect	slope			
Solanum aculeatissimum	bio_2	bio_7	t_caco3	t_ph_h2o	aspect	alt	eastness		
Solanum anguivi	bio_1	bio_3	bio_7	t_gravel	s_cec_clay	slope	aspect	alt	
Solanum campylacanthum	bio_1	bio_4	bio_15	t_caco3	s_cec_clay	slope	northeness	alt	
Solanum hispidum	bio_1	bio_2	t_ph_h2o	t_gravel	northess	eastness			

Taxa	Environmental variables used for species distribution modelling							
Solanum incanum	bio_1	bio_3	t_ph_h2o	t_sand	aspect	northness		
Solanum nigrum	bio_1	bio_4	bio_19	t_sand	t_ph_h2o	aspect	northeness	
Solanum panduriforme	bio_1	bio_3	t_gravel	s_gravel	t_caco3	aspect	slope	
Solanum richardii subsp. richardii	bio_4	bio_15	s_gravel	t_cec_clay	slope	northness		
Solanum richardii	bio_1	bio_7	bio_19	s_ph_h2o	s_gravel	slope	eastness	
Solanum schumannianum	bio_2	bio_4	t_sand	s_cec_clay	slope	eastness		
Solanum tarderemotum	bio_1	bio_3	bio_7	t_gravel	t_ph_h2o	alt	northeness	eastness
Solanum terminale	bio_2	bio_3	bio_15	t_gravel	t_cec_clay	slope	eastness	
Solanum terminale subsp. terminale	bio_4	bio_15	t_sand	s_cec_clay	alt	northness		
Solanum torvum	bio_4	bio_12	t_sand	s_cec_clay	alt	northness		
Sorghum bicolor subsp. arundinaceum	bio_1	bio_4	t_gravel	t_ph_h2o	aspect	slope		
Sorghum versicolor	bio_1	bio_19	t_ph_h2o	t_oc	slope	alt		
Vigna frutescens	bio_1	bio_15	t_ece	s_gravel	slope	alt		
Vigna unguiculata var. unguiculata	bio_1	bio_2	t_sand	t_caco3	alt	eastness		
Vigna pygmaea	bio_4	bio_15	t_sand	t_ph_h2o	slope	northness		
Vigna platyloba	bio_1	bio_15	t_oc	t_caco3	slope	alt		
Vigna oblongifolia	bio_2	bio_19	s_gravel	s_ph_h2o	aspect	nortness		
Vigna phoenix	bio_2	bio_10	t_cec_clay	slope	aspect			
Vigna heterophylla subsp .ambacensis	bio_14	bio_16	t_caco3	t_ref_bulk	alt	aspect		
Vigna unguiculata subsp. dekindtiana	bio_4	bio_15	t_ph_h2o	t_oc	aspect	slope		

Environmental variables used for species distribution modelling

Таха	Environmental variables used for species distribution modelling							
Vigna unguiculata subsp. spontanea	bio_2	bio_3	bio_15	t_oc	t_bs	eastness	aspect	
Vigna gazensis	bio_7	bio_15	t_ece	s_ph_h2o	aspect	slope		
Vigna vexillata subsp.angustifolia	bio_1	bio_7	t_oc	t_cec_clay	eastness	alt		
Vigna vexillata var.vexillata	bio_1	bio_3	bio_7	t_oc	t_bs	alt	eastness	
Vigna comosa	bio_7	bio_15	t_ph_h2o	s_gravel	slope	northness		
Vigna luteola	bio_1	bio_15	t_ece	s_ph_h2o	eastness	northness	alt	
Vigna racemosa	bio_3	bio_12	t_cec_clay	t_oc	slope	alt		
Vigna reticulata	bio_7	bio_15	t_ph_h2o	t_caco3	slope	aspect		

Sources 1.Bioclimatic data https://www.worldclim.org/bioclim

2. Edaphic data and geophysical data (Harmonised soil data base): <u>http://www.fao.org/soils-portal/soil-survey</u> (Fischer et al., 2008).

Таха	Number of Pixels at present climatic conditions	Number of pixels projected with taxa in 2030 RCP4.5	Number of pixels gained or lost in 2030	Number of pixels projected with taxa in 2050 RCP4.5	Number of pixels gained or lost in 2050	Number of pixels projected with taxa in 2070 RCP4.5	Number of pixels gained or lost in (2070)
Coffea ligustroides S.Moore	854	160	-694	103	-751	144	-710
<i>Coffea mufindiensis</i> Hutch Ex Bridson subsp. <i>australis</i>	1856	1855	-1	1278	-578	1028	-828
Ipomoea pileata Roxb	710	716	6	190	-520	170	-540
Oryza longistaminata A.Chev.& Roehr	2847	690	-2157	4473	1626	4711	1864
Solanum anguivi Lam	646	513	-133	161	-485	127	-519
Solanum schumannianum Dammer	2872	2373	-499	2551	-321	2847	-25
Solanum terminale Forssk. subsp. terminale	3648	2611	-1037	2447	-1201	2112	-1536
Sorghum bicolor (L.) Moench subsp. arundinaceum	2299	1828	-471	3722	1423	3795	1496
Vigna comosa Baker	611	1225	614	273	-338	488	-123
Vigna frutescens A.Rich. subsp. frutescens	2318	3096	778	641	-1677	501	-1817
<i>Vigna oblongifolia</i> A. Rich. var. <i>parviflora</i> (Baker) Verdc.	1059	1241	182	1619	560	1603	544
Vigna racemosa (G. Don) Hutch. & Dalziel	46	2286	-560	2478	-368	2085	-761
Vigna unguiculata (L.)	1067	1265	198	182	-885	140	-927
Vigna vexillata (L.) A.Rich. subsp.angustifolia	1284	1848	564	230	-1054	165	-1119

Supplementary Table 6a: Change in coverage of the 14 priority taxa under climate change scenarios RCPs 4.5 from 2030 to 2070

Таха	Number of pixels at present climatic conditions	Number of pixels projected with taxa in 2030 RCP8.5	Number of pixels gained or lost in 2030	Number of pixels projected with taxa in 2050 RCP8.5	Number of pixels gained or lost in 2050	Number of pixels projected with taxa in 2070 RCP8.5	Number of pixels gained or lost in 2070
Coffea ligustroides S.Moore	854	107	-747	42	-812	19	-835
<i>Coffea mufindiensis</i> Hutch Ex Bridson subsp. <i>australis</i>	1856	1202	-654	1051	-805	791	-1065
Ipomoea pileata Roxb	710	219	-491	145	-565	104	-606
Oryza longistaminata A.Chev.& Roehr	2847	4309	1462	4953	2106	5288	2441
Solanum anguivi Lam	646	210	-436	100	-546	82	-564
Solanum schumannianum Dammer	2872	2637	-235	2698	-174	2528	-344
Solanum terminale Forssk. subsp. terminale	3648	2710	-938	2452	-1196	2236	-1412
Sorghum bicolor (L.) Moench subsp. arundinaceum	2299	3561	1262	4060	1761	4227	1928
Vigna comosa Baker	611	372	-239	154	-457	175	-436
Vigna frutescens A.Rich. subsp. frutescens	2318	759	-1559	NA	NA	135	-2183
<i>Vigna oblongifolia</i> A. Rich. var. <i>parviflora</i> (Baker) Verdc.	1059	1189	130	1519	460	1463	404
Vigna racemosa (G. Don) Hutch. & Dalziel	46	2232	-614	2563	-283	2531	-315
Vigna unguiculata (L.)(wild types)	1067	226	-841	108	-959	80	-987
Vigna vexillata (L.) A.Rich. subsp.angustifolia	1284	282	-1002	128	-1156	86	-1198

Supplementary Table 2b: Change in coverage of the 14 priority taxa under climate change scenario RCP 8.5 from 2030 to 2070

CHAPTER 5

GENOTYPING FOR DROUGHT TOLERANCE AMONG WILD AND CULTIVATED ORYZA GENEBANK ACCESSIONS FROM MALAWI.

ABSTRACT

Drought is one of the rice production challenges in Malawi and has often reduced grain yield by more than 50%. With increased water pumping costs in irrigation schemes, farmers have opted to either stop growing or reducing its cultivation affecting its annual production in the country. The availability of drought tolerant rice varieties could significantly restore and increase rice productivity. Thirteen simple sequence repeats (SSRs) markers were used to genotype for drought tolerance 130 Oryza germplasm accessions (wild and cultivated species) from the Malawi Plant Genetic Resources Centre (MPGRC) in order to increase the availability of drought tolerant lines in the national rice-breeding programme. The results indicate that 62% of the accessions were amplified at >9 loci. The associated SSRs include RM472, RM242, RM72, RM28166, RM219 and RM212 and these are linked with grain yield, earliness, and root length characteristics. High significant differences (P<0.001) were noted on genetic differentiation within populations and within individual accession with 50% molecular variance explaining allelic distance within individual accession. The accessions identified will provide initial pool of potential germplasm to be further screened for drought tolerance for improving rice production in Malawi. Relatively high heterozygosity and low in breeding coefficients noted in SSRs RM472, RM28166 and RM212 indicate the existence of variations within Malawi's rice germplasm that can be tapped in development of elite drought tolerant lines. RM472 was highly polymorphic for all accessions and its ability to detect the highest number of alleles makes it a potential marker for further drought tolerant screening.

Key words: Oryza accessions, drought tolerant, germplasm, rice improvement, SSR.

5.1. INTRODUCTION

Rice is the second main cereal crop grown in Malawi with a cultivation area of between 480,000 and 600,000 hectares of land (MoAIWD, 2012) and with an estimated annual production of 2 million tonnes (CCARDESA, 2020). The crop is versatile in use, has a well-defined value chain, is regarded as one of the highest economic value crops produced in Malawi (MoAIWD, 2012) and is listed under the forex earners crops of Malawi (FAO, 2019). Because of its high economic value, rice has potential to boost local economies of most small and medium scale farmers in Malawi (Before *et al.*, 2018, Magreta, 2011). However, like many other crops in Malawi, rice productivity has been negatively impacted by droughts (Mzengeza, 2010, Before *et al.*, 2018).

Due to erratic rains, Malawi experiences droughts every year in some parts of the country especially in the Shire Valley and Lakeshore areas. Although not quantified in terms of yield, droughts is reported to significantly reduce rice grain yield in Malawi (Before *et al.*, 2018, Mzengeza, 2010) and causes annual fluctuations of rice production. For rice grown with supplemental irrigation, reduction of water levels negatively affects rice plant development and growth (Mzengeza, 2010). In rice irrigation schemes, water has become a scarce commodity due to high competition by users as well as costs associated with pumping the water (MoAIWD, 2012, Kumwenda *et al.*, 2015). Due to this, farmers have often relied on partial irrigation where the crop is irrigated in its early growth stage and supplemented by rainwater at later growth stages. Nevertheless, in rain fed rice production, moisture management strategies (Daccache *et al.*, 2015) like mulching, and close spacing have been adopted.

The current total production stands at an average of 150,000 tonnes per annum, which is, much less than its potential. High yield (6 tonnes/ha) rice varieties were released in order to reduce the production gap (DARS, 2019). Breeding for high yielding alone is likely not sufficient enough to bridge this production gap as yield performance is influenced by a combination of factors such as genetic, environmental and the interaction between genetic and the environment (Vanniarajan et al., 2012). National rice-breeding programmes sourced some drought tolerant parental lines from Uganda (NERICA4) and African Rice Institute, Senegal and are currently under evaluations in all rice agro ecological zones (Cornwell Iman, 2021, personal communication). Malawi Plant Genetic Resources Centre (MPGRC) holds a collection of rice accessions (cultivated and wild species) collected from across all ricegrowing areas of Malawi and adapted to local conditions. This pool of diversity is meant for use by breeders in an effort to increase the availability of improved rice varieties in Malawi in away addressing some of the rice production constraints (Before *et al.*, 2018; Mzengeza, 2010). Collected from all rice growing areas (all rice agro ecological zones), which include Shire Valley, and Lakeshore areas (known drought prone areas of Malawi), it is anticipated that some of the accessions have developed drought tolerant associated traits, such as high number of tillers per hill, deep rooting system, yielding ability and earliness among others. To facilitate use of this rice germplasm, MPGRC characterized some of these accessions using morphological markers that include days to 50% flowering, number of tillers per hill and yielding ability. In selecting for drought tolerance, yield ability under different stress conditions is considered a principal trait in a number of crops. However, trait like grain yield has low heritability (Lang and Buu, 2008) which makes it difficult to select for under phenotypic characterization. Use of molecular markers helps identify multiple genes

associated with drought being in itself a polygenic trait (Ramadam et al., 2015) and hence shorten the screening period. DNA based molecular markers for drought tolerance will help identify genomic locations that control drought tolerance in rice (Lanceras et al., 2004, Ramadam et al., 2015,). These have also been used to understand the mechanisms of drought tolerance in plants and traits of association (Lanceras et al., 2004). As compared to morphological markers, DNA based molecular markers are not subject to environmental modification and are easy to select for given their abundance and ability to detect genotype differences at molecular level (Ramadam et al., 2015). SSRs markers are highly polymorphic, heterozygous and co-dominant making them more reliable in mapping for genetic variation in rice ((Ni et al., 2002, Lapitan et al., 2007, Faridul Islam et al., 2012). Because of high discriminating power, SSRs markers have frequently been used to detect genetic diversity among different germplasm sources, drought tolerance and or water stress marker association in rice improvement (Lapitan et al., 2007, Vanniarajan et al., 2012,). This study was designed to genotype rice accessions for drought tolerance in order to identify accessions potentially with drought tolerant genes and promote the same in rice breeding programme in Malawi. The study further mapped the distribution of drought tolerant alleles across the country to aid generation of rice trait specific core collections.

5.2. MATERIAL AND METHODS

5.2.1. Accessions used and sampling strategy

The study used 130 rice accessions (95 cultivars of indica) and 35 wild species) including those of wild species of *Oryza punctata*, *O. barthii* and *O. longistaminata*. The accessions were sampled from all rice agro-ecological zones in Malawi. Selection of the samples was guided by the passport data of the accessions. Duplicate samples were excluded. However,

accessions with the same coordinates but with different grain characteristics and vernacular names (names given by farmers based on their attributes) were included. Sampled accessions were not necessarily proportionate across the agro-ecological zones, but rather the sampling ensured incorporation of at least 10 accessions from each rice agro-ecological zone except for *O. punctata* and *O. longistaminata* since they had a few samples under *ex situ* but was applied for *O. barthii*. The accessions included were those collected from low altitude areas (20 accessions) (<50 to 250 meters above sea level), 23 accessions from medium altitude areas (areas lying between 500-750 meters above sea level), 37 accessions from medium to high altitude areas that include areas lying between >800-1500 meters above sea level and 50 samples collected from high altitude areas (areas >1500 meters) above sea level.

5.2.2. Plant tissue preparation and DNA extraction

Leaf samples were taken from 30 days immature leaves from 15 plants per accession planted in the screen house at MPGRC. Silica gel was used to dry the leaf material. Upon drying, the samples were stored in airtight and waterproof packaging materials to check out moisture. DNA was extracted from 25 mg of plant material; and this yielded a suitable concentration of DNA for input into the fragment analysis polymerase chain reaction (PCR). However, in the case of MW5101, MW5086, MW5104, MW1625 and MW4856, these samples were used neat in the PCR, as they were below the usual 10 ng/µl concentration. 25 mg of plant material was initially lysed using the kit lysis buffer and a Qiagen TissueRuptor. Once lysed the material was extracted using a chemagic MSMI instrument and chemagic Plant DNA extraction kit reagents. This automated system uses a magnetic bead based method. The samples were quantified using a Nanodrop ND-1000 Spectrometer Analysis.

5.2.3. SSRs used, Polymerase chain reaction (PCR) mix, thermal cycling conditions and PCR products

The PCRs were set up using a 10 pmol/µl mix of the relevant primer pairs ((0.5μ l) and 25 ng of input DNA (in a total of 2.5 µl), along with a standard all in one Applied Biosystems AmpliTaq Gold 360 Master-Mix (5 µl) and Molecular Biology Water (2 µl). A total of 13 SSRs DNA primers (RM201, RM72, RM212, RM410, RM315, RM3825, RM28166, RM242, RM328, RM472, RM260, RM219 and RM302) were used to amplify the sample DNA fragments and the primer pair sequences of these can be obtained on (<u>http://www.gramene.org</u>). These primers were selected based on their linkage to yield and drought related traits, high polymorphism and relatively high number of effective alleles (Ramadam *et al.*, 2015). All forward primers were labelled with the 6FAM dye and the reverse primers were unlabelled. The thermal cycling conditions are as noted below (Table 5.1) with just one primer set deviating from the standard 55 °C annealing temperature.

	Temp (degrees C)	Time	
	94	3 m	Initial denaturation
	94	30 s	Further denaturation
30 CYCLES	55 (most) or 51.8 (RM260)	30 s	Primer annealing
	72	1 m	Primer elongation
	72	30 m	Primer extension
	15	HOLD	

Table 5. 1: Polymerase chain reaction and thermal cycling conditions

N.B. just one primer pair required a non-standard annealing temperature (RM260) which annealed at 51.8 $^{\circ}C$.

5.2.3.1. Electrophoresis Set-Up

The PCR products were diluted 1 in 3 and 0.5 μ l of this dilution was added to a loading cocktail. The loading cocktail contained Hi-Di Formamide (9 μ l) and the size standard LIZ500

 $(0.5 \,\mu$ l). The electrophoresis was performed on an Applied Biosystems 3730xl DNA Analyser and the data was viewed in Applied Biosystems GeneMapper software.

5.3. DATA ANALYSIS

Peaks for the amplified accessions were scored for each SSR marker generating both codominant data in the form of genotype by fragment size and a matrix of binary data where data was numerically coded as 0 and 1 for a single locus. This data was then analysed using GenAlex6.5 software (Peakall and Smouse, 2006, Peakall and Smouse, 2015). Both frequency and genetic distance based analyses were obtained. Analysis of molecular variance (AMOVA) was used to estimate hierarchical genetic variation among populations based on Excoffier *et al.* (1992), Huff *et al.* (1993) and Peakall *et al.* (1995) procedures. A standardised F-statistics (FST) was calculated for the genetic distance among *Oryza* accessions based on Meirmans (2006). Allele frequency Fx=(2Nxx+Nxy)/2N for a single locus was calculated where Nxx represents number of XX homozygous individuals, and Nxy represents number of XY heterozygous individuals, where Y can be any other allele. N is the number of samples (Hartl and Clark, 1997; Frankham, 2004).

Genetic diversity through partitioning (Shannon index) was also calculated among the accessions. Correlations between diversity and allelic frequency were calculated to estimate the strength of association. Statistical differences among accessions were tested with F-statistics performed in GenAlex software 6 and 6.5 version (Peakall and Smouse, 2006, 2012).

G-statistics calculations were done by random permutations through Shannon partition option to avoid increasing the type I error (Peakall and Smouse, 2012). Finally, spatial diversity of potential drought tolerant alleles was analysed in DIVA-GIS 7.5.0 version (http://www.diva-

gis.org) (Hijmans *et al.*, 2012) and the distribution and diversity of potential drought tolerant alleles across rice collection sites were mapped.

5.4. **RESULTS**

5.4.1. Samples realised due to sampling strategy and sites of collection.

The study used 130 Oryza accessions, 95 accessions being cultivars of indica and 35 accessions from *Oryza* wild relatives of *O. longistaminata* (7), *O. barthii* (22) and *O. panctata* (6) sourced from Malawi Plant Genetic Resources Centre (MPGRC), a national repository centre of plant germplasm in Malawi. Samples yielded enough quality DNA sufficient for multiplexing.

5.4.2. PRC products and amplification

Ninety (97) alleles were detected across 13 loci of SSRs for 130 Oryza accessions. Almost all accessions were amplified on one or two locations of the base pair region. Allele number per locus ranged from 7 to 23 with the lowest alleles generated by RM201 and RM410 SSRs and the highest numbers associated with RM472 (23 alleles) and RM242 (21 alleles). Marker RM72 generated 14 alleles, RM28166, RM219 and RM212 generated 12 alleles, RM3825 generated 10 alleles while the rest had 9 alleles. The allele size ranged from 68 to 238 base pairs (bp) with 16 alleles lying between 68–100 bp, 18 alleles lying between 100–140 bp, 37 alleles lying between 150–190 bp and rest were above 200 bp.

In terms of drought tolerance amplification, all accessions were amplified at one or more loci. Accessions MW1155, MW5266 and MW5283 recorded the highest number (12) of alleles being amplified followed by 18 accessions which were amplified at 11 loci and these include MW1700 (11), MW1753 (11), MW1804 (11), MW1825 (11), MW39 (11), MW4700 (11), MW5054 (11), MW5062 (11), MW5088 (11), MW5106, MW5111, MW525, MW526, MW5269, MW5271, MW5285, MW5352 and MW5362. Thirty accessions were amplified 10 times, another 30 accessions were amplified 9 times and 15 accessions had 8 loci amplified. Accessions MW4657 (3 loci), MW4855 (4 loci), MW1702 (5 loci), MW4862 (5 loci) and MW4872 (5 loci) had the least number of loci being amplified. The remaining 27 accessions had loci range of 6–7. In this study, cultivated accessions were considered as population one (P1) and the wild species accessions assigned as population two (P2). By population, the cultivated accessions had a high mean number (9) of amplified and private alleles compared to wild species accessions (Figure 5.1). Consequently, P1 exhibited relatively high heterozygosity than P2 (Figure 5.1) but with similar number of effective alleles (Ne) and sHa information index (I). By locus, SSRs RM472, RM212 and RM28166 exhibited high values of heterozygosity (Ho) (0.6–0.7), low in breeding coefficients (Fis) (0.0–0.2) within individuals and relatively high genetic differentiation among populations (Figure 5.1 and Table 5.3).

5.4.3. Genetic diversity and differentiation among accessions.

By locus, the highest allelic frequency was observed in RM472 with Shannon diversity index (sHa) of 2.53 followed by RM242 with sHa of 2.16. The lowest allelic frequency was noted in RM201 (sHa=1.07) followed by RM410 (sHa=1.29). By Population (cultivated versus wild species), cultivated accessions had high allelic frequency at loci RM472 (sHa 2.198), RM219 (sHa 1.972) and at RM242 (sHa=1.78). For wild species, sHa was highest at RM72 (2.365) followed by RM472 (2.196), RM28166 (2.114) RM242 (1.887) and RM212 (1.765).

There was relatively small variation observed on allelic patterns between and within the two populations (Figure 5.1 and Table 5.3) as indicated by their standard errors (Table 5.2. In terms of genetic differentiation, results of AMOVA estimated by microsatellite distance matrix for R statistics indicated that the genetic differences among populations was 41%,

among individual variation was 28% and within individual was 31% and this was significant at P(<0.001). Results of molecular variance due to allelic distance for F-statistics indicated that 50% of variance was within individuals, 31% among individuals variation and 19% variation among populations at P(<0.001) significant levels for all F-statistics parameters (FIS,FIT, FST). Nei's unbiased genetic distance indicated a value of 0.699 for the cultivated accessions and 1 for wild species accessions.

Positive and strong correlations were also noted between heterozygosity and allelic frequency r=0.5–0.9 confirming that the observed 50% molecular variance within individual accessions was a true reflection of the variation existing in our germplasm. P2 had a high number of effective alleles (Ne=3.94) than P1 (Ne=3.34). A small difference (sHa=0.1) in Shannon information index was noted between populations (P1, sHa=1.45 and P2, sHa=1.34), however P1 had high average number (9.16) of total and private alleles.

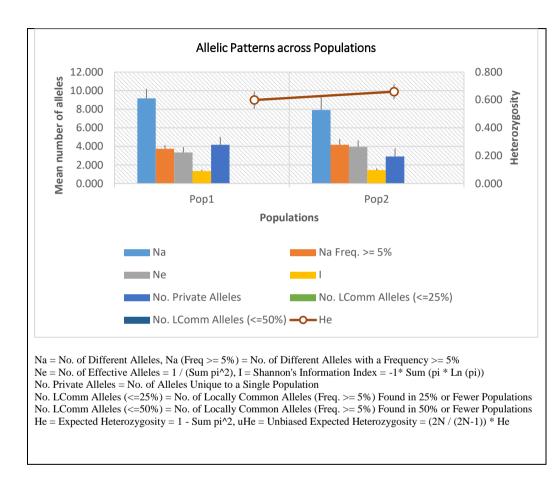


Table 5. 2: Standard errors of genetic differentiation among Oryza accessions from Malawi

Standard Error (SE) values	Standard	Error	(SE)	values
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Population	Pop1	Pop2
Na	1.036	1.448
Na Freq. >= 5%	0.392	0.626
Ne	0.570	0.710
I	0.168	0.178
No. Private Alleles	0.860	0.866
No. LComm Alleles (<=25%)	0.000	0.000
No. LComm Alleles (<=50%)	0.000	0.000
Не	0.062	0.054

Figure 5. 1: Allelic pattern across populations (cultivated P1 and wild species P2) for the codominant data by step by step method and their standard errors

Mean N	Mean Na	Mean Ne	Mean cNe	Mean Ho	Hs	Ht	Fis	Fst	Gis	Dest	P (Gst)
114.0	11	6.2	4.0	0.3	0.8	0.8	0.6	0.1	0.6	0.5	0.0
102.0	5	2.1	2.1	0.1	0.5	0.6	0.8	0.1	0.8	0.1	0.0
130.0	17	6.2	6.1	0.7	0.8	0.9	0.2	0.1	0.2	0.8	0.0
130.0	8	2.7	2.4	0.4	0.6	0.7	0.3	0.2	0.3	0.8	0.0
130.0	9	4.9	3.9	0.6	0.7	0.8	0.2	0.1	0.2	0.5	0.0
125.0	9	3.5	2.1	0.2	0.5	0.7	0.6	0.3	0.7	0.9	0.0
130.0	14	3.8	3.8	0.4	0.7	0.9	0.5	0.1	0.5	0.9	0.0
130.0	9	3.4	3.1	0.7	0.7	0.8	0.0	0.1	0.0	0.6	0.0
115.0	7	2.6	2.6	0.3	0.6	0.6	0.5	0.0	0.6	0.0	1.0
46.0	8	4.6	4.1	0.1	0.8	0.8	0.8	0.1	0.9	-0.5	1.0
105.0	4	1.7	1.7	0.0	0.4	0.4	0.9	0.0	0.9	0.0	0.1
109.0	4	2.0	1.7	0.0	0.4	0.5	1.0	0.1	1.0	0.2	0.0
	102.0 130.0 130.0 130.0 125.0 130.0 130.0 115.0 46.0 105.0	114.0 11 102.0 5 130.0 17 130.0 8 130.0 9 125.0 9 130.0 14 130.0 9 130.0 7 46.0 8 105.0 4	114.0116.2102.052.1130.0176.2130.082.7130.094.9125.093.5130.0143.8130.093.4115.072.646.084.6105.041.7	114.0116.24.0102.052.12.1130.0176.26.1130.082.72.4130.094.93.9125.093.52.1130.0143.83.8130.093.43.1115.072.62.646.084.64.1105.041.71.7	114.0 11 6.2 4.0 0.3 102.0 5 2.1 2.1 0.1 130.0 17 6.2 6.1 0.7 130.0 8 2.7 2.4 0.4 130.0 9 4.9 3.9 0.6 125.0 9 3.5 2.1 0.2 130.0 14 3.8 3.8 0.4 130.0 9 3.4 3.1 0.7 115.0 7 2.6 2.6 0.3 46.0 8 4.6 4.1 0.1 105.0 4 1.7 1.7 0.0	114.0 11 6.2 4.0 0.3 0.8 102.0 5 2.1 2.1 0.1 0.5 130.0 17 6.2 6.1 0.7 0.8 130.0 8 2.7 2.4 0.4 0.6 130.0 9 4.9 3.9 0.6 0.7 125.0 9 3.5 2.1 0.2 0.5 130.0 14 3.8 3.8 0.4 0.7 130.0 9 3.4 3.1 0.7 0.7 115.0 7 2.6 2.6 0.3 0.6 46.0 8 4.6 4.1 0.1 0.8 105.0 4 1.7 1.7 0.0 0.4	114.0 11 6.2 4.0 0.3 0.8 0.8 102.0 5 2.1 2.1 0.1 0.5 0.6 130.0 17 6.2 6.1 0.7 0.8 0.9 130.0 8 2.7 2.4 0.4 0.6 0.7 130.0 9 4.9 3.9 0.6 0.7 0.8 125.0 9 3.5 2.1 0.2 0.5 0.7 130.0 14 3.8 3.8 0.4 0.7 0.9 130.0 14 3.8 3.1 0.7 0.7 0.8 115.0 7 2.6 2.6 0.3 0.6 0.6 46.0 8 4.6 4.1 0.1 0.8 0.8 105.0 4 1.7 1.7 0.0 0.4 0.4	114.0116.24.00.30.80.80.6102.052.12.10.10.50.60.8130.0176.26.10.70.80.90.2130.082.72.40.40.60.70.3130.094.93.90.60.70.80.2125.093.52.10.20.50.70.6130.0143.83.80.40.70.90.5130.093.43.10.70.70.80.0115.072.62.60.30.60.60.546.084.64.10.10.80.80.8	114.0116.24.00.30.80.80.60.1102.052.12.10.10.50.60.80.1130.0176.26.10.70.80.90.20.1130.082.72.40.40.60.70.30.2130.094.93.90.60.70.80.20.1125.093.52.10.20.50.70.60.3130.0143.83.80.40.70.90.50.1130.093.43.10.70.70.80.00.115.072.62.60.30.60.60.50.046.084.64.10.10.80.80.80.1105.041.71.70.00.40.40.90.0	114.0116.24.00.30.80.80.60.10.6102.052.12.10.10.50.60.80.10.8130.0176.26.10.70.80.90.20.10.2130.082.72.40.40.60.70.30.20.3130.094.93.90.60.70.80.20.10.2125.093.52.10.20.50.70.60.30.7130.0143.83.80.40.70.90.50.10.5130.093.43.10.70.70.80.00.10.0115.072.62.60.30.60.60.50.00.646.084.64.10.10.80.80.80.10.9105.041.71.70.00.40.40.90.00.9	114.0116.24.00.30.80.80.60.10.60.5102.052.12.10.10.50.60.80.10.80.1130.0176.26.10.70.80.90.20.10.20.8130.082.72.40.40.60.70.30.20.30.8130.094.93.90.60.70.80.20.10.20.5125.093.52.10.20.50.70.60.30.70.9130.0143.83.80.40.70.90.50.10.50.9130.093.43.10.70.70.80.00.10.00.6115.072.62.60.30.60.60.50.00.60.046.084.64.10.10.80.80.80.10.9-0.5105.041.71.70.00.40.40.90.00.90.0

Table 5. 3: Genetic differentiation among Oryza accessions estimated by G statistics and related base level statistics by locus as estimated over populations.

Mean Na = Mean No. of Different Alleles Over Pops ,

Mean Ne = Mean No. of Effective Alleles Over Pops. Ne = $1/(1-\text{He}) = 1 / (\text{Sum pi}^2)$.

Mean cNe = Mean No. of Effective Alleles Over Pops. cNe = 1/(1-Hs), where Hs is the average He over k pops (per locus).

Mean Ho = Mean Observed Heterozygosity over k pops. Ho = (Sum(No. of Hets / N))/k.

Hs = Mean Expected Heterozygosity He over k pops. Hs = $(Sum(1 - Sum pi^2))/k$, where pi = pop allele frequency.

Ht = Total Expected Heterozygosity. Ht = 1-Sum pai², where pai = average pop allele frequency.

Fis = Inbreeding coefficient within individuals. Fis = (Hs-Ho)/Hs.

Fst = Inbreeding coefficient within subpopulations, relative to total = genetic differentiation among populations. Fst = (Ht-Hs)/Ht

Gis = Inbreeding coefficient within individuals, adjusted for bias. Gis = (cHs-Ho)/cHs., Dest = Jost's estimate of differentiation

5.4.4. Distribution of potential drought tolerant alleles across Malawi

Figure 5.2 highlights the distribution and diversity of potential drought alleles of Oryza accessions across rice collections sites and it clearly shows that the alleles are distributed across the country. However, highest diversity was noted in the Southern Region of Malawi (sHa range 3.6–4). Based on their frequency of occurrence (Figure 5.3), these alleles were grouped into three categories. Category one are alleles with sHa index range of 2.0–2.9; these are unique alleles only observed once per site but being distributed on a large area (spanning from the Southern to the Northern Region of Malawi) (Figure 5.2). The second category includes alleles with sHa index range of 2.9–3.3. Relatively, these were observed on a larger area (across all rice growing areas) but still have low frequency of occurrence being observed only once or twice per site. The last group are those highlighted in red (sHa>3); these alleles were observed at a relatively small area in the Southern Region of Malawi but with high frequency of occurrence (Figure 5.3) indicated by their sHa diversity index (Figure 5.2). Correlation analysis between potential drought tolerant alleles and geographical distribution of the genotyped accessions showed positive and strong correlations (r=0.5-1.0).

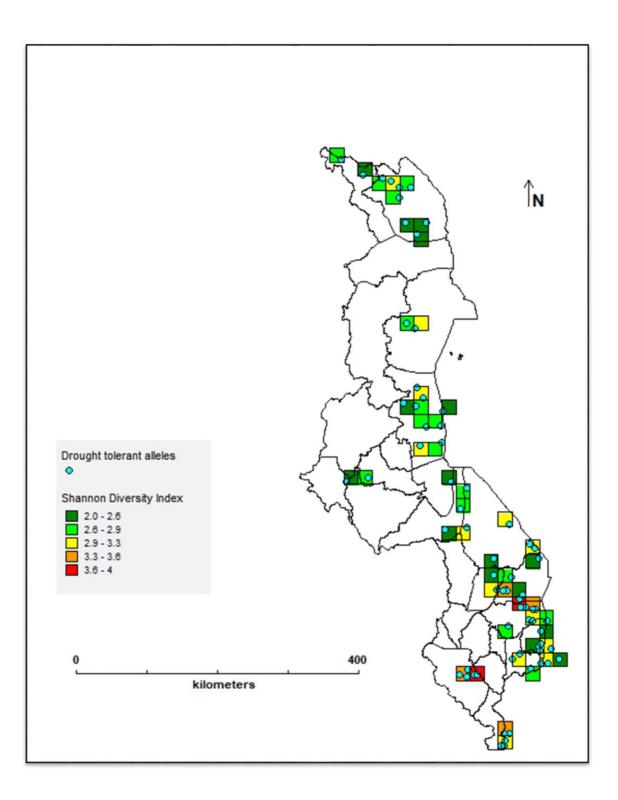


Figure 5. 2 Diversity index and distribution of potential drought tolerant alleles of *Oryza* accessions sourced from Malawi Plant Genetic Resources Centre.

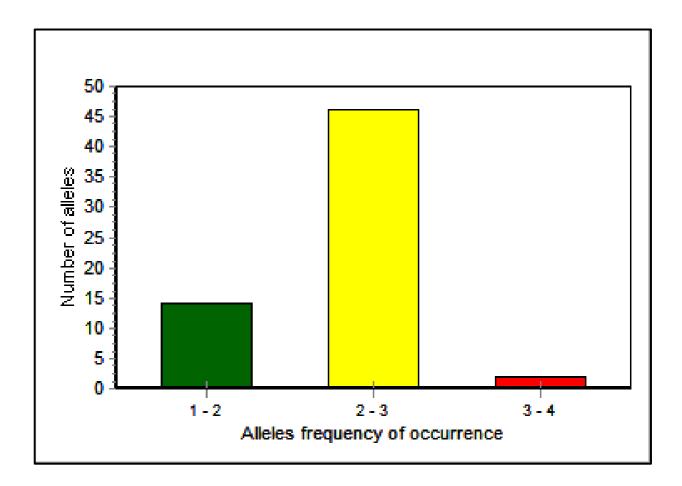


Figure 5. 3 On site occurrence of potential drought tolerant alleles among *Oryza* accessions

5.5. **DISCUSSIONS**

The results of the study indicate that drought tolerant alleles in Malawi's Oryza accessions are randomly distributed across the country. In total, 97 alleles were detected by 13 SSRs. Some variation was noted in the number of alleles amplified per accession and per loci. The number of alleles per locus ranged from 7 to 23 loci and that of per accession ranged from 3 to 12 alleles and this means that there is a probability of finding accessions with drought tolerance genes. However, the rate of amplification was relatively high in 62% of the accessions with the least amplified number of loci being eight. Accessions MW1155, MW5266 and MW5283 were amplified 12 times and followed by accessions MW1700 MW1753, MW1804, MW1825, MW39 MW4700, MW5054, MW5062, MW5088, MW5096, MW5106, MW5111, MW525, MW526, MW5269, MW5271, MW5285, MW5352 and MW5362 which were amplified at 11 loci. SSRs RM472, RM242, RM72, RM28166, RM219 and RM212 were more polymorphic for the genotypes used having generated an average of 17 alleles per loci. The highest polymorphic SSR was RM472 and it generated 23 alleles and the least alleles were produced by RM201 (mean alleles of 5) and RM315 and RM328 with mean number of four alleles.

Alleles associated with drought tolerance were detected in almost all accessions in varying frequencies. The above accessions were amplified relatively more compared to other accessions. The least number of amplified alleles were noted in accessions MW4657 (3), MW4855 (4 loci) and five loci amplified in MW1702, MW4862 and MW4872.

Cultivated accessions had higher numbers (9) of amplified alleles, they also exhibited high heterozygosity and low in breeding coefficients in SSRs RM472, RM212 and RM28166 within individual accessions compared to wild species. High molecular variance (50%) was

observed within individual accessions for both cultivated and wild accessions. Significant differences in genetic differentiation parameters and positive and strong correlation coefficients (r=0.5-0.9) between allelic frequency and heterozygosity was noted among individual accessions. This confirms the existence of some degree of variation among the genotyped accessions. Genetic variation is the basis for breeding in any plant species so the availability of this variation among our accessions provides an opportunity for selection by breeders to improve the current rice varieties and for the development of new rice lines.

In terms of allelic distribution, the results showed that potential drought tolerant alleles (both common and unique alleles) were distributed across all rice agro-ecological zones (low to high altitudes areas). However, the frequency of occurrence of these alleles per site is low. A subset of these alleles will need to be conserved despite their wide adaptation and could be bred for multiple traits. Alleles with high frequency of occurrence but only observed in few sites were observed in the Southern Region of Malawi (low to medium lying areas), in collection sites from Nsanje, Chikwawa, Zomba and Phalombe Districts. Nsanje and Chikwawa are known drought prone areas in Malawi while some parts of Zomba, Thyolo and Phalombe experience seasonal droughts. Positive and strong correlations noted between geographical distribution of the accessions and potential drought tolerance alleles is an indication that accessions collected from these sites contain traits associated with drought tolerance.

These were also the sites observed with high diversity of alleles based on their Shannon diversity index. Being drought prone areas, it is likely that the accessions evolved to adapt to the local conditions and therefore the probability of occurrence of drought tolerance genes is high. Therefore, these might include rare and unique alleles specifically adapted to harsh

climatic conditions. With issues of climate change, accessions collected from these sites will be useful in breeding for climate change adaptation.

The mechanism of drought tolerance is complex and difficult to understand since drought is a polygenic trait (Lang and Buu, 2008, Qu et al., 2008). Because of this, breeders have had difficulty breeding for drought tolerant rice varieties (Courtois et al., 2000). Therefore, the existence of accessions with potential drought tolerant genes makes Malawi rice germplasm potential genetic material for rice improvement. Use of drought tolerant varieties will boost upland rice production and reduce a burden of relying on irrigation considering that Malawi has a sub-tropical climate and a short and erratic rainfall season (an average of 3 months of sufficient rains). Cultivation of drought tolerant varieties becomes an additional approach to climate change adaptation in Malawi noting that most rice growing areas are impacted by climate change. The 13 SSRs markers used are associated with traits linked to drought tolerance in rice (Radmad et al., 2015). For examples, SSRs RM201, RM72, RM410, RM242 and RM328 were associated with root length characteristics (Qu et al., 2008; Lang and Buu, 2008; Courtois et al., 200); and RM28166, RM472, RM260, RM219 were associated with maximum root length, root dry weight and grain yield (Dixit et al., 2012; Kanbar and Shashidhar, 2011 and the remainder were associated with QTLs linked to drought tolerant traits (Yue et al., 2006). It is therefore, expected that accessions amplified by these SSRs have traits associated with drought tolerance on one or more loci.

This study recommends the following accessions for field drought tolerance screening: cultivated accessions include MW1155, MW5266, MW5283 MW1700, MW1804, MW1825, MW39, MW4700, MW5054, MW5062, MW5088, MW5096, MW5106, MW5111, MW525,

MW526, MW5269, MW5271, MW5285, MW5352, MW5362 and wild species accession of barthii (MW1753), having being amplified at more than 10 loci by the above SSRs.

Cultivated accessions MW1686, MW1823, MW4702, MW4703, MW4712, MW4729, MW4757, MW5059, MW5075, MW5076, MW5079, MW5080, MW5086, MW5094, MW5100, MW5104, MW5105, MW5106, MW5111, MW5112, MW5113, MW524, MW5256, MW5274, MW5276, MW5277, MW5278, MW534, MW555, MW5560 and wild species accession of barthii (MW4867) were amplified at 9 loci. These should also be considered for field drought tolerance screening whenever resources are available as they could also be linked to one or more drought tolerant traits.

5.6. CONCLUSIONS

There are drought tolerant genes in the Oryza accessions included in this study. This has been deduced by the detection of the alleles amplified by SSRs linked to drought tolerant traits. When drought tolerant alleles were mapped, it was observed that drought prone areas of Malawi (the Lower Shire Valley and Lake Shore areas) had high number and frequency of occurrence of drought tolerant alleles. The genotyped accessions exhibited some level of genetic variation providing an opportunity for selection. What is more interesting is that these are locally adapted genetic material and will not require adaptation selection. The percentage of variation within individual accessions is a high indication of low level of relatedness but high heterozygosity that will allow segregation in early generation upon crossing. Although the recommendation for field screening is on 22 accessions with the highest amplified frequency, ideally 53 accessions should be considered for further field screening whenever screening infrastructure is available. These accessions were amplified at ≥ 9 loci indicating some possibility of drought tolerant association. However, we recommend close collaboration

with Africa Rice Institute during the screening process of these accessions to benefit from their rich experience of Africa Rice Institute in rice breeding and infrastructure.

Lastly, this study has highlighted the need to do pre-breeding studies on the germplasm conserved at seed genebanks in order to promote its use without which it becomes not relevant to national breeding programmes. Such kind of studies are therefore recommended to other crops whose germplasm is conserved in national genebanks. Discovery of elite germplasms with novel traits will enhance the national breeding programmes in respective crop commodities and promote use of the same by breeders.

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CHAPTER 6

GENERAL DISCUSSIONS

About 100 food crops are known to be cultivated in Malawi but only a few (maize, rice, beans soya bean, cowpeas) are cultivated on large scale. Small-scale farmers are the main food producers in Malawi but their capacity to produce is limited by a number of factors that include small land holding size, low farm inputs and poor rains (that is associated with floods and/or droughts). Due to low yields, Malawi experiences food shortages every year in some parts of the country. The carryover effect of this has been an increased rate of deforestation due to charcoal burning and tree logging as alternative sources of income and living when farming fails. Overexploitation of this nature has a negative bearing on biodiversity and the associated ecosystems. Malawi's biodiversity offers a number of ecosystem services (Environmental Affairs Department, 2015) and benefits to a rural people so loss of these means loss of these valuable ecosystems services. Forests provide habitat for CWR and we now know that 73% of the diversity of priority taxa analysed is passively conserved in protected areas (PAs), most being Forest Reserves (Khaki Mponya *et al.*, 2020). Conservation of CWR in Malawi has potential to offer the following:

- Conservation of CWR potential for crop improvement in such traits of economic importance as drought tolerance, resistant to pests and diseases can help improve crop productivity in Malawi. Increased crop yields means increased food availability and cash income from the surplus.
- Rural people directly use CWR as wild food. Some CWR are a source of income to farmers; they are harvested and sold in local markerts by farmers. Boosting local

people's economy has got a positive bearing on biodiversity. Their conservation will therefore enhance ecosystem services they offer to the community.

- Enhancing conservation of CWR *in situ* will go along with habitat management and hence ensure conservation of other forms of biodiversity and maintain our ecosystem.
- *Ex situ* conservation of CWR will increase availability of these to users and facilitate use in crop improvement hence improved crop productivity.

6.1 CWR occurrence and diversity

In Malawi, the diversity of CWR is distributed across the country with the Southern Region having the highest number of taxa followed by the Northern Region and Central Region (Chapter 2) for the taxa analysed. The potential distribution exhibited similar taxa diversity pattern as observed distribution. The reason for this could be that most collectors and botanists target areas of diversity during collection missions and it is therefore likely that, the diversity of taxa not included in this study could exhinit similar diversity parten if it were analysed. About 90% of the taxa in the South and Northern Region occur in PAs while most diversity in the Central Region occurs outside PAs, in cultivated land and some in towns and close to the developed areas. These taxa need to be collected and conserved ex situ for their continued survival before their habitat is destroyed. About 66% of CWR taxa diversity hotspots are captured in 36 PAs (Chapter 3) but the diversity that could be considered stable and cost effective for *in situ* conservation occurs in the PAs of Zomba, Mulanje and South Viphya Forest Reserves, Nyika, Kasungu and Lengwe National Parks. It was noted that Malawi shares much of its CWR diversity with the SADC region. For instance, out of the 466 taxa of CWR occurring in Malawi, >30 % also occur in other countries of the SADC region. The diversity in the national inventory captures taxa that are a priority for conservation in Malawi. These taxa also included those, which underpin the SADC region and global food security (Allen *et al.*, 2017; Vincent *et al.*, 2013) putting Malawi at an important position of germplasm exchange within the SADC region and globally.

6.2. Previous conservation status of CWR in Malawi

CWR did not receive significant conservation attention in Malawi previously, both by the MPGRC and other related conservation institutions, primarily due to information gaps in their occurrence status, their geographical distribution, and their conservation gaps both *in situ* and *ex situ*. The country had no checklist and inventories that could guide their systematic conservation review (Chapter 2). Although the occurrence of CWR were reported in country reports such as the State of Plant Genetic Resources for Malawi (FAO, 1996 and FAO, 2008), this information was sketchy and unquantified making it difficult for CWR conservation planning. MPGRC made several efforts to organize general collections of CWR in Malawi but almost 80% of these missions failed due to lack of the information on what CWR occur in Malawi, issues of identification (failure to differentiate CWR from other weedy species) as well as general lack of information about their distribution and phenological cycle. This greatly limited conservation of CWR *ex situ* and *in situ*.

Of all the known CWR occurring in Malawi, MPGRC was only able to collect and conserve in its seed genebank three *Oryza* wild species (*O. punctata, O. longistaminata* and *O. barthii*) and a few samples of unidentified *Vigna* species. CGIAR specialists guided the first joint collection missions for *Oryza* species especially from International Rice Research Institute (IRRI) whose research mandate is focused on rice. Such collaboration empowered MPGRC and guided it with subsequent collections. There was almost nothing done under *in situ* conservation, as the MPGCR was limited in capacity to undertake such an initiative.

Reporting and monitoring of the CWR diversity was practically impossible due to such information gaps. It was also difficult to report on the same to the biodiversity monitoring bodies. Most of the country's reports on CWR conservation relied on experts' knowledge from the Departments of Forest and Parks and Wildlife and this information was seldom updated due to lack of inventories from such Departments, relying on all-inclusive periodic wild species surveys that might capture some CWR in the wild.

In terms of policy and strategies for conservation, issues of CWR and conservation of PGR in general are barely mentioned in the National Agricultural Policy (NAP) (Malawi Government, 2016), however, strategic conservation actions are included in NBSAP (Environmental Affairs Department, 2015). NBSAP covers all forms of biodiversity as a National working document for biodiversity management in Malawi and does not provide a detailed roadmap of conservation of CWR as such still leaves issues of CWR conservation and PGR hanging. In view of this, MPGRC with support from FAO–TCP programme engaged with a consultant to develop a national PGR Conservation strategy that aimed to guide conservation of PGR of selected crops (DARS, 2014-draft) as way of promoting use of PGR in crop improvement.

This strategy was not implemented due primarily to lack of integration of the strategic actions and the prevailing PGR use priorities in breeding. Additionally, the implementation of the strategy was derailed by lack of comprehensive awareness of the importance of the strategy on food security and why partners, especially breeders, were key to achieving the strategic actions included in the strategy. The other bottleneck to its implementation was lack of involvement of stakeholders in problem analysis. More also in this draft strategy, there was no inclusion of how CWR were to be systematically conserved and later on used in crop improvement. However, the underlying cause was lack of information about CWR taxa occurrence, distribution, and the state of their conservation in the wild that could reveal their conservation gaps. This PhD study was therefore designed to provide such information to guide the systematic conservation of CWR.

6.3. Project achievements and contributions to sustainable conservation of CWR in Malawi

The approach to this study aimed at addressing the information gaps on the status of conservation and use of CWR in Malawi, which was the limiting factor for use and systematic conservation of CWR in the country. The information generated aimed at informing conservation strategy formulation as well as policy decisions at national level and productive for an avenue of collaboration at regional and global levels. These steps in addressing the above challenges followed those outlined in the Interactive Toolkit for CWR Conservation Planning (Magos Brehm *et al.*, 2017).

6.3.1. Development of a national CWR inventory

The focus was to develop a working list of priority CWR taxa that could be conserved under limited resources. The first step was the development of a national CWR checklist which was further prioritized by a team of national stakeholders based on conservation needs for Malawi (details of the procedures followed are described in Chapter 2), namely conservation status, potential use for crop improvement and threats levels. The CWR checklist, which comprised of 446 taxa and the prioritized inventory that included 277 taxa were developed for the first time in Malawi. The checklist and the inventory will guide the systematic conservation of CWR in Malawi as already demonstrated. These documents are now available for use by the national conservation authorities advocating for CWR conservation. Additionally, Malawi has

benefited from this study already as the CWR inventory has already guided two CWR collection missions, which would not have been possible previously due to lack of information. *Ex situ* collections provide a back-up that would prevent loss of CWR in the wild.

The Darwin Initiative SADC CWR project "Bridging agriculture and environment: Southern African crop-wild-relative regional network" initiated in April 2019 and expected to end by April 2022 is one of the good examples of direct benefit from this PhD study. The project aims at promoting active and sustainable conservation of CWR in Malawi and the National Checklist and Inventory provide useful guide to active conservation of the same. Another example of the project that has benefited from this study is the Shire Valley Transformation Programme (https://www.afdb.org/en/documents/document/malawi-shire-valley-

transformation-program-phase-1-svtp-1-appraisal-report-107435), a National Programme with a Natural Resources Management Component aiming at managing the catchment area of the Shire River, one of the perennial water bodies in Malawi. This project has an objective of conducting IUCN Red List assessments of species at national level and MPGRC is privileged to include CWR taxa in these assessments. This has been possible because of the availability of the National Checklist and Inventory without which, it would not have been possible to participate in this exercise.

6.3.2. In situ and ex situ conservation gaps analyses of CWR

The aim of this study was to identify *in situ* and *ex situ* conservation gaps of the priority taxa with the aim of proposing sustainable conservation measures. The study developed maps on the observed and potential taxa distribution and these will guide future *ex situ* and *in situ* conservation actions. The potential taxa distribution area closely resembled taxa observed distribution area suggesting that the geographic coverage of CWR taxa in Malawi is relatively

more than what was observed (Chapter 3 and Khaki Mponya *et al.*, 2020). Priority sites for *in situ* conservation (potential for genetic reserves) were identified as Zomba, Mulanje and South Viphya Forest Reserves and Nyika, Liwonde and Kasungu National Parks having large number of priority taxa. Hotspots outside PA where non-PA reserves could be designated include areas in Dedza and Ntcheu Districts. Priority sites for *ex situ* collections include hotspots in Dedza and Ntcheu Districts and the six PAs mentioned above.

Collection from these sites will ensure a broad range of taxa is captured and a back-up of the diversity present in PAs is maintained *ex situ*. This study was so significant for Malawi because it generated data that was not available and enable Malawi to engage with national, regional and global partners in conservation of CWR. The information generated by this study is already in use for conservation planning in Malawi as the country plans to develop its first National Conservation Strategy for CWR. This information will help in defining effective conservation measures for the threatened taxa, provides a platform for networking with other stakeholders as well as help engagement with policy makers and partners in conservation of CWR and plant diversity in general. Further, complementarity analyses were undertaken on a network of PAs to estimate the amount of ecogeographic diversity already passively conserved in these PAs.

The emphasis for this study was to encourage conservation of a broad range of CWR diversity in their natural environment that allows for taxa continued evolution and to conserve taxa with recalcitrant seeds that could not be conserved under *ex situ*.

Taxa diversity representativeness analyses were also conducted in this study using the CAPFITOGEN Tools (Para-Quijano *et al.*, 2016). The aim was to identify the gaps in representativeness of the ecogeographic diversity of CWR that is being conserved under *ex*

situ at MPGRC and international gene banks (Chapter 3). Through these analyses, gaps in ecogeographic diversity representativeness of the taxa conserved *ex situ* were noted and actions to bridge the gaps were proposed. Areas and taxa for *ex situ* collections were identified and prioritised.

In situ conservation gaps were identified and measures to address the gaps were proposed. An ecogeographic land characterization (ELC) map was created for Malawi which divided the country into 27 ecogeographic zones based on edaphic, geophysical and climatic variables. These zones were further classified into four categories based on their frequency on the ELC map as well as based on the frequency of collections done per ecogeographic zone. The ELC map and classes helped to identify the most common CWR ecogeographic diversity in Malawi, the ecogeographic diversity that was collected and the diversity that is rare and not represented under *ex situ*. For instance, the study found out that ELC zones 0 and 8 have relatively high representation of their ecogeographic diversity in MPGRC and international genebanks than the rest of the ecogeographic zones. The ELC map (Chapter3) provides us with a reliable tool for collection of non-represented taxa and rare ecogeographic diversity can be easily targeted (Parra-Quijano *et al.*, 2011).

6.3.3 Climate change impact and conservation of CWR in Malawi

This study focused on assessing the future impact of climate change on richness and distribution of the priority taxa noting that climate change is one of the drivers of biodiversity loss in Malawi (Malawi Government, 2010). The study estimated the medium- and long-term impact of climate change on priority CWR taxa to inform policy decisions. The projection period is in line with most sectoral planning in Malawi and was selected to allow for easy mainstreaming and monitoring of the proposed conservation actions. Incorporation of

recommended actions to address climate change impact on CWR in Malawi in the most practical period, allows for easy evaluation and revisions of the strategies and management plans that guide conservation of priority CWR.

The distribution of 44 priority CWR taxa were modelled and these included taxa that had enough occurrence data. Modelling was done in MaxEnt (Maximum Entropy), an algorithm for ecological modelling of species. A combination of edaphic, geophysical, and bioclimatic variables specific for the occurrence and adaptation of each taxon were used in the modelling process. Nevertheless, due to lack of environmental information from some sites where the taxa were observed, only 14 taxa had stable and transferable potential distributions models and therefore climate change impact was modelled on these taxa only.

Climate impact was modelled with an ensemble of three (3) greenhouse gas general circulation models (GCMs); bcc_csm1_1_m, csiro_access1_0 and gfdl_esm2m using future bioclimatic variables available on www.ccafs-climate.org/data. Projections were made for 2030, 2050 and 2070 based on two representative circulation pathways (RCPs) 4.5 (Smith and Wigley, 2006; Clarke *et al.*, 2007; Wise *et al.*, 2009) and 8.5 (Rao and Riahi, 2006 and Riahi *et al.*, 2007). The GCMs used are those recommended for climate modelling studies in Southern Africa and were selected based on their performance on previous climate change studies conducted in Malawi (Chibwana *et al.*, 2014; England *et al.*, 2017, Mittal *et al.*, 2017). Taxa outside PA will be most impacted come 2070. The most impacted taxa will on average lose 75% of their area of occupancy (AOO) by 2070 under both climatic scenarios considered for this study. Taxa such as *Coffea ligustroides*, *Vigna unguiculata* (wild species) and *V. vexillata.* subsp. *angustifolia* will lose more than 80% of their AOO. Based on IUCN criterion AC3 for the threat assessment, these taxa will fall in the Critically Endangered threat category.

All these taxa have potential for crop improvement (Chapter 2 and Mponya *et al.*, 2020) and therefore must be targeted for *ex situ* collections. However, it was projected that PAs will be least impacted with climate change as shown by the continued projections of hotspots across the projection period.

Additionally, taxa turnover was calculated for the projected period under limited migration and non-migration scenarios. More taxa migration is expected in 2070 for the projection period under both limited and non-limited migration scenarios. Habitats that were most hit by climate change impact were noted and suggestions to safeguard the priority CWR occurring in such sites were provided. Sites least hit by climate change were also noted and sites potential for genetic reserve designation were proposed. Complementary PAs that will be suitable for the conservation of the priority taxa in the next 10–50 years were identified and actions to modify their management practices to adapt to forth coming changes due to climate change impact were suggested in Chapter 4.

6.3.4. Mapping drought tolerance in cultivated and wild Oryza accessions

The final part of this study was to demonstrate the potential use of CWR in crop improvement by identifying drought tolerant alleles in the *Oryza* accessions conserved at the MPGRC as a way of justifying their immediate conservation. This study employed use of DNA molecular technique, Polymerase Chain Reaction (PCR), and use of DNA molecular markers, simple sequence repeats (SSRs), to identify presence of drought tolerant alleles among cultivated *Oryza* accessions and accessions of three wild relatives of *O*ryza (*O. barthii, O. longistaminata* and *O. punctata*). The distribution of these alleles were mapped using GIS tools to aid development of core collections based on the ecogeographic distribution maps. *Oryza* is the second most important cereal crop in Malawi that has its relatives occurring in

the country. Rice production has largely been affected with erratic and insufficient rainfall in Malawi and drought is one of the major production constraints of rice in Malawi. In the past, most farmers relied on rains and supplemental irrigation. But due to insufficient rainfall, there is a drop in water levels and this reduced the amount of water supply to rice fields. For farmers with access to irrigation facility (those involved in rice irrigation schemes), water pumping costs have become unaffordable as more power is required to pump water for rice growing than previously. Being a crop of economic and food security importance in Malawi, rice has a number of value chains with a growing industry. Therefore, in order to boost its production, research to develop drought tolerant varieties is underway. The MGRC holds rice germplasm including three CWR collected from across the country and these provide potential source of breeding materials. To facilitate the use of conserved germplasm, there was a need to genotype for drought tolerance. Collections used in this study were sampled across Malawi where Oryza is cultivated and where wild species occur. This facilitated establishment of genetic relatedness (diversity) of cultivated rice with its wild relatives in Malawi as well as formation of trait specific core collections of rice germplasm.

DNA was extracted from immature leaf samples of 130 accessions of *Oryza* species. PCR was run on the DNA extracted from the samples. With the aid of 13 SSRs, loci linked to drought tolerant genes were detected. Diversity analyses on the samples were done based on this data.

6.4. Project limitations

This study established a baseline of CWR research in Malawi and being the first time to study CWR in Malawi, the project required collation of substantial amount of data. Some data that could be useful were not included in some studies like the conservation gaps and climate change analyses due to in adequate georeferencing information. Nevertheless, the study used data from all potential data holding institutions (those holding plant data for Malawi) and this provides a true reflection of the conservation status of CWR in Malawi. The CAPFITOGEN tool GEOQUAL was also utilized to improve the quality of the data used in this study. However, follow up studies should take advantage of the recommendations from this PhD project on adding more data through field surveys (Chapter 3 and Khaki Mponya *et al.*, 2020).

Another area considered to be important in a different way is genotyping. Here, the main challenge was the choice of the software used to perform the actual genotyping analysis. It was challenging to download and install the software for the analysis of the genotyping outputs. The software required specific configuration of the computer to enable its installation. Due to this another analytical software was used. Nevertheless, the DNA SSRs markers used were reported heterozygous, with high levels of diversities in a number of studies (Ramadam *et al.*, 2015; Vanniarajan *et al.*, 2012; Lapitan *et al.*, 2007) and their selection was well guided. Therefore, the data generated from this genotyping will be useful to breeders and will guide conservation and utilization of this germplasm in Malawi and beyond. Since the data generated is huge, it requires sufficient amount of time to analyze it and fully utilize the outputs.

6.5. Recommendations from this PhD study

The recommendations suggested from this PhD study are not the end in themselves but rather recognize the role of stakeholders at every level of conservation planning. Their implementation must therefore consider stakeholders inclusion in decision-making. Such recommendations include:

- <u>Development of a national strategic and action plan for the conservation and utilization</u> of <u>CWR (NSAP)</u>. The information generated by this study is sufficient to develop NSAP and guide the management of the priority CWR populations. Through this study,
 - a national CWR checklist and a national inventory were developed, with national stakeholders defining the criteria for prioritization see (Chapter 2),
 - diversity analyses were carried out on the priority taxa, *in situ* and *ex situ* conservation gaps analyses were performed (Chapter 3),
 - climate change impact analysis on species distribution and richness was carried out s well (Chapter 4) and
 - genetic analysis was performed on some priority taxa to promote use (Chapter
 6).

The details of all these analyses form the content of the technical background document that accompanies the NSAP (Magos Brehm *et al.*, 2019) and therefore MPGRC and stakeholders can adapt this.

- <u>Secure collection of vulnerable, threatened and endemic taxa for *ex situ* backup and taxa with potential for crop improvement.</u>
 - Endemic taxa. These include Coffea mufindiensis Bridson subsp. pawekiana
 (Bridson) and C. arabica L. wild types, Eragrostis fastigiata Cope., E. sylviae
 Cope. and Plectranthus mandalensis Baker. These are endemic to Southern
 Region of Malawi. C. mufindiensis Hutch ex Bridson subsp. lundaziensis

Bridson and *Setaria grandis* Stapf are near endemic and were observed in the Northern Region of Malawi. Collectors should use distribution maps and potential distribution maps to survey new locations.

- Threatened taxa. These include *Coffea arabica* L. (wild types) and *C. salvatrix* Swynnerton & Phillipson assessed as Endangered (EN), *Prunus africana* (Hook.f.) Kalkman, and *C. ligustroides* S. Moore and *Oryza longistaminata* A. Chev. & Roehras as Vulnerable (VU), *Siphonochilus aethiopicus* (Schweinf.) B.L.Burtt assessed as Critically Endangered (CR), and rare taxon *O. punctata* known to occur in Lengwe National Park.
- **Priority sites for collections.** Hotspots outside protected areas should be targeted first as these taxa will be impacted most by climate change. These include sites in Dedza and Ntcheu Districts and the boundary between Ntchisi and Dowa Districts and then rare adaptive environments (ecogeographic zones) and ELC map in Chapter 3 could guide this process.
- <u>Active in situ conservation</u>. Designate *in situ* reserves in Zomba, South Viphya and Mulanje Forest Reserves and Lengwe, Kasungu and Nyika National Parks and lobby for evaluations of the management plans of these sites. The first targets should be Zomba Forest Reserve (representing warm and cold conditions), Nyika (representing cold and humid environment) and Lengwe National Parks (representing hot and dry environment) as these have relatively wide taxa diversity and the diversity in these PAs represent broad range of ecogeographic diversity being located across Malawi. Considerations should be paid to the following during the evaluation about whether genetic reserves could be implemented in these sites:

- Taxa populations and its distribution within the reserve biosphere.
- Size of the reserve biosphere including sketch map versus taxa populations' structure, distribution and the diversity.
- Current standards of the management practices of the biosphere reserves.
- Formulation of national multi-stakeholders committee that will oversee the designation and monitoring of the populations.
- Initiate negotiations for border expansion for the suitable complementary PAs whose diversity spans beyond the set boundaries and this should only be considered if the diversity of CWR in question is not conserved within the borders of the PAs.
- <u>Develop a germplasm use strategy.</u> To promote use of taxa in crop improvement the following should undertaken:
 - Plan for retrieval of the *ex situ* collections of the taxa held at international genebanks but have no duplicates at MPGRC. These should be duplicated with the SADC Plant Genetic Resources Centre (SPGRC) as well.
 - Make agreements with International Consultative Group on International Agricultural Research (CGIARs) such as the African Rice Center and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to make crosses of the potential taxa and the elite parents. For instance, the agreement with the African Rice Center would be centered on making crosses of drought tolerant lines between *O. barthii* and cultivated rice varieties and

bacterial blight and brown hopper resistant lines by making crosses between *O. punctata* and cultivated rice varieties. Agreement with ICRISAT could be on developing *Striga asiatica* resistant sorghum lines by crossing between *S. arundinaceum* with popular sorghum varieties in Malawi. These can then be sent to breeders to use them in improving such traits.

• Also check if the selected PAs meet the minimum standards for genetic reserves (Iriondo *et al.*, 2012)

6.6. Further studies

The amount of energy, resources and commitment invested in this project cannot be undermined looking at the type and nature of the work done. However, time and resources are always a limitation and might, in one way or the other, have limited some work. As such, there are a number of suggestions for further studies:

• Field surveys to validate the occurrence of priority CWR taxa

As mentioned earlier, some data was left out due to insufficient amount of georeferencing information but from the potential distribution modelling, it was observed that there was a lot of diversity of CWR in Malawi. This means that there is more chance to collect more data from these potential areas. Therefore, field surveying should be one of the areas to extend in order to generate more data. Field surveys will form part of the ground truthing of the current status of the taxa in the wild and the occurrence data to be collected will be useful for red listing of the priority taxa as it was discovered that some taxa have restricted geographic distribution and others are threatened (Mponya *et al.*, 2020). Taxa with fewer populations (1–4) in both PAs and

outside the PAs should also be assessed in order to establish their current status and design sustainable measures for their conservation.

• IUCN red listing

The project revealed that most of the populations of priority CWR occur in non-PA raising concerns for their survival (Chapter 3). In addition to this, low populations were noted for the taxa that occur in PAs. Further, there has been a dramatic change in land use for the past three decades in Malawi. The country has seen an increase in agricultural production as well as infrastructural development hence destroying several habitats that could be potential for occurrence of priority CWR. Further, threat assessments will help validate the status of CWR in Malawi especially for those reported as threatened at global level and endemic to Malawi.

• Predictive characterization

Predictive characterization was not one of the recommendations from the studies conducted, but can be used as a preliminary method for identifying potential germplasm for breeding. These can then be validated using genetic studies. Predictive characterization could also be useful comparative study with the molecular analyses and help us validate our results.

Conduct economic benefits analysis of conserving CWR by farmers

It is always difficult to advocate for *in situ* conservation of CWR when you do not know if there will be economic benefits, whether directly or indirectly, for the landowners. This exercise, if conducted, will enhance conservation of the priority

CWR in the agricultural landscape and encourage farmers to continue conserving these in their landscapes and on farms.

• Awareness creation of the value of CWR to farmers, breeders, policy makers, conservationists and other stakeholders.

Understanding the value of CWR will enhance its conservation and use. This can generate interests among users to use them in their breeding programmes as well as among donors who could be interested to support their conservation and use. This should involve the following:

- Document traits of economic importance associated with the priority CWR;
- Put use case studies together to demonstrate how CWR were used in crop improvement programmes and their contribution to food security, nutrition, health, and economy in general. For example, a case like that of Mexican corn, potato and tomatoes which benefited from genes from the wild relatives might be illustrated;
- Engage with breeders to promote use of these CWR as well as national stakeholders to start recognizing the value of CWR in both ecosystem based management and food security;
- Constantly engage with PA managers, local and international partners to lobby for revisions of PA management plans. In Malawi, three PAs are currently revising their management plans and this could be an opportunity to advocate for the conservation of CWR in these areas.

6.7. Conclusion

This PhD project contributes to the development of the national strategic action plan for the conservation and use of CWR in Malawi, which MPGRC is facilitating. Each of the studies included respond to specific strategic actions to be included in the conservation strategy. Proposed strategic actions are aligned with those outlined in NBSAP (Environmental Affairs Department, 2015). This then contributes to the achievement of global biodiversity conservation targets (Aichi targets). This PhD project is significant to Malawi as it managed to address most basic and preliminary information gaps on CWR conservation in the country. Further, the studies have created an opportunity for engaging with other stakeholders on CWR conservation having now known of the conservation status of CWR in Malawi.

It has also helped us ddefine further actions that are required. Largely, it has created a networking platform that was previously missing. With such information and findings, this has put conservation of CWR in Malawi a step further.

Malawi should take advantage of the Darwin Initiative SADC CWR project "**Bridging agriculture and environment: Southern African crop-wild-relative regional network**" to mobilize additional field data, engage with other stakeholders to develop the much-needed CWR conservation strategy and advocate for active *in situ* conservation. Malawi should also take advantage of the capacity enhancement training on CWR conservation planning offered by experts in the project to build its capacity in CWR conservation.

As a model, Malawi has now started with the designation of two potential PAs as genetic reserves for priority CWR taxa. For this to be successful, MPGRC needs to work in close collaboration with PA managers and utilize the existing conservation network-the Malawi

Biodiversity Information Management Forum (BIMF)-that includes all nature conservation and biodiversity data users' institutions to strengthen its capacity.

Additionally, promoting use of CWR is key to its sustainable conservation as such strengthening and maintaining user interface is crucial in creating the impact. It is therefore, recommended to keep on engaging CWR users at all levels.

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