

**GENETIC DIVERSITY AND SCREENING OF COWPEA (*Vigna unguiculata* L.
Walp.) GENOTYPES FOR DROUGHT TOLERANCE IN SOUTH AFRICA**

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ABSTRACT

A survey was carried out to assess the farmers' production constraints, traits and preferred cowpea varieties. A semi-structured questionnaire was used in a survey in Buhera District, Zimbabwe, in March and April of 2018. Women farmers dominated the survey as they were 52% of the surveyed population, while men occupied 48% of the total population. All participants concurred that cowpeas were used for domestic consumption. Eighty-three percent of the farmers cited shortage, unavailability, and cost of fertiliser, 16% of the farmers acknowledged that they do not have access to quality seed, and 1% cited labour as the major constraints in cowpea production. Farmers ranked heat (86%), drought (10%), and soil fertility (4%) as the most important abiotic factors. Ninety-one percent of the farmers' ranked rust as the major disease, while 2% ranked storage rot, 1% ranked anthracnose, and 1% ranked downy mildew. Eighty-one percent of the farmers ranked aphids as the main pests, while 3% ranked thrips, 3% ranked legume borers, and 2% ranked pod borers. Fifty-two percent of the farmers preferred varieties that are resistant to diseases such as rust, whereas 48% were not concerned about diseases. For qualitative traits, 50% of the farmers had no specific colour preference, 32% preferred white colour, 14% brown colour, 3% red colour, and 1% tan colour. Ninety-four percent of interviewed farmers were not concerned about the pod shape, 3% preferred the kidney shape, 2% preferred the spherical shape and 1% preferred the globular shape. Ninety-nine percent of farmers agreed that they needed high yielding varieties per unit area and only 1% were unsure. For quantitative traits such as grain size, pod size, plant height, and head size, the preferences of farmers varied. Forty-four percent of the farmer respondents preferred larger cowpea grains, while 56% were not concerned about the size of the grain. A paltry 2% of the farmers were interested in pod size, while 98% did not regard it as important. Thirteen percent of the farmers were interested in climbing varieties, while 87% considered high grain yield as of the utmost importance. The top ranked accessions were CBC1, IT 18, and "*Chibundi Chitsvuku*", respectively, while the least ranked was "*Kangorongondo*". The survey showed that there is a need to breed for both biotic and abiotic factors, such as drought and moisture stress, as well as against pests and diseases. In order to assess the genetic diversity and population structure among cowpea accessions, diversity arrays technology (DArTSeq) genotype by sequencing technique was used. A total of 85 cowpea accessions (45 from the International Institute of Tropical Agriculture [IITA], 25 from South Africa, and 15 from Zimbabwe) were investigated in this study using 284 single-nucleotide polymorphisms (SNPs). Fifty-one percent of SNPs were polymorphic across the 85 accessions and fulfilled the selection

criteria. The genetic distance, estimated based on Nei's index among genotypes, ranged from 0.14 to 0.44, with a mean value of 0.35. The polymorphic information content value ranged from 0.024 to 0.50, with a mean value of 0.25. Twenty-six percent of the genotypes had genetic diversity values greater than 0.40, suggesting that the genotypes were moderately genetically diversified. A high gene flow (N_m) of 4.89 was observed between Zimbabwean and South African accessions indicating the high germplasm exchange among the neighbouring countries. The analysis of molecular variance revealed highly significant variation among individual accession and low variation within individuals. The accessions showed significant ($p < 0.001$) but low levels of differentiation among the geographic regions. Cluster analysis of the 85 accessions generated by the unweighted pair group method with arithmetic mean (UPGMA) procedure clustered the accessions into three distinct groups. The clustering patterns showed that accessions collected from the same geographic regions tend to cluster in the same groups. SNP data analysis indicated the existence of high levels of genetic diversity among cowpea accessions collected from southern Africa and Nigeria. However, the low genetic variation within individuals suggested the need to further widen the genetic base of the crop. Molecular data should be backed by morphological data to link the SNP markers that are associated with desirable agronomic attributes, such as high tolerances to biotic and abiotic stress factors. Sixty cowpea genotypes were screened for seedling drought tolerance in screen houses. Of these, 33 accessions were from the IITA in Nigeria, 19 accessions were from the Agricultural Research Council – Grain Crops in South Africa, and eight accessions were from smallholder farmers in Buhera District, Zimbabwe. Bartlett's test of sphericity was significant at $p < 0.05$, while the Kaiser-Meyer-Olkin measure of sampling adequacy was 77, indicating sufficient items for each factor. A principal component analysis (PC) showed that number of pods (NP), seeds per pod (SP), survival count (SC), pod weight (PWT), and stem wilting in week one (WWK1) had the most significant contributions to genetic variability in cowpea accessions to drought tolerance, as well as yield after stress imposition. Based on the PC, accessions IT 07-292-10, RV 343, and IT 95K-2017-15 had the maximum variability for NP, SP, SC, PWT, and WWK1 after drought imposition. There were significant differences among most drought-related traits at the seedling stage with the exception of environment \times genotype on days to emergence (DTE), stem greenness at week 1 (SGWK1) after the imposition of water stress and also among replicates in terms of DTE. A total of 37 cowpea accessions from both screen houses were found to be tolerant to drought, while 23 were susceptible. From the results, it was observed that the accessions had similar responses to drought. The findings of the study provided a useful tool for screening and determining the accessions that are drought tolerant and susceptible at

the seedling stage. Finally, it was necessary to conduct association mapping for drought tolerance at the seedling stage and yield-related traits of cowpeas. The cowpea accessions were analysed for their possible population structure using STRUCTURE 2.3.4, and the peak of delta K in the greenhouse showed the existence of seven sub-populations, whereas the peak of delta K in the glasshouse indicated the presence of six sub-populations. One SNP marker, 14083649|F|0-9 was associated with number of pods (NP) with a p value <0.001 . Fifty SNP markers were associated with pod weight (PWT) at $p < 0.001$. Four SNP markers, 14074781|F|0-16, 100047392|F|0-36, 14083801|F|0-28 and 100051488|F|0-49 were associated with ASPD at $p < 0.001$. SNP markers, 14074781|F|0-16, 14083801|F|0-28 and 100051488|F|0-49 were associated with PL at $p < 0.001$. Five SNP markers, 100047392|F|0-36, 14083801|F|0-28, 100072738|F|0-34, 14076881|F|0-49 and 14076881|F|0-49 were associated with PWDTH at $p < 0.001$. The major difference that was observed in association mapping in the two environments was due to temperature variations. The 67 SNP markers identified can be used in cowpea molecular breeding to select for AVSPD, NP, PL, PWDTH, PWT, and RR through marker assisted selection (MAS).

DECLARATION OF INDEPENDENT WORK

DECLARATION WITH REGARDS TO INDEPENDENT WORK

I, GABRIEL VUSANIMUZI NKOMO, identity number _____ and student number _____, do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree DOCTOR TECHNOLOGIAE: AGRICULTURE, is my own independent work; complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

SIGNATURE OF STUDENT

DATE

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DEDICATION

I dedicate this thesis to my beloved wife Beatrice Fadzai Kungwara and my late father Hlupani David Nkomo.

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CHAPTER 1

1.1: Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important food and fodder legume crop in the tropical and sub-tropical climates of the Middle East, Asia, Southern Europe, Africa, and the Americas (Singh *et al.*, 1997). Global cowpea production is cultivated on about 14.5 million ha, with an annual production of over 7.4 million t (FAOSAT, 2017). The top three producers of cowpeas in Africa comprise Nigeria (2.6 million t), Niger (950,000 t), and Burkina Faso (405,000 t) (FAO, 2017). Sub-Saharan Africa dominates in cowpea production, with 96% (4.9 million t) of the total produce (Nedumaran *et al.*, 2015). In southern Africa, by area size, South Africa and Zimbabwe are among the top producers of cowpeas, and production is mainly for subsistence in marginally low-input environments. In Zimbabwe, cowpea is produced in Buhera, Mudzi, Rushinga, and other low-lying districts (Sparg, 2017), whereas in South Africa, it is produced in Limpopo, Mpumalanga, North West, and KwaZulu-Natal.

Cowpea is an important grain legume and it is used for various purposes, ranging from medicinal, dietary, and as a green manure in crop production. The leaves can be cooked while green or in dried form. Immature pods and dried seeds can also be used as vegetables. This crop can grow in harsh conditions considered marginal for crops such as maize (da Silva *et al.*, 2018). It has the ability to fix atmospheric nitrogen and withstand drought and water logging. Cowpea is an excellent cover crop and can add nitrogen to the soil and improve soil structure. Less than 500 mm of annual rainfall can raise the crop to maturity (Boukar *et al.*, 2019). In some areas where annual rainfall is high, planting is performed to coincide with the peak period of rainfall during the vegetative phase or flowering stage, so that pod-drying can take place during dry weather. Adequate rainfall is important during the flowering/podding stage. However, cowpea is adapted to withstand serious moisture stress by limiting its growth (especially leaf growth) and reducing its leaf area by changing its leaf orientation and closing its stomata (Freitas *et al.*, 2017). Flower and pod abscission during severe moisture stress also serves as a growth-restricting mechanism. Currently, on-farm grain yields in Africa reach only 10%–30% of their biological yield potential, primarily due to insect attacks, disease, and drought (Okonya and Mass, 2014).

Although cowpea is considered as being more drought tolerant than many other crops, its productivity is negatively affected by prolonged droughts and high temperatures attributed to

the effects of climate change (Hall, 2012). Some climate models have predicted the increased severity and frequency of droughts in Sub-Saharan Africa (Mwale *et al.*, 2017). The improvement of drought-tolerance in crops is one of the strategies to cope with the changing climate. When cowpea is often subjected to drought stress in both the seedling and terminal growth stages; this causes a substantial reduction in grain yield, as well as biomass production (Olajide and Ilori, 2017). In susceptible genotypes cellular integrity is lost more rapidly leading to premature cell death and subsequently tissue death under drought stress at seedling stage (Muchero *et al.*, 2008) Hence, there is a need for the deliberate breeding and selection of genotypes that are well-adapted to endure water deficit conditions during critical reproductive stages.

At the inception of any breeding programme, it is important to understand the genetic basis of the inheritance of targeted traits, as this helps selection decisions (Olajide *et al.*, 2017). Success in selection for any particular trait is at the discretion of the extent of genetic diversity availed to the breeder. Genetic diversity plays a crucial role in the survival and adaptability of species. Knowledge of genetic diversity is essential for the conservation of genetic resources and improvement of crop plants through breeding (Rao and Hodgkin, 2002). Various techniques are used to assess the presence of genetic diversity in crop plants. The techniques include agromorphological, molecular, and biochemical markers. Molecular markers are preferred as they are less affected by the influence of environment. Diversity Arrays Technology Pty Ltd (DArT, Canberra, ACT, Australia) has developed a cost-effective, sequence-independent and ultra-high-throughput marker systems. DArT markers are developed through a microarray hybridisation method, which produces thousands of polymorphic loci in a single assay (DArTSeq, 2018). Numerous marker technologies have been used in studies of genetic diversity in cowpeas. Some of the markers that have been used include microsatellites, randomly amplified polymorphic DNA (RAPD), chloroplast microsatellites and next generation sequencing (NGS) (Nadeem *et al.*, 2018). The major problems associated with previously developed marker systems were low marker density, poor genome coverage, and low cost effectiveness. Diversity Arrays Technology (DArTSeq, 2018) provides an ultra-high-throughput low-cost assays that facilitate the genotyping of individuals with the use of a large number of high-density markers that cover the entire genome. Robbana *et al.* (2019) used DArTseq-based SNP markers to confirm the genetic identity of 196 durum wheat lines collected in Tunisia. Barilli *et al.* (2018) used DArTSeq to understand the genetic control

underlying pea rust resistance from a cross between two *Pisum fulvum* accessions, IFPI3260 and IFPI3251.

Understanding farmers' requirements and trait preferences, as well as their farming systems, is essential for the wide adoption of newly developed crop varieties and production technologies (Hoffmann *et al.*, 2007). A survey was thus important to understand farmers' concerns and how they would adapt to newly introduced cowpea varieties for food and nutritional security.

1.2: Justification of Research

The overall productivity of cowpea is very low, with the average yield (particularly in Africa) ranging from 100 to 400 kg/ha (Chiulele, 2010; Alidu, 2019); under research conditions, yields of more than 1,500 kg/ha for grain and 2,500 kg/ha fodder can be achieved (Singh *et al.*, 2003). This is because of several biotic and abiotic constraints. Critical among these factors is the increasing incidence of droughts due to the changing climate in most parts of sub-Saharan Africa.

The incidence and intensity of droughts is predicted to be increased, threatening food security in Africa, particularly in the smallholder sector, which relies on rain-fed agriculture and accounts for 90% of staple food production (IPCC, 2007; Serdeczny *et al.*, 2019). Increased rainfall variability, in terms of its onset, cessation, amount, and distribution in the smallholder-sector, coupled with low-productive genotypes, low soil fertility, and low adaptation to improved soil and crop management practices, have resulted in low yields of less than 1 t/ha-, and, in some cases, complete crop failure (Fanadzo *et al.*, 2010; Karim *et al.*, 2018). Poor crop management practices and the lack of appropriate weather information make smallholder farming vulnerable to climate change.

Moisture stress causes both determinate and indeterminate cowpea varieties to cut short their flowering time with improper seed maturity. During the most severe droughts, leaves can wilt, show marginal scorch, or prematurely drop from the plant and die. Despite the tremendous efforts of plant breeders in developing improved drought-tolerant varieties, the occurrence of early and/or late season drought negatively affects cowpea growth and yield (Owusu *et al.*, 2018). This is further compounded by the limited number of drought-tolerant cowpea varieties in both South Africa and Zimbabwe. Therefore, most smallholder farmers use retained seed from previous harvests, often attaining very low yields.

Smallholder farmers both in Zimbabwe and South Africa face many challenges in the production of legumes such as cowpeas. One challenge is low yield, which is caused by several

factors, such as the use of retained seed, limited availability of proper inputs through agro-dealers, and hesitancy of farmers to use fertilisers during production (Schilt, 2013; Sparg, 2017). Farmers are reluctant to buy fertiliser and certified seeds. The recycled seeds of these legumes has exhausted its production potential, and yields keep decreasing ((N2 Africa, 2013). Thus, it is important to routinely carry out surveys of agricultural extension to better understand farmers' production systems and ensure the sustainability of agricultural projects (Moyo and Salawu, 2018).

According to Calzadilla *et al.* (2013), the population in Sub-Saharan Africa could double by 2050, increasing agricultural consumption by 2.8% annually until 2030, and by 2.0% annually from 2030 to 2050. There is, therefore, a need to develop high-yielding cowpea varieties available to farmers in order to ensure food and nutritional security. It is imperative to improve the drought tolerance of crops under the changing climate (Ali and Dov, 2017). Currently, there are no economically viable technological means to facilitate crop production under drought conditions. However, the development of crops tolerant to drought stress might be a promising approach, which could help in meeting food demands. The development of crops with enhanced drought resistance, among other things, require knowledge of the physiological mechanisms and genetic controls of the contributing traits at different plant developmental stages.

1.3: Research Objectives

The aim of this study is to contribute to increased food production in southern Africa through screening cowpea for drought tolerance and determine farmer's production constraints and preferred cowpea lines.

The specific objectives of the study were as follows:

1. To determine farmers' perceptions on the major constraints limiting cowpea production and identify their preferences regarding cultivars and traits.
2. To determine the genetic diversity of cowpea using genotyping by- sequencing- technique.
3. To screen cowpea germplasm for drought tolerance at the seedling stage.
4. To conduct association mapping for drought tolerance and yield related traits in cowpea at the seedling stage.

References

- Ali, I., and Dov, P., 2017. Yields and Forage Nutritive Quality of High-Yielding Dual Purpose Cowpea (*Vigna unguiculata* L. Walp.) Varieties in the Sahelian Low-Input Cropping System African Journal of Agriculture Research 12:2969-2975.
- Alidu, M.S., 2019. Evaluation of Planting Dates on Growth and Yield of Three Cowpea [*Vigna unguiculata* (L) Walp.] Genotypes in Northern Ghana. Advances in Research 18(4): 1-14.
- Barilli, E., Cobos, M.J., Carrillo, E., Kilian, A., Carling, J., and Rubiales, D., 2018. A High-Density Integrated DArTseq SNP-Based Genetic Map of *Pisum fulvum* and Identification of QTLs Controlling Rust Resistance. Frontiers in Plant Science. 9:167. doi: 10.3389/fpls.2018.00167.
- Boukar, O., Belko, N., Chamathi, S., Togola, A., Batiemo, J., Owusu, E., Haruna, M., Diallo, S., Umar, M.L., Olufajo, O., and Fatokun, C., 2019. Cowpea (*Vigna unguiculata*): Genetics, Genomics and Breeding. Plant Breeding 2019; 138:415–424. <https://doi.org/10.1111/pbr.12589>.
- Calzadilla, A., Zhu, T., Rehdanz, K., Tol, R.S.J., and Ringler, C., 2013. Economy Wide Impacts of Climate Change on Agriculture in Sub-Saharan Africa. Ecological Economics 93:150-165.
- Chiulele, R.M., 2010. Breeding Cowpea (*Vigna unguiculata* (L.) Walp) for Improved Drought Tolerance in Mozambique. PhD Plant Breeding Thesis. University of Kwazulu Natal, South Africa: 33-51.
- DArTSeq, 2018. Technology and Resources. <https://www.diversityarrays.com/technology-and-resources/dartseq/>.
- da Silva, AC., da Costa Santos, D., Teixeira Junior, D.L., da Silva, P.B., dos Santos, R.C., and Siviero, A., 2018. Cowpea: A Strategic Legume Species for Food Security and Health. IntechOpen 48-57: <http://dx.doi.org/10.5772/intechopen.79006>.
- De Freitas, R.M.O., Dombroski, J.L.D., De Freitas, F.C.L., Nogueira, N.W., and Pinto, J.R.D., 2017. Physiological Responses of Cowpea under Water Stress and Rewatering In No-Tillage and Conventional Tillage Systems. Revista Caatinga 30:3 pp.559-567. <https://doi.org/10.1590/1983-21252017v30n303rc>.

Fanadzo, M., Chiduza, C., Mnkeni, P.N.S., van der Stoep, I., and Stevens, J., 2010. Crop Production Management Practices as a Cause for Low Water Productivity at Zanyokwe Irrigation Scheme. *Water South Africa* 36: 27-36.

FAOSTATS, 2017. Production Quantities of Cowpeas, Dry By Country. Average 1994 – 2017. <http://www.fao.org/faostat/en/#data/QC/visualize>.

Hall, A.E., 2012. Phenotyping Cowpea for Adaptation to Drought. *Frontiers in Physiology* 3:1-8. <https://doi.org/10.3389/fphys.2012.00155>.

Hoffmann, V., Probst, K. and Christinck, A., 2007. Farmers And Researchers: How Can Collaborative Advantages Be Created In Participatory Research And Technology Development? *Agriculture and Human Values* 24:355-368. <https://doi.org/10.1007/s10460-007-9072-2>.

Intergovernmental Panel on Climate Change Fourth Assessment Report: Climate Change, 2007. https://www.ipcc.ch/publications_and_data/ar4/syr/en/mains3-3-2.html.

Karim, T.D.A., Sanoussi, A., Maârrouhi, I.M., Falalou, H., and Yacoubou, B., 2018. Effect of Water Deficit at Different Stages of Development on the Yield Components of Cowpea (*Vigna unguiculata* L. Walp) genotypes. *African Journal of Biotechnology* 17(9), pp. 279-287. doi: 10.5897/AJB2017.16347.

Muchero, W., Ehlers, J.D., and Roberts, P.A., 2008. Seedling Stage Drought-Induced Phenotypes and Drought-Responsive Genes in Diverse Cowpea Genotypes. *Crop Sci.* 48:541–552. doi: 10.2135/cropsci2007.07.0397.

Moyo, R., and Salawu, A., 2018. A Survey of Communication Effectiveness by Agricultural Extension in the Gweru District of Zimbabwe. *Journal of Rural Studies* 60 (2018): 32–42. <http://dx.doi.org/10.1016/j.jrurstud.2018.03.002>.

Mwale, S.E., Ochwo-Ssemakula, M., Sadik, K., Achola, E., Valentor, A.O., Gibson, P., Edema, R., Singini, W., and Rubaihayo, P., 2017. Response of Cowpea Genotypes to Drought Stress in Uganda. *American Journal of Plant Sciences* 8:720-733. doi.org/10.4236/ajps.2017.84050.

N2 Africa, 2013. Limitations in the Production of Legume Crops in Zimbabwe <https://n2africa.org/limitations-production-legume-crops-zimbabwe>.

Nadeem, M.A., Nawaz, M.A., Shahid, M.Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G., and Baloch, F.S., 2017. DNA Molecular Markers in Plant Breeding: Current Status and Recent Advancements in Genomic Selection and Genome Editing. *Biotechnology and Biotechnological Equipment*. doi.org/10.1080/13102818.2017.1400401.

Nedumaran, S., Abinaya P., Jyosthnaa P., Shraavya, B., Rao, P., and Bantilan, C., 2015. Grain Legumes Production, Consumption and Trade Trends in Developing Countries. Working Paper Series No 60. ICRISAT Research Program, Markets, Institutions and Policies. Patancheru 502 324, Telangana, India: International Crops Research Institute for the Semi-Arid Tropics: 64.

Okonya, J.S., and Maass, B.L., 2014. Potential of Cowpea Variety Mixtures to Increase Yield Stability in Subsistence Agriculture: Preliminary Results. *International Journal of Agronomy* 2014:1- 7. <https://doi.org/10.1155/2014/515629>.

Olajide, A.A., and Ilori, C.O., 2017. Effects of Drought on Morphological Traits in Some Cowpea Genotypes by Evaluating Their Combining Abilities. *Advances in Agriculture* 2017:1-10. <https://doi.org/10.1155/2017/7265726>.

Owusu, E.W., Akromah, R., Denwar, N.N., Adjebeng-Danquah, J., Kusi, F., and Haruna, M., 2018. Inheritance of Early Maturity in Some Cowpea (*Vigna unguiculata* (L.) Walp.) Genotypes under Rain Fed Conditions in Northern Ghana. *Advances in Agriculture* 2018:1-10. <https://doi.org/10.1155/2018/8930259>.

Rao, V.R., and Hodgkin, T., 2002. Genetic Diversity and Conservation and Utilisation of Plant Genetic Resources. *Plant Cell, Tissue and Organ Culture* 68.pp 1-19. <https://doi.org/10.1023/A:101335901>.

Robbana, C., Kehel, Z., Naceur, M.B., Sansaloni, C., Bassi, F., and Amri, A., Genome-Wide Genetic Diversity and Population Structure of Tunisian Durum Wheat Landraces Based on DArTseq Technology. *International Journal of Molecular Science*. Volume 20(6). 1352; <https://doi.org/10.3390/ijms20061352>.

Schilt, C., 2013. Limitations in the Production of Legume Crops in Zimbabwe. <http://www.n2africa.org/content/limitations-production-legume-crops-Zimbabwe>.

Serdeczny, O., Adams, S., Baarsch, F., Coumou, D., Robinson, A., Hare, W., Schaeffer, M., Perrette, M., and Reinhardt, J., 2017. Climate Change Impacts In Sub-Saharan Africa: From Physical Changes to Their Social Repercussions. *Regional Environmental Change* 17(6):1585-1600. doi: 10.1007/s10113-015-0910-2.

Singh, B., Ajeigbe, H., Tarawali, S.A., Fernandez-Rivera, S., Abubakar, M., 2003. Improving the Production and Utilization of Cowpea as Food and Fodder. *Field Crops Research*. 84:169-177.

Singh, B. B., Cambliss, O. I., and Sharma, B., 1997. Recent Advances in Cowpea Breeding. In: Singh B. B., Mohan Raj, D. R., Dashiell, K., Jackai, L. E. N. (Eds.) *Advances In Cowpea Research*. Ibadan: IITA: 30-49.

Sparg, L., 2017. Against The Odds: Smallholder Farmers and Agriculture Biodiversity in South Africa. <https://acbio.org.za/wp-content/uploads/2017/02/South-Africa-Field-Report-2017.pdf>.

CHAPTER 2

LITERATURE REVIEW

2.1: Origin of cowpeas

Cowpea originated in Africa though the exact place of domestication is uncertain. Ng and Marechal (1985) consider Ethiopia, Central Africa, Central and South Africa and West Africa as probable centers of domestication. Da Silva *et al.* (2018) contend that the crop originated in Africa. Apart from nutrients, cowpea has dietary fibre, antioxidants and polyunsaturated fatty acids (PUFA) and polyphenols (Da Silva *et al.*, 2018). However, it is generally agreed that the cowpea is of African origin as conspecific wild forms are found in Africa.

2.2: Cowpea production worldwide

According to FAOSTAT, (2017) cowpea was grown on an estimated 11 million ha in Africa in 2017 with the bulk of production occurring on 10.6 million ha in West Africa, particularly in Niger, Nigeria, Burkina Faso, Mali and Senegal. More than 7.4 million tons of dried cowpeas are produced worldwide, with Africa producing nearly 5.2 million (<https://www.iita.org/cropsnew/cowpea/>). Nigeria, being the largest producer and consumer, which accounts for 61% of production in Africa and 58% worldwide. Fifty-two percent of Africa's production is used for food, 13% as animal feed, 10% for seeds, 9% for other uses, and 16% is wasted. According to FAOSTAT (2017), over 87% of cowpeas is produced in Africa. However, in South America, Brazil increased cowpea cultivation and the country is now in third place in terms of global area and production.

2.3: Importance of cowpea

The main use of cowpeas is food as dry grain or fresh peas or immature pods. However the dry grains have more resistance to terminal drought than the fresh peas or immature pods. Cowpea grains have substantial quantities of protein, carbohydrate, vitamins, and fiber (Hall, 2012). The amino acid and vitamin profiles of cowpea grain complement those of cereals. According to Hall (2012), in the subsistence farming areas where it is mainly grown, cowpeas is the cheapest main source of protein. Cowpeas has many beneficial uses as animal feed, human consumption and income generation (IITA, 2009).

In some cases, the pods of cowpeas are harvested when they are full-sized, but not before they dry out and then the grains are cooked and eaten as fresh peas. Since 1985, the consumption of cowpeas as fresh peas has also become important in the semi-arid Sahelian zone of Africa especially Senegal (Hall, 2012). Some extra-early maturing cowpea varieties have been introduced, which provide food during the hungry period of August and September. The hungry period is just before the main harvests of pearl millet, sorghum, and dry grains of traditional varieties of cowpeas and peanuts, which begin in October. Consumption of cowpeas as fresh peas has been increased in the Sahelian zone as extra-early cowpea cultivars are being made available to farmers. The Sahel region stretches from Senegal on the Atlantic coast, through parts of Mauritania, Mali, Burkina Faso, Niger, Nigeria, Chad, Sudan and Eritrea (Suleiman, 2017).

Immature pods of cowpea cultivars are consumed as fresh in Kenya (African green beans), Trinidad (bodie beans), and Southeast Asia (yard-long beans) (Hall, 2012). The fresh leaves of cowpeas are also consumed in sauces, especially in East Africa. Another major use of cowpeas in the Sahelian zone of Africa is as hay, after the pods have been harvested. Hay is used to maintain draft animals during the long dry season in the Sahel and to fatten rams and goats in preparation for various festivals (Hall, 2012). Cowpeas have high protein and low fat content, which is important in the prevention of diverse metabolic and cardiovascular diseases (Goncalves *et al.*, 2016).

Cowpeas are also important as a rotation crop with cereals as they enhance the fertility of the soil with respect to nitrogen and phosphate, thereby benefiting subsequent cereal crops. It has been observed that some cowpea lines prevent the emergence of the seeds of *Striga hermonthica*, which parasitizes pearl millet, sorghum, and maize (Samejima and Sugimoto, 2018). Lee and Thierfelder, (2017) notes that intercropping with cowpeas has been known to suppress and smother weeds in dryland farming systems in the smallholder farming sector. Certain lines can also suppress the populations of the nematode *Scutellonema cavenessi*, which is a major pest of pearl millet, sorghum, and peanut in the Sahel (Hall, 2012). In some cropping systems, cowpeas can be used as cover crops and these can suppress pests, many plant pathogens including nematodes and increase carbon sequestration (Wang and McSorley, 2018).

2.4 Constraints of cowpea production

Several biotic and abiotic factors such as insect pests, diseases (fungal, viral and bacterial), poor soil fertility, metal toxicity and drought contribute to the reduction of cowpea yield potential in sub Saharan Africa (SSA) (Saka *et al.*, 2018; Boukar *et al.*, 2018; Gomes *et al.*, 2019). Other factors contributing to low yield in SSA include lack of improved varieties that can withstand these stresses and lack of adequate production practices and inputs needed for higher productivity and profitability. This yield gap therefore can be bridged if improved varieties and production practices are available to farmers through participatory on-farm training and evaluation with farmers (Ajeigbe *et al.*, 2010). Among the abiotic factors, drought is one of the constraints limiting cowpea production in Southern Africa (Boukar *et al.*, 2018).

2.4.1: Effect of drought on cowpea production

Drought has been reported as a major constraint in semiarid tropics due to erratic rainfall in the beginning and towards the end of the rainy season (Olajide *et al.*, 2017). The crop is often subjected to drought stress in both seedling and terminal growth stages and this causes the substantial reduction in grain yield as well as biomass production. Iwuagwu *et al.* (2017), reported that the effects of drought vary and depend on the intensity, developmental stage and duration of stress as well as the adaptive strategy that the plant possess to tolerate this stress. During the vegetative phase, water deficit causes leaf and plant growth reduction; alteration in the process of nutrient absorption due to low water availability in the environment; increase in stomatal resistance, and ultimately, a decrease in gaseous exchange between the environment and the plant (Boukar *et al.*, 2018).

Similarly, Abidoeye (2004), reported reduction in leaf production and/or increase in leaf senescence and abscission due to water stress. Some cowpea varieties are heat sensitive are affected by night temperatures above 35 °C as their flowers abort due to poor pollination, resulting in poor seed and pod set (Boukar *et al.*, 2018). Water stress leads to a decrease in plant water content, turgor reduction and results in a decrease in cellular expansion (Iwuagwu *et al.*, 2017). Thus, there is need for establishment of an absolute need for effective breeding programs that develop high yielding and well-adapted varieties for water deficit conditions. Olajide *et al.* (2017) noted that drought is one of the most important abiotic constraints threatening food security in the world. This is because the likelihoods of African farmers

depend on rain-fed agricultural systems that are seriously affected during periods of severe drought thus drought is serious in Africa (Oladipo, 2008). Drought conditions can either be intermittent when they occur at one or more intervals during the crops' growing period or terminal when there is progressive decrease in available soil moisture content resulting in severe drought (Ibitoye, 2015).

2.5 Strategies used to mitigate drought

Various adaptive measures are used by farmers to mitigate the effects of drought. From a survey conducted in the smallholder farming sector, Umdale *et al.* (2014), observed that most farmers preferred to change their cropping calendar to adjusted planting or to use drought tolerant crops to mitigate the drought impacts. In some instances farmers resort to keeping the land unsown after the possibility of droughts so as to minimise seed and inputs losses.

Some farmers have opted to cultivate early maturing varieties. These early- maturing varieties are considered climate- smart cultivars as they can have the ability to escape terminal drought as well as pests and diseases damage that normally occur later in the cropping season (Owusu, *et al.*, 2018). Early maturing cultivars are important in agro-ecological zones with short growing seasons usually in the arid and semiarid tropics where cowpea genotypes that mature between 55 and 60 days are ideal for cultivation. Their greatest advantage is that they provide food sooner than any other crop, thus shortening the hunger period (Owusu *et al.*, 2018).

According to Oxfam (2016), farmers in Zimbabwe have resorted to using various methods to mitigate drought. In marginal districts of the country, farmers are opting towards more drought-tolerant alternatives such as indigenous crops like sorghum, pearl millet, cowpeas and groundnuts. Farmers believe that these old and forgotten crops are better adapted to dry conditions. Farmers are also planting a combination of crops and varieties per crop to minimize risk. This minimises crop failure as some varieties are likely to yield even under low moisture conditions. Smallholder farmers' deal with unpredictable rainfall and pests by combining early-maturing seeds with late- maturing seeds. That way they spread the risk of crop failure. Even if the late maturing varieties fail under water stress conditions, those that mature earlier might get modest yields during the first half of the season. Finally in some instances, during drought

years, farmers may be forced to replant two to four times (Oxfam, 2016). However, this coping strategy has the disadvantage of farmers failing to access sufficient seed on time.

2.5.1: Vegetative- stage drought resistance

Cowpeas were shown to own substantial resistance to vegetative-stage drought in California (Turk *et al.*, 1980). In an experiment, cowpea seeds were planted into a dry soil profile with simply enough water to allow germination and emergence, plants survived for 43 days under very hot dry summer conditions with no rain but were badly stunted. Most of other crop plants would have been killed with the intensity of drought that the cowpea plants were exposed. The stunted plants were irrigated on the 44th day and recovered rapidly, producing very high grain yields of 4,000 kg ha⁻¹ by 107 days after sowing. A control treatment that had received optimal irrigation every week produced a similar grain yield during the same period. Subsequently, cowpeas have been observed to survive vegetative-stage droughts in the Sahelian zone that killed pearl millet and peanut plants growing in the same field (Hall, 2012).

This survival of vegetative-stage droughts by cowpeas was associated with the maintenance of higher leaf water status than pearl millet (Petrie and Hall, 1992). Cowpeas exhibit very small changes in leaf water potential when subjected to drought and very little osmotic adjustment (Shackel and Hall, 1983). Cowpeas also have stomata that are very sensitive to soil drying, partially closing before any changes in leaf water potential are detected (Shackel and Hall, 1983). When cowpea plants are subjected to drought in the field conditions, their leaves do not usually wilt but tend to orient more vertically, tracking the sun in a manner that minimizes the interception of radiation (Shackel and Hall, 1979). These mechanisms contribute to the distinctive ability of cowpeas to survive extreme vegetative-stage droughts that kill most alternative crop plants.

A screening technique for survival of drought at the seedling-stage was developed that uses a shallow soil layer in boxes (Singh and Matsui, 2002). When 190 diverse cowpea breeding lines from IITA were screened with this technique, only 22% were found to be susceptible to drought. This suggests that most current cowpea cultivars may have resistance to vegetative-stage drought. Inheritance studies indicated that the susceptibility was due to a single recessive gene (Singh and Matsui, 2002). In another screening test, it was shown that cowpeas survived seedling-stage drought longer than all other species tested: lablab beans, Bambara groundnuts, peanuts, pearl millet, sorghum, greengram, blackgram, maize, and soybeans (Hall, 2012).

According to Hall, (2012) there are two conditions where the resistance of cowpeas to vegetative-stage drought is not effective. The first condition is when the cowpea plants are infected with soil born disease called ashy stem blight disease (*Macrophomina phaseolina*). This is because the resistance of cowpeas to vegetative-stage drought breaks down and the plants die. This disease organism is widespread and causes severe damage to cowpea seedlings growing in the hot, dry soil conditions that often occur in the Sahelian zone and in Botswana. The second condition is when the cowpea plants have been attacked by the lesser corn stalk borer (*Elasmopalpus lignosellus*) and killed young cowpea plants that would have been subjected to drought while plants in well-watered treatments were not affected. Varietal resistance to this pest is not yet available (Hall, 2012).

2.5.2: Cowpeas response to drought

Nadeem *et al.*, (2019), observed that root length, fibrous root system, density and rooting depth are promising factors that are useful for screening genotypes for drought stress tolerance. Duc *et al.*, (2015), highlighted that early flowering, podding and maturity are mechanisms that are very useful in mass screening. The UC-Riverside and the Institut Sénégalais de Recherches Agricoles (ISRA) has bred extra-early cowpea cultivars that has very short growth cycles to escape drought. According to Hall (2012), the ideotype involves combining vegetative-stage drought resistance with erect plant habit and early synchronous flowering beginning on low nodes on the main stem. These cultivars do not spread much during the vegetative-stage, as a result they can be planted at close spacing (50 cm between rows and 25 cm between seeds). Close spacing also enhances the synchrony of pod production and earliness of harvest.

UC-Riverside has also bred the cultivar Ein El Gazal by crossing an erect early California cultivar that has resistance to vegetative-stage drought and an erect early line from Senegal. These lines were then tested in the Sahelian zone at Louga, Senegal, in 1982 (Hall, 2012). Line UCR 1- 12-3 (Ein El Gazal) began flowering in 35 days and produced 1,091 kg ha⁻¹ of dry grain by 55 days from sowing with a rainfall of only 181 mm under hot conditions in a short season (Hall and Patel, 1985). Other crops grown in the Louga area in 1982, pearl millet, peanuts, and traditional cowpeas which had 90-day cycles, produced virtually no grain. In the same year at a wetter location (Bambey, Senegal, with 452 mm rain), Ein El Gazal produced 2,406 kg ha⁻¹ of dry grain by 60 days from sowing (Hall and Patel, 1985) indicating it had high yield potential. This line also performed well in the Sahelian zone of the Sudan (Hall and Patel, 1985) where it was released as a registered cultivar (Elawad and Hall, 2002). An early erect

cultivar that matures in 60–65 days, Vuli-1, was bred for cultivation during the short rains in Tanzania (Mligo and Singh, 2007).

Ein El Gazal and Melakh have three advantages: (i) they can survive vegetative-stage droughts; (ii) they can produce significant grain in locations and years when the rainy season is very short (iii) they produce fresh pods beginning about 45 days from sowing, which is during the hungry period in the Sahel region. However, the major disadvantages of these extra-early erect cowpea cultivars are: (i) they must be sown at close spacing (ii) they produce very little hay; and (iii) they can be devastated by a late-season drought (Thiaw *et al.*, 1993) and other biotic stresses.

2.5.3: Resistance to mid-season drought

There are various studies regarding tolerance to mid-season drought. For instance, line 58–57 was selected from a landrace growing around the Senegal River and it has a relatively short cycle, begin flowering 41 days after sowing and reach maturity in 75 days (Li *et al.*, 2001). It has a spreading habit and experiences sequential flowering, which may partially account for its resistance to mid-season drought. Line 58–57 is a dual-purpose line that produces hay as well as grain. Mouride was bred by Institut Senegalais de Recherches Agricoles (ISRA) by crossing 58–57 with an associate erect breeding line and this was done in Senegal in 1992 (Cisse *et al.*, 1995). Mouride is a semi-erect line that reaches maturity in 65 days after sowing. It has a yield potential of 3,000 kg ha⁻¹ and has considerable ability to resist mid-season drought. Mouride conjointly also has resistance to two seed-borne diseases, charcoal rot and *Macrophomina* as well as the plant parasitic weed *Striga gesnerioides* and cowpea weevil.

A delayed-leaf-senescence (DLS) trait was observed in cowpea that enables the plants to maintain a green canopy after the first flush of pods mature and then produce a second flush of flowers and pods (Gwathmey *et al.*, 1992b). When genotypes with this attribute were subjected to a mid-season drought throughout the first flowering period, they produced a considerable second flush of pods and grain (Gwathmey and Hall, 1992a). Early erect lines with DLS trait were bred, selected and tested and produced a first flush of 2,000 kg ha⁻¹ of grain in about 65 days from sowing and then a second flush production of an additional 1,000 kg ha⁻¹ of grain by 95 days from sowing (Hall *et al.*, 1997). Cultivars with both earliness and DLS trait may be most useful in the longer rainy season than in occasional mid-season drought season.

The mechanism of DLS attribute leads to the build-up of larger reserves of carbohydrates within the base of the stem (Gwathmey *et al.*, 1992a) and within the roots. The trait is most expressed when *Fusarium solani* f. sp. *Phaseoli* that causes leaf senescence of cowpeas during pod maturation is present in the soil. According to Gwathmey *et al.* (1992b), *Fusarium solani* f. sp. *Phaseoli* is expressed by plants in soils where cowpeas have been grown in rotations for several years in many locations. DLS trait can be selected effectively with advanced lines and appears to have simple inheritance (Ismail *et al.*, 2000).

Some dual-purpose erect cowpea cultivars that reach maturity in 85 days have been bred for use in the Savanna zone (Dingkhun *et al.*, 2006). These can produce 1,500–2,500 kg ha⁻¹ grain and 3,000–5,000 kg ha⁻¹ of hay when sown at moderately close spacing. The high hay production is strongly associated with the ability to continue producing leaves after the grain has matured. Consequently, it is assumed that these cultivars have the DLS trait.

Goufo *et al.* (2017), investigated osmo-adaptation in leaves and roots using photosynthetic traits, water homeostasis, inorganic ions, and primary and secondary metabolites. The multifactorial analyses indicated allocation of high quantities of amino acids, sugars, and proanthocyanidins into roots that were assumed to be linked to growth and initial stress perception. Physiological and metabolic changes developed in parallel with drought/recovery responses showed a progressive acclimation of the cowpea plant to stress. Out of the 88 metabolites studied, proline, galactinol, and a quercetin derivative responded most to drought as highlighted by multivariate analyses, and their correlations with yield indicated beneficial effects. These metabolites accumulated in roots, suggests a more conservative strategy to cope with drought in the aerial parts (Goufo *et al.*, 2017).

2.5.4: Use of other cowpea cultivars

In regions where rainfall is variable and intermittent droughts, a single type of cowpea cultivar is not ideal as it does not meet needs of farmers. It is thus important to breed more than two types of cultivars so that farmers grow at least two types every year to enhance the chances of significant grain and hay production (Hall, 2004). Extra-early erect cultivars such as Ein El Gazal that have synchronous flowering and mature within 60 days from sowing escape late-season drought and also have resistance to vegetative-stage drought can be grown. Ein El Gazal is an early maturing cowpea variety. Although early erect cultivars provide useful food during the hungry period, they are devastated by mid-season drought and also produce little hay.

Spreading cultivars like 58–57 with successive flowering and a medium-cycle maturity within 75 days, have substantial resistance to mid-season drought and vegetative-stage drought (Hall, 2004). They manufacture useful quantities of grain and hay, however when the growing season is very short, and produce little grain. Ibitoye, (2015), observed that crosses TVu6707 x TVU9797, TVu9693 x TVu7778, and TVu9693 x TVu9797 had more grain yield and seeds per pod and thus were very useful candidates for screening for drought tolerance.

A comparison was made between varietal intercrops consisting of alternating rows of extra-early erect cultivars and medium-cycle spreading cultivars with sole crops of the same cultivars within the Sahelian zone of Senegal (Thiaw *et al.*, 1993). The varietal intercrops made additional grain and hay under dry conditions with infertile soil and were more stable than any of the sole crops of cowpeas that were tested. In screening for drought tolerance at seedling stage for 23 cowpea varieties, Muhammad *et al.* (2015) observed that five varieties, that is Kanannado, Danila, IT07K-297-13, IT03K-378-4 and Aloka local were highly tolerant to drought. In an experiment to identify reliable morpho physiological parameters for selecting drought tolerant cowpea varieties, it was found out that Danila was the most tolerant variety (Pele *et al.*, 2016). In years in which a mid-season drought happens, the extra-early erect cultivar becomes stunted while the medium-cycle spreading cultivar grows into space and produces a lot of grain and hay yields of the intercrop. In years with short rainy season, the extra-early erect cultivars produce a lot of grain, while the medium-cycle spreading cultivars produce abundant hay for the intercrop but little grain (Thiaw *et al.*, 1993).

2.6: Genetic diversity in cowpeas based on molecular markers

Genetic diversity is the variation of heritable characteristics present in a population of one species and serves as a way for populations to adapt to changing environments (Xu *et al.*, 2016). It is applicable to wild populations as well as domesticated strains, which generally have lower levels of diversity. Genetic variation can be caused by mutation (which can create entirely new alleles in a population), chromosomal manipulation (polyploidization, chromosomal abnormalities such as deletions and inversions) and recombination between homologous chromosomes during meiosis (which reshuffles alleles within an organism's offspring). With more variation, it is more likely that some individuals in a population will possess allelic variants of alleles that are suited for the specific environment. Wamalwa *et al.* (2016), evaluated genetic diversity from 19 accessions of cowpeas from the national genebank of

Kenya. The accessions clustered into two major groups namely Ethiopian and Australian. These cowpea accessions showed high levels of divergence to the accessions from Western Kenya. From this evaluation diversity is important as it can be used to improve other existing cowpeas accession through further selection and breeding.

According to Nadeem *et al.* (2018), molecular markers are nucleotide sequences which are investigated through the polymorphism present between the nucleotide sequences of different individuals. Molecular markers are classified into various groups on the basis of: (1) mode of gene action (co-dominant or dominant markers) (2) method of detection (hybridization- based molecular markers or polymerase chain reaction (PCR) - based markers and (3) mode of transmission (paternal organelle inheritance, maternal organelle inheritance, bi-parental nuclear inheritance or maternal nuclear inheritance. These markers are widely used because of their high prevalence and expression in different stages of the organisms (Kodrostami and Rashimi, 2015).

An ideal DNA marker should be co-dominant, evenly distributed throughout the genome, highly reproducible and highly polymorphic. Batiemo *et al.* (2018), notes that markers based on single nucleotide polymorphisms (SNPs) are increasingly being used in molecular genetics in recent years. This is due to their abundance in the genomes and their amenability for high-throughput detection and automation in many genotyping platforms (Mammadov *et al.*, 2012). Among these platforms is the LGC genomics', United Kingdom which provides DNA sequence data to scientists using Kompetitive Allele Specific PCR (KASP). SNP markers are progressively being employed for a large number of genetic as well as diversity studies. Such studies are reported in pea (Deulvot *et al.*, 2010), cowpea (Huynh *et al.*, 2013; Egbadzor *et al.*, 2014), and cassava (Thompson, 2013). SNPs provide a large number of markers to be used in diversity studies or in marker assisted breeding. SNPs are co-dominant markers and they are most often linked to genes. Thus, they are the foremost enticing genetic markers in genetic studies (Jiang, 2013). The use of SNPs might also facilitate clustering of germplasm that help breeders to have enlightened alternatives of parents for breeding functions. SNP markers therefore facilitate decision making once the variability within the germplasm is identified.

Alghamdi *et al.* (2018), used sequence-related amplified polymorphisms (SRAP) markers to assess genetic variability in seven cowpea landraces using seed storage proteins. Igwe *et al.* (2017), used inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) polymorphic markers to assess the genetic diversity in eighteen *Vigna unguiculata* L. (Walp) accessions.

Mafakheri *et al.* (2017), characterised 32 cowpea genotypes for molecular markers using a set of 22 Simple Sequence Repeat (SSR) primer pairs. In their study a total of 186 alleles were detected with an average of 2 alleles for each locus, and genetic distance between genotypes was estimated 0.0066.

Xiong *et al.* (2016), observed that the level of genetic diversity tends to have a positive correlation with the geographic origin from which the accessions were collected. In their study of genetic diversity and population structure of 768 cowpea types from 56 countries, Xiong *et al.* (2016), found that there was high genetic diversity of accessions from South and West Africa, which had 7 and 8 countries respectively, based on the number of countries from which the accessions were collected. Similarly, the lowest genetic diversity of accessions was from Europe with only 2 countries. In analysing genetic diversity of 370 landraces, Fatokun *et al.* (2018), observed that cluster one had 115 accessions from the largest number of countries and thus the highest genetic diversity, heterozygosity and polymorphic information content. Cluster two was made up of 102 accessions, of which 90 percent were from West and Central Africa.

DArTseq, provides a good choice as a high throughput marker genotyping platform that can develop a relatively large number of polymorphic markers to build dense genetic maps with low-cost investments (<http://www.diversityarrays.com/>). Wide coverage of the genome and the high-density genetic maps based on DArTseq™ technology increases the power of QTL detection. Additional advantages of DArTseq™ technology are its suitability for polyploid species as well as the possibility of developing rapidly for virtually any genome. As a result, DArTseq-derived markers are currently used in more than 400 species (<http://www.diversityarrays.com/>), being very popular among crops with the non-sequenced genomes.

Several researchers have used DArTseq™ technology to investigate genetic diversity across different crop species. Balochi *et al.* (2017), evaluated the genetic diversity of durum wheat landraces using DArTseq and SNP analysis. A total of 9,568 DArTseq and 20,661 SNP markers were used to characterize the genetic characteristic of 91 durum wheat landraces. Killian *et al.* (2016), applied DArTseq™ technology to pineapple in order to understand the genetic relationships between the genotypes. SNP markers were used in characterization of 113 cowpea accessions comprising of 108 from Ghana and abroad (Egbadzor, *et al.*, 2014). The results revealed 477 SNPs, out of which 458 were polymorphic. Fatokun *et al.* (2018), used

genotyping by sequencing on 298 lines from the loosely composed mini core collection of 370 landraces in cowpeas to assess genetic diversity and population structure. The study revealed three different clusters.

2.7: Molecular breeding in cowpeas

A genetic map of seven populations of cowpea recombinant inbred lines (RILs) based on SNPs has been reported by Muchero *et al.*, (2009a). Consensus genetic linkage maps give a genomic framework for quantitative attribute loci identification, map-based cloning, assessment of genetic diversity, association mapping, and applied breeding in marker-assisted selection schemes. In orphan crops with limited genomic resources such as cowpea [*Vigna unguiculata* (L.) Walp.] ($2n=2x=22$), the use of transcript-derived SNPs in genetic maps provides opportunities for automated genotyping and estimation of genome structure based on synteny analysis. Muchero *et al.*, (2009) further reports that SNP mining from 183,118 ESTs sequenced from 17 cDNA libraries yielded 10,000 high-confidence SNPs from which an Illumina 1,536-SNP GoldenGate genotyping array was developed and applied to 741 recombinant inbred lines from six mapping populations. Approximately 90% of the SNPs were technically successful, providing 1,375 dependable markers. Of these, 928 were incorporated into a consensus genetic map spanning 680 cM with 11 linkage groups and a median marker distance of 0.73 cM. Pan *et al.*, (2017) identified 34,868 SNPs that were distributed in the cowpea genome based on the (restriction-site associated DNA sequencing) technique using a population of 170 accessions developed from two cowpea bi-parental crosses. Of these, 17,996 reliable SNPs were assigned to 11 consensus linkage groups (LGs). The length of the genetic map was 1,194.25 cM in total with a mean distance of 0.066 cM/SNP marker locus. Using this map and also the F2:3 population, combined with the CIM (composite interval mapping) method, eleven quantitative trait loci (QTL) of yield-related trait were detected on seven LGs (LG4, 5, 6, 7, 9, 10, and 11) in cowpea.

2.8: Association mapping in cowpeas

For effective selection in cowpea, it is imperative to have knowledge on genetic variability, estimate character association and heritability of important agronomic traits. These estimates are important and help in the design of selection strategies for cowpea breeding programs.

Kouam *et al.* (2018), studied character associations, phenotypic and genotypic divergence for yield and yield related quantitative traits among 30 cowpea landraces in Cameroon. The results indicated that broad sense heritability was high with 20 traits out of 25 having heritability values greater than 70%. The highest heritability was for 100 seed weight (98.15%) and the lowest for shoot weight (41.38%). At 0.05 probability level, grain yield correlated significantly with 21 out of the 24 other quantitative traits.

In order to control aphids (*Aphis craccivora*) in cowpeas, Qin *et al.* (2017), studied and identified SNP markers associated with aphid resistance in 338 aphid resistant cowpea accessions. They observed that two SNP markers, C35011941_894 and Scaffold30061_3363, were strongly associated with aphid resistance across three models with the log of odds (LOD) score of 2.5. In identifying genes that control seed coat and pod tip colours, Herniter *et al.* (2018), identified in their analysis MYB113 genes on chromosome Vu05, which belong to the R2R3 MYB class of transcription factors. Further, it was observed that the expression of the MYB113 genes showed that *Vigun05g039400* and *Vigun05g039500* were highly expressed in developing seeds while *Vigun05g039300* was highly expressed in pods, flowers and leaves.

In cowpea, salinity has become an increasing threat to production, and Ravelombola *et al.* (2018), identified SNPs associated with salt tolerance at germination and seedling stages. A total of 1049 SNPs postulated from genotyping-by-sequencing were used for association analysis. Three SNPs, Scaffold87490_622, Scaffold87490_630, and C35017374_128 were highly associated with salt tolerance at germination stage. Seven SNPs, Scaffold93827_270, Scaffold68489_600, Scaffold87490_633, Scaffold87490_640, Scaffold82042_3387, C35069468_1916, and Scaffold93942_1089 were found to be associated with salt tolerance at seedling stage. These markers can be applied as a tool for selecting salt tolerant lines to be included in breeding programs of this crop.

2.9: Surveys in Agriculture Extension

Ngaka and Zwane (2018), concur that surveys in agriculture are important in order to assist decision makers in future planning to strengthen extension and advisory services. Extension services is one of the policy instruments which can be used to solve the food insecurity challenges. The active involvement of farmers is important in understanding of underlying decision-making factors for farmers to adopt or not-to-adopt agricultural innovations.

Ndoro *et al.* (2017), carried out a survey in Buhera District to evaluate the impact of master farmers training on farmers' finger millet productivity in Buhera District, considering the farmers' gender, institutional and income factors. It was observed that master farmer training had an influence on decision to grow finger millet. Maruzani (2014), carried out a survey to assess problems faced by rural women in Buhera District of Manicaland South Province of Zimbabwe. The study showed that poverty resulting from the depletion of natural resources and gender inequality were the underlying causes to the problems that rural women are facing. Mujaju and Mazvimavi (2019), assessed seed security in four most vulnerable districts in Zimbabwe. These districts included Mudzi, Binga, Buhera and Mwenezi, which are always prone to droughts. Results indicated that farmers needed good quality drought tolerant seeds and that there is a need for extension training on selection, grading and post-harvest management of good quality local and recycled seed. Mubaiwa *et al.* (2018), carried out a survey in seven districts in semi-arid regions of rural Zimbabwe to gather knowledge on current production and utilization of Bambara groundnut, to assess its role in providing sustainable food and nutrition security for rural populations and to determine priorities. The survey targeted farmers in Uzumba, Binga, Buhera, Mudzi, Pfungwe, Lower Gweru and Bikita. Farmers in these districts use various processing techniques that include boiling, soaking, roasting and milling.

Masegela and Oluwatayo (2018), evaluated value chain mapping and marketing efficiency of smallholder cowpea farmers in Capricorn and Waterberg districts of Limpopo province in South Africa. The study revealed that 66% of the smallholder cowpea farmers were market efficient and 34% were market inefficient. It was also revealed that women were more involved in cowpea production than men. Maseko *et al.* (2018), assessed production and utilization in South Africa of African leafy vegetables. The assessment revealed that there is lack of suitable production systems, innovative processing, and value-adding techniques that promote utilisation of African leafy vegetables. It was also observed that African leafy vegetables are food for the poor among the youth and urban folks, while, among the affluent, they are highly regarded as being nutritious. In order to ascertain constraints and varietal trait preferences of sorghum producers in South Africa, Mofokeng *et al.* (2016), carried out a survey in Limpopo Province of South Africa. From the survey, farmers indicated that they grew both improved and local varieties. Bird damage, parasitic weeds, drought and storage pests were cited as the major obstacles threatening sorghum production in the area.

Technologies that do not meet farmers' preferences, objectives and conditions are less likely to be adopted (Sharma *et al.*, 2016). Farmers are more likely to assess a technology with criteria and objectives that are different from criteria used by scientists. However, farmers' and scientists' criteria for technology assessment are complementary and essential for effective research and technology development. Acceptance of the technology as a new farming method in the community will have an impact on its continued promotion and existence in the community. In this context, participation is crucial. Participatory research allows incorporation of farmers' indigenous technical knowledge, identification of farmers' criteria and priorities and definition of research agenda. There is a need to train farmers on new technology and innovations to improve their appreciation of the methods, especially in areas where farmers have limited opportunities for and exposure to formal education (Ntshangase *et al.*, 2018). Extension information is very vital and needs to be carefully disseminated in a way that is well understood by even those with little formal education.

Thompson *et al.* (2018), evaluated producers' perspectives of four key precision agriculture technologies (variable rate fertilizer application, precision soil sampling, guidance and auto steer, and yield monitoring) in terms of the benefits they provide to their farms. The results indicated that farmers' perceptions of the benefits derived from various precision agriculture technologies are diverse. Most farmers reported the benefits to be yield improvement or cost reduction. The reasons for differing perceptions was that farmers need information and benefits that accrue from an adoption of a technology to use.

Lunduka *et al.* (2017), carried out a survey to assess the impact of smallholder farmers' adoption of drought tolerant maize varieties on total maize production in two districts of Chiredzi and Chipinge in south-eastern Zimbabwe. Results showed that 93% of the households were growing improved maize varieties and that 30% of the sampled households were growing drought tolerant maize varieties. Total maize yield was 436.5 kg/ha for a household that did not grow drought tolerant maize varieties and 680.5 kg/ha for households that grew drought tolerant maize varieties. Maredia *et al.* (2019), evaluated farmer perception in seed quality in bean and seed auctions in Tanzania and Ghana. The study concluded that there was a need to increase the availability of qualitatively better performing seed that is affordable to smallholder farmers. It was also observed that there is a need to lower the cost of producing higher quality certified seed so that more farmers have access to the seed as this helps to have more vibrant seed system. So by integrating farmers' concerns and conditions into agricultural research, the

developed technologies may get widely adopted, resulting in more productive, stable, equitable and sustainable agriculture. Further, understanding farmers' preferences is important for successful adoption of improved varieties.

References

Abidoye, T.O., 2004. Effects of Soil Moisture Content on Growth and Yield of Cowpea (*Vigna unguiculata* (L.) Walp). Msc Agriculture Dissertation, University of Ilorin, Nigeria.

Ajeigbe H.A., Singh, B.B., Adeosun, J.O., and Ezeaku, I.E., 2010. Participatory On-Farm Evaluation of Improved Legume-Cereals Cropping Systems for Crop-Livestock Farmers. Maize-Double Cowpea in Northern Guinea Savanna Zone of Nigeria. African Journal of Agriculture Research 5: 2080-2088.

Alghamdi, S.S., Khan, M.A., Migdadi, H.M., El-Harty, E.H., Afzal, M., and Farooq, M., 2018. Biochemical and Molecular Characterization of Cowpea Landraces Using Seed Storage Proteins and SRAP Marker Patterns. Saudi Journal of Biological Sciences (2018). <https://doi.org/10.1016/j.sjbs.2018.09.004>.

Bates, L.M and Hall, A.E., 1981. Relationships between Leaf Water Status and Transpiration of Cowpea with Progressive Soil Drying. Oecologia 53.No. 3 pp. 285-289.

Balochi, F.S., Alasaleh, A., Shahid, M.Q., Ciftci, V., Saenze de Miera, L.E., Aaasim, M., Nadeem, M.A., Aktas, H., Özkan, H., and Hatipoğlu, R., 2017. A Whole Genome DArTseq and SNP Analysis for Genetic Diversity Assessment in Durum Wheat from Central Fertile Crescent. PLoS ONE 12(1):e0167821.doi:10.1371/journal.pone.016782.

Batieno, T.B.J., 2014. Breeding For Drought Tolerance in Cowpea [*Vigna Unguiculata* (L.) Walp.] Using Marker Assisted Backcrossing. PhD Thesis. West Africa Centre for Crop Improvement School of Agriculture College of Basic and Applied Sciences University of Ghana Legon.

Batieno, T.B.J., Souleymane, O., Tignegre, Huynh, B., Kusi, F., Poda, S.L., Close, T.J., Roberts, P., Danquah, E., Ofori, K., and Oudraogo, T.J., 2018. Single Nucleotide Polymorphism (SNP)-Based Genetic Diversity in a Set of Burkina Faso Cowpea Germplasm. African Journal of Agriculture Research 13: 978-987. doi: 10.5897/ajar2018.13121.

Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batiemo, J., Owusu, E., Haruna, M., Diallo, S., Umar, M.L., Olufajo, O., and Fatokun, C., 2018. Cowpea (*Vigna unguiculata*): Genetics, Genomics and Breeding. *Plant Breeding* 2019; 138:415–424. <https://doi.org/10.1111/pbr.12589>.

Cisse, N., Ndiaye, M., Thiaw, S., and Hall, A. E., 1995. Registration of Mouride Cowpea. *Crop Science*. 35, 1215–1216.

Da Silva A.C., Santos, D.C, and Junior, D.L.T., 2018. Cowpea: A Strategic Legume Species for Food Security and Health. *IntechOpen Chapter 3*. doi: 10.5772/intechopen.79006.

Deulvot, C., Charrell, H., Marty, A., Jacquin, F., Donnadieu, C., Lejeune-Hénaut, I., Burstin, J., and Aubert, G., 2010. Highly-Multiplexed SNP Genotyping for Genetic Mapping and Germplasm Diversity Studies in Pea. *BMC Genomics* 11:468-477.

Dingkhun, M., Singh, B.B., Clerget, B., Chantereau, J., and Sultan, B., 2006. Past, Present And Future Criteria to Breed Crops For Water-Limited Environments in West Africa. *Agriculture and Water Management* 80: 241–261.

Dube, E., and Fanadzo, M., 2013. Maximising Yield Benefits From Dual-Purpose Cowpea. *Food Security* 5:10.1007/s12571-013-0307-3.

Duc, G., Agrama, H., Bao, S., Berger, J., Bourion, V., De Ron, A.M., Gowda, C.L.L., Mikic, A., Millot, D., Singh, K.B., Tullu, A., Vandenberg, A., Patto, M.C.V., Thomas D. Warkentin, T.D., and Zong, X., 2015. Breeding Annual Grain Legumes for Sustainable Agriculture: New Methods to Approach Complex Traits and Target New Cultivar Ideotypes. *CRC. Critical Reviews in Plant Sciences* 34:381–411. doi: 10.1080/07352689.2014.898469.

Egbadzor, K.F., Ofori, K., Yeboah, M., Aboagye, L.M., Opoku-Agyeman, M.O., Danquah, E.Y., and Offei, S.K., 2014. Diversity in 113 Cowpea [*Vigna unguiculata* (L.) Walp] Accessions Assessed With 458 SNP Markers. *Springer Plus* 3:541.

Elawad, H.O.A., and Hall, A. E., 2002. Registration of Ein El Gazal Cowpea. *Crop Science* 42: 1745–1746. [10.2135/cropsci2002.1745](https://doi.org/10.2135/cropsci2002.1745).

FAOSTAT, 2017. <http://www.fao.org/faostat/en/#data/QC>.

Fatokun, C., Girma, G., Abberton, M., Gedil, M., Unachukwu, N., Oyatomi, O., Yusuf, M., Rabbi, I., and Boukar, O., 2018. Genetic Diversity and Population Structure of a Mini-Core Subset from The World Cowpea (*Vigna unguiculata* (L.) Walp.) Germplasm Collection. *Scientific Reports* 8:16035. doi 10.1038/41598-018-34555-9.

Gomes, A.M.F., Nhantumbo, N., Ferreira-Pinto, M., Ferreira-Pinto, M., Massinga, R., Ramalho, J.C., and Ribeiro-Barros, A., 2019. Breeding Elite Cowpea [*Vigna unguiculata* (L.) Walp] Varieties for Improved Food Security and Income in Africa: Opportunities and Challenges. *IntechOpen*. doi: dx.doi.org/10.5772/intechopen.84985.

Gonçalves A., Goufo P., Barros A., 2016. Cowpea (*Vigna unguiculata* L. Walp) A Renewed Multipurpose Crop for a More Sustainable Agri-Food System. *Nutritional Advantages and Constraints. Journal of the Science of Food and Agriculture* 96: 2941-2951. <https://doi.org/10.1002/jsfa>.

Goufo, P., Moutinho-Pereira, J. M., Jorge, T. F., Correia, C.M., Oliveira, M.R., Rosa, E.A.S., Carla, A., and Trindade, H., 2017. Cowpea (*Vigna unguiculata* L. Walp.) Metabolomics: Osmoprotection as a Physiological Strategy for Drought Stress Resistance and Improved Yield. *Frontiers in Plant Science* 8:586. <http://doi.org/10.3389/fpls.2017.00586>.

Gwathmey, C.O., and Hall, A.E., 1992a. Adaptation to Midseason Drought of Cowpea Genotypes with Contrasting Senescence Traits. *Crop Science* 32:773–778.

Gwathmey, C.O., Hall, A.E., and Madore, M.A., 1992b. Adaptive Attributes of Cowpea Genotypes with Delayed Monocarpic Leaf Senescence. *Crop Science*. 32:765–772.

Hall, A.E., and Patel, P.N., 1985. Breeding for Resistance to Drought and Heat, in *Cowpea Research, Production, and Utilization*, Eds S. R. Singh and K. O. Rachie .New York: John Wiley and Sons, 137–151.

Hall, A.E., Thiaw, S., Ismail, A.M., and Ehlers, J. D., 1997. Water-use efficiency and drought adaptation of cowpea, in *Advances in Cowpea Research*, eds Singh B. B., Mohan Raj D. R., Dashiell K. E., Jackai L. E. N., editors. (Ibadan, IITA), 87–98.

Hall, A. E., 2004. Breeding For Adaptation to Drought and Heat in Cowpea. *European Journal of Agronomy* 21: 447–454. doi:10.1016/j.eja.2004.07.005.

Hall, A.E., 2012. Phenotyping Cowpeas for Drought Adaptation. *Frontiers in Physiology* 3: 155. doi:10.3389/fphys.2012.00155.

Herniter, I.A., Muñoz-Amatriaín, M., Lo, S., Guyo, Y.N., and Close, T.J., 2018. Identification of Candidate Genes Controlling Black Seed Coat and Pod Tip Color in Cowpea (*Vigna unguiculata* [L.] Walp. *G3: Genes, Genomes and Genetics* 8: 3347-3355; <https://doi.org/10.1534/g3.118.200521>.

Huynh, B.L., Close, T.J., Roberts, P.A., Hu, Z., Wanamaker, S., Lucas, M.R., Chiulele, R., Cisse, N., David, A., Diop, N.N., and Ehlers, J.D., 2013. Gene Pools and the Genetic Architecture of Domesticated Cowpea. *Plant Genome* 6:8.

Ibitoye, D., 2015. Performance of Cowpea [*Vigna unguiculata* (L.) Walp] Hybrids under Drought Induced and Well-Watered Conditions. TEEAL Research Paper Competition: 1-19.

Igwe, D.O., Afiukwa, C.A., Ubi, B.E., Ogbu, K.I., Ojuederie, O.B., and Ude, G.N., Assessment of Genetic Diversity In *Vigna unguiculata* L. (Walp) Accessions Using Inter-Simple Sequence Repeat (ISSR) And Start Codon Targeted (Scot) Polymorphic Markers. *BMC Genetics* 18, Article number: 98.

IITA, 2009. Cowpea Project for Africa Research and Development. International Institute of Tropical Agriculture. Available at: <http://www.iita.org/crop/cowpea>.

Ismail, A.M., Hall, A. E., and Ehlers J. D., 2000. Delayed-Leaf-Senescence and Heat-Tolerance Traits Mainly Are Independently Expressed in Cowpea. *Crop Science* 40:1049–1055. doi:10.2135/cropsci2000.4041049x.

Iwuagwu, M.O., Ogonnaya, C.I., and Onyike, N.B., 2017. Physiological Response of Cowpea [*Vigna unguiculata* (L.) Walp.] To Drought: The Osmotic Adjustment Resistance Strategy. *Academic Journal of Science* 07(02):329–344.

Jiang, GL., 2013. Molecular Markers and Marker-Assisted Breeding in Plants. In: Anderson SB (ed.) *Plant Breeding from Laboratories to Field*. Croatia: In Technology 45-83.

Kilian, A., Sanewski, G., and Ko, L., 2016. The application of DArTseq technology to pineapple. *Acta Horticulturae*. 1111, 181-188 doi: 10.17660/ActaHortic.2016.1111.27 <https://doi.org/10.17660/ActaHortic.2016.1111.27>.

Kodrostami, M., and Rashimi, M., 2015. Molecular Markers in Plants: Concepts and Applications. Review Article. https://researchgate.net/publication/282954774_Molecular_Markers_in_Plants_Concepts_and_Applications.

Kouam, E.B., Ngompe-Deffo, T., Anoumaa, M., Pasquet, R.S., 2018. Preliminary Study on Character Associations, Phenotypic and Genotypic Divergence for Yield and Related Quantitative Traits among Cowpea Landraces (*Vigna unguiculata*) from the Western Highland Region of Cameroon. *Open Agriculture* 3:84-97. doi: <https://doi.org/10.1515/opag-2018-0009>.

Lee, N., and Thierfelder, C., 2017. Weed Control under Conservation Agriculture in Dryland Smallholder Farming Systems of Southern Africa. A Review. *Agronomy for Sustainable Development* 37: 48. <https://doi.org/10.1007/s13593-017-0453-7>.

Li, C., Fatokun, C.A., Ubi, B., Ubi, B., Singh, B.B., and Scoles, G.J., 2001. Determining Genetic Similarities and Relationships among Cowpea Breeding Lines and Cultivars by Microsatellite Markers. *Crop Science* 41:189-197. doi:10.2135/cropsci2001.411189x.

Lunduka, R.W., Mateva, K.I., Magorokosho, C., and Manjeru, P., 2017. Impact of Adoption of Drought-Tolerant Maize Varieties on Total Maize Production in South Eastern Zimbabwe. *Climate and Development* 11:1, 35-46. doi: 10.1080/17565529.2017.1372269.

Mafakheri, K., Bihamta, M.R., and Abbasi, A.R., 2017. Assessment of Genetic Diversity in Cowpea (*Vigna unguiculata* L.) Germplasm Using Morphological and Molecular Characterisation. *Cogent Food and Agriculture* 3:1.

Mammadov, J., Aggarwal, R., Buyyarapu, R., and Kumpatla, S., 2012. SNP Markers and Their Impact on Plant Breeding. *International Journal of Plant Genomics*: 1-12.

Maredia, M.K., Shupp, R., Opoku, E., Mishili, F., Reyes, B., Kusolwa, P., Kusi, F., and Kudra, A., 2019. Farmer Perception and Valuation of Seed Quality: Evidence From Bean and Cowpea Seed Auctions in Tanzania and Ghana .Agricultural Economics 2019:1–13.<https://doi.org/10.1111/agec.12505>.

Maruzani, N., 2014. Problems Faced by Rural Women in Buhera District of Manicaland South Province of Zimbabwe. Journal of Emerging Trends in Educational Research and Policy Studies (JETERAPS) 5(3): 370-376.

Masegela, C., and Oluwatayo, I., 2018. Value Chain Mapping and Marketing Efficiency of Smallholder Cowpea Farmers in Capricorn and Waterberg Districts of Limpopo Province. The 56th Annual Conference of Agriculture Economics of South Africa. 25-27 September 2018.

Maseko, I., Mabhaudhi, T., Tesfay, S., Araya, H.T., Fezzehazion, M., and Christian Phillipus Du Plooy, C.P., 2018. Review. African Leafy Vegetables: A Review of Status, Production and Utilization in South Africa. Sustainability 2018, 10: 16.

Mligo, J. K., and Singh, B. B., 2007. Registration of Vuli-1 Cowpea. Crop Science 47:437–438.

Mofokeng, M.A., Shimelis, H., Tongoona, P., and Laing, M.D., 2016. Constraints and Varietal Trait Preferences of Sorghum Producers in South Africa. Journal of Tropical Agriculture 54 (1): 7-15, 2016.

Mubaiwa, J., Fogliano, V., Chidewe, C., Bakker, E.J., and Linnemann, A.R., 2018. Utilization of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) for Sustainable Food and Nutrition Security in Semi-Arid Regions of Zimbabwe. PLoS ONE 13 (10): e0204817. <https://doi.org/10.1371/journal.pone.0204817>.

Muchero, W., Ehlers, J.D., Close, T.J., and Roberts P.A., 2009a. Mapping QTL For Drought Stress-induced Premature Senescence and Maturity in Cowpea [*Vigna unguiculata* (L.) Walp.]. Theoretical and Applied Genetics 118:849–863.

Muchero W, Diop, N.N., Bhat, P.R., Fenton, R.D., Wanamaker, S., Pottorff, M., Hearne S., Cisse, N., Fatokun, C., Ehlers, J.D., Roberts, P.A., and Close, T.J., 2009b. A Consensus Genetic Map of Cowpea [*Vigna unguiculata* (L) Walp.] and Synteny based on EST-derived SNPs. Proceedings of National Academy of Sciences USA 106: 18159–18164.

Muhammad, I., Ramlatu, M.A., and Zakari, B.G., 2015. Screening of Selected Varieties of Cowpea Seedlings [*Vigna unguiculata* (L.) Walp.] For Drought Tolerance. *Journal of Biology and Nature* 5:31-38.

Mujaju, C., and Mazvimavi, K., 2019. Seed Security Assessment of the 4 most Vulnerable Districts in Zimbabwe. [Agroecologyconference.co.za/wp-content/uploads/2019/02/Claid-Mujaju-Seed-security-assessment-Zimbabwe.pdf](http://agroecologyconference.co.za/wp-content/uploads/2019/02/Claid-Mujaju-Seed-security-assessment-Zimbabwe.pdf).

Nadeem, M.A., Nawaz, M.A., Shahid, M.Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G. Baloch, F.S., 2018. DNA Molecular Markers in Plant Breeding: Current Status and Recent Advancements in Genomic Selection and Genome Editing. *Biotechnology and Biotechnological Equipment*. 32:2, 261-285.doi: 10.1080/13102818.2017.1400401.

Nadeem, M., Li, J., Yahya, M., Sher, M., Ma, C., Wang, X., and Qiu, L., 2019. Research Progress and Perspective on Drought Stress in Legumes: A Review. *International Journal of Molecular Science* 10:2541.doi: 10.3390/ijms20102541.

Ndoro, O., Mvumi, C., Matizanadzo, P., and Manyiwo, S.A., 2017. Impact of Master Farmer Training on Smallholder Farmers' Rapoko *Eleusine Coracana* (L.) Productivity in Buhera District, Zimbabwe. *Journal of Sustainable Development in Africa* Volume 19 Number 3.

Ng, N.Q., and Marechal, R., 1985. Cowpea Taxonomy, Origin and Germplasm. In: Singh SR, Rachie, K.O., (Eds) *Cowpea Research, Production and Utilization*. John Wiley and Sons, Ltd., Chichester, NY. 11–21.

Ngaka, M. J., and Zwane, E.M., 2018. The Role of Learning Networks in Agricultural Extension Service Delivery: A Survey in the Nine Provinces of South Africa. *South African Journal of Agricultural Extension* Volume 45 Number 2: pp 26-37. <http://dx.doi.org/10.17159/2413-3221/2017/v45n2a398>.

Ntshangase, N.L., Muroyiwa, B., and Sibanda, M., 2018. Farmers' Perceptions and Factors Influencing the Adoption of No-Till Conservation Agriculture by Small-Scale Farmers in Zashuke, KwaZulu-Natal Province. *Sustainability* 10:555.

Odeseye, A.O., Amusa, N.A., Ijagbone, I.F., Aladele, S.E., and Ogunkanmi, L.A., 2018. Genotype by Environment Interactions of Twenty Accessions of Cowpea [*Vigna unguiculata* (L.) Walp.] Across Two Locations in Nigeria. *Annals of Agrarian Sciences* 4:481-489. doi: 10.1016/j.aasci.2018.03.001.

Oladipo, E., 2008. Climate Change and Sustainable Livelihoods: Greening Options for Nigeria. Report of the First National Environmental Summit on the Theme: Greening the Environment for Sustainable Development 83-95.

Olajide A.A., and Ilori O.C., 2017. Genetic Variability, Performance and Yield Potentials of Ten Varieties of Cowpea (*Vigna unguiculata* (L.) Walp) Under Drought Stress. *Legume Genomics and Genetics* 8: 17-25 (doi: 10.5376/lgg.2017.08.0003).

Olubnmi, I.D., 2015. Genetic Analysis of Drought Tolerance in Cowpea (*Vigna unguiculata* (L.) Walp.). Phd Thesis. University of Ghana.

Owusu, E.Y., Akromah, R., Denwar, N.N., Adjebeng-Danquah, J., Kusi, F., and Haruna, M., 2018. Inheritance of Early Maturity in Some Cowpea (*Vigna unguiculata* (L.) Walp.) Genotypes under Rain Fed Conditions in Northern Ghana. *Advances in Agriculture* Volume. 2018, Article ID 8930259, 10 pages, 2018. <https://doi.org/10.1155/2018/8930259>.

Oxfam, 2016. Our Seeds: Lessons Learnt from The Drought. Voices of Farmers in Zimbabwe. <https://www-cdn.oxfam.org/s3fs-public/bn-our-seeds-food-security-zimbabwe-151216-en.pdf>.

Pan, L., Wang, N., Wu, Z., Guo, R., Yu, X., Zheng, Y., Xia, Q., Gui, S., and Chen, C., 2017. A High Density Genetic Map Derived from RAD Sequencing and Its Application in QTL Analysis of Yield-Related Traits in *Vigna unguiculata*. *Frontiers in Plant Science* 8:1544. doi: 10.3389/fpls.2017.01544.

Pele, F.D., Yeboah, A., Buari, M.S., and Kofi, E.J., 2016. Morpho-Physiological Parameters Used in Selecting Drought Tolerant Cowpea Varieties Using Drought Index. *African Journal of Agriculture* 3: 125-134.

Petrie, C. L., and Hall, A. E., 1992. Water Relations in Cowpea and Pearl Millet under Soil Water Deficits: Contrasting Leaf Water Relations. *Australian Journal of Plant Physiology* 19: 577–589.

Qin, J., Shi, A., Mou, B., Bhattarai, G., Yang, W., Weng, Y., and Motes, D., 2017. Association Mapping of Aphid Resistance in USDA Cowpea (*Vigna unguiculata* L. Walp.) Core Collection Using SNPs. *Euphytica* 213: 36. <https://doi.org/10.1007/s10681-016-1830-z>.

Ravelombola W., Shi A., Weng Y., Mou B., Motes D., Clark, J., Chen, P., Srivastava, V., Qin, J., Dong, L., Yang, W., Bhattarai, G., and Sugihara, Y., 2018. Association Analysis of Salt Tolerance in Cowpea (*Vigna unguiculata* (L.) Walp) At Germination and Seedling Stages. *Theoretical and Applied Genetics* 131: 79–91.

Saka, J.O., Agbeleye, O.A., Ayoola, O.T., Lawal, B.O., Adetumbi, J.A., and Oloyede-Kamiyo, Q.O., 2018. Assessment of Varietal Diversity and Production Systems of Cowpea (*Vigna unguiculata* (L.) Walp.) In Southwest Nigeria. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* Vol. 119 No. 2 (2018): 43–52. <https://doi.org/10.17170/kobra-2018121864>.

Samejima, H., and Sugimoto, Y., 2018. Recent Research Progress in Combatting Root Parasitic Weeds. *Biotechnology and Biotechnological Equipment* 32: (2) 221-240. doi: 10.1080/13102818.2017.1420427.

Shackel, K. A., and Hall, A. E., 1979. Reversible Leaflet Movements In Relation To Drought Adaptation of Cowpeas, *Vigna unguiculata* (L) Walp. *Australian Journal of Plant Physiology* 6: 265–276.

Shackel, K. A., and Hall, A. E., 1983. Comparison of Water Relations and Osmotic Adjustment in Sorghum and Cowpea under Field Conditions. *Australian Journal of Plant Physiology* 10: 423–435.

Sharma, N., Sharma, A., and Sharma, J.P., 2016. Farmers' Preferences to Varietal Attributes as an Indicator for Acceptance and Adoption of Aromatic Rice (*Oryza sativa*) Varieties. *Indian Journal of Agricultural Sciences* 87:51-55.

Singh, B. B., and Matsui, T., 2002. Cowpea Varieties For Drought Tolerance, In Challenges and Opportunities for Enhancing Sustainable Cowpea Production, eds C. A. Fatokun, S. A. Tarawali, B. B. Singh, P. M. Kormawa, and M. Tamò (Ibadan: IITA) 287–300.

Suleiman, M.D., 2017. Sahel Region, Africa. <http://theconversation.com/sahel-region-africa-72569>.

Thiaw, S., Hall, A. E., and Parker, D. R., 1993. Varietal Intercropping and the Yields and Stability of Cowpea Production in Semiarid Senegal. *Field Crops Research* 33: 217–233.

Thompson, R., 2013. Genetic Analysis of Postharvest Physiological Deterioration in Cassava (*Manihot esculenta* Crantz) Storage Roots. PhD Thesis. University of Ghana.

Thompson, N.M., Bir, C., Widmar, D.A., and Mintert, J.R., 2018. Farmer Perceptions of Precision Agriculture Technology Benefits. *Journal of Agriculture and Applied Economics*. 1-22.

Turk, K. J., Hall, A. E., and Asbell, C. W., 1980. Drought Adaptation of Cowpea. Influence of Drought on Seed Yield. *Agronomy Journal* 72: 413–420.

Umdale, P., Ichikawa, Y., Manandhar, S., Ishidaira, H., and Kiem A.S., 2014. Farmers' Perception of Drought Impacts, Local Adaptation and Administrative Mitigation Measures in Maharashtra State, India. *International Journal of Disaster Risk Reduction* 10: 250-269

Wamalwa, E.N.I., Muoma, J., and Wekesa, C., 2016. Genetic Diversity of Cowpea (*Vigna unguiculata* (L.) Walp.) Accession in Kenya Gene Bank Based on Simple Sequence Repeat Markers. *International Journal of Genomics* 2016. <https://doi.org/10.1155/2016/8956412>.

Wang, K.H., and McSorley, R., 2018. Management of Nematodes with Cowpea Cover Crops <http://edis.ifas.ufl.edu>.

Xiong, H., Shi, A., Mou, B., Qin, J., Motes, D., Lu, W., Ma, J., Weng, Y., Yang, W., and Wu, D., 2016. Genetic Diversity and Population Structure of Cowpea (*Vigna unguiculata* L. Walp). *PLoS ONE* 11: e0160941. doi: 10.1371/journal.pone.0160941.

Xu, P., Jiang, Y., Xu, J., Li, J., and Sun, X., 2016. Genomics in the Common Carp. *Genomics in Aquaculture* 2016, Pages 247-274.

CHAPTER 3

FARMERS' PRODUCTION CONSTRAINTS, PERCEPTIONS, AND PREFERENCES OF COWPEA IN BUHERA DISTRICT, ZIMBABWE.

ABSTRACT

Many smallholder farmers face a lot of challenges in crop production, especially under rapidly changing climatic conditions. A survey was carried out to assess farmers' production constraints, traits, and preferred cowpea varieties. A semi-structured questionnaire was used in a survey of Buhera District, Zimbabwe, in March and April of 2018. Women farmers dominated the survey as they were 52% of the surveyed population, while men occupied 48% of the total population. Eighty-three percent of farmers cited the shortage, unavailability, and cost of fertiliser. Sixteen per cent of farmers acknowledged that they do not have access to quality seeds, and 1% cited labour as the major constraint in cowpea production. Cowpea yield varied from 100 to 500 kg/ha. However, 48% of farmers harvested 200 kg/ha. As for abiotic factors, farmers ranked heat (86%), drought (10%), and soil fertility (4%) as the most important abiotic factors. Ninety-one percent of farmers ranked rust as the most destructive disease, while 2% ranked storage rot, 1% ranked anthracnose, and 1% ranked downy mildew. Eighty-one percent of farmers cited aphids as the main pests, while 3% ranked thrips, 3% ranked legume borers, and 2% ranked pod borers as other pests. Fifty-two percent of farmers preferred varieties that are resistant to diseases such as rust, whereas 48% were not concerned about diseases. As for qualitative traits, 50% of farmers had no specific colour preference, 32% preferred white colour, 14% preferred brown colour, 3% preferred red colour, and 1% preferred tan colour. For quantitative traits, such as grain size, pod size, plant height, and head size, the preferences of farmers varied. Ninety-nine percent of the farmers interviewed preferred cowpea varieties that are bred for drought tolerance, as Buhera District is frequented by intermittent droughts. Farmers' experience in growing cowpeas ranged from 5 to 30 years. The top ranked accessions were CBC1, IT 18, and *Chibundi Chitsvuku*, while the least ranked was *Kangorongondo*. Identified constraints to cowpea farming included education, insect pests, diseases, drought, weeds, harvesting difficulties and a lack of extension. The survey showed that there is a need to breed for both biotic and abiotic factors such as drought and moisture stress, as well as against pests and diseases.

Key words: Buhera District, cowpeas, production constraints, variety preference, Zimbabwe

3.1: Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] has been grown and used in Africa for many years and is considered to be the single most important pulse in the dry areas of tropical Africa (FAOSTAT, 2018). This is because cowpea is able to thrive in challenging environmental conditions, such as sandy soils, and with little rainfall. It provides strong support to the livelihoods of many small-scale farmers through its contributions to their nutritional security, income generation, and soil fertility enhancement (Mfeka, 2017).

Cowpea is an important grain and fodder legume grown around the world. It is a dual-purpose grain–legume crop, providing food for both humans and livestock. Cowpea is identified as a potential crop to diversify food production, minimise production input by improving soil fertility, and improve the seed micronutrients, thereby improving human nutrition (Mfeka, 2017). It contains about 24% protein, 62% carbohydrate, and a minute amount of other nutrients (Olalekan and Bosede, 2010). High protein and carbohydrate contents with relatively low fat content and a complementary amino acid pattern to that of cereal grains make cowpea an important nutritional food in the human diet. Cowpea use worldwide is gaining prominence because of its exerted health beneficial properties, including anti-diabetic, anti-cancer, anti-hyperlipidaemic, anti-inflammatory, and anti-hypertensive properties (Chathuni *et al.*, 2018). The presence of compounds, such as soluble and insoluble dietary fibre, phytochemicals, and proteins and peptides, in cowpea have been cited as preventing chronic diseases in humans.

Asiwe (2009) observed that the research and production of cowpea has been neglected in South Africa in the last three decades because of a lack of funding and interest of researchers to work on improving the crop. Therefore, cultivated varieties are unimproved and the lack of knowledge of good agronomic practices increase the limitations to cowpea production. It was also observed that cowpea yields among farmers in Africa are generally low, averaging 275 kg/ha per season (FAOSTAT, 2017).

Surveys and interviews can help on information exchange so that farmers and consumers have more options. Through participatory plant breeding, many different lines can be released that meet both local and market preferences for taste and other seed traits (Witcombe *et al.*, 2005). Farmer-developed local varieties are an important resource and logical starting point for plant breeding programmes that seek to strengthen these diverse systems. There is a need for cooperation between scientists and farmers in evaluating plant material and in establishing

plant-breeding goals through participatory research. In this regard, extension has been shown to be more effective when client-oriented, focusing on supporting farmer experimentation and participatory approaches (Johnson *et al.*, 2003). There are numerous opportunities to support farmer experimentation and local adaptation to—and adoption of—technical options. This process can be promoted through education and policy initiatives.

Participatory approaches to extension that involve farmer experimentation are essential for the adoption and adaptation of legumes to local environments. Collection of landraces and a diverse germplasm are also essential to enhance local variations in genetic resource options that can support farmer experimentation. Sibiya *et al.* (2013), concur that it is important to determine farmers preferred traits in crop varieties or involve farmers in a variety of selection processes. This ultimately enhances the potential for adoption of the varieties in the respective communities where studies are conducted. The agronomic and socio-economic requirements of smallholder farmers and consumers in marginal or stressed environments are very broad and diverse to be satisfied by a limited number of genotypes; hence, plant breeding must be able to make use of and maintain the crop diversity that is available from farmers (Nkongolo *et al.*, 2008). Through surveys and interviews a lot of valuable scientific information has been gathered that can be very helpful in developing sustainable breeding programmes.

A survey approach on farmers' production constraints, perceptions, and preferences using a semi-structured questionnaire was carried out i) to determine farmers' perceptions on major constraints limiting cowpea production and ii) to identify preferences regarding cultivars and traits. The hypothesis tested was that farmers are very much aware of production constraints and that they prefer certain traits and cultivars in cowpeas.

3.2: Materials and Methods

A survey was carried out to assess the farmers' production constraints and traits and types of cowpea varieties that they prefer. Random sampling was used to select participants at ward level and a semi-structured questionnaire was used. The questionnaire had four components: demographic information, cowpea farming systems (farm size, land allocated to cowpea and other main crops, and varieties grown), cowpea production constraints, and trait preferences of cowpea varieties.

3.3: Description of the study area

The study was carried out in March and April of 2018 in Buhera District of Manicaland Province, in Zimbabwe. The coordinates of Buhera District are: 19° 19' 57.00"S, 31° 26' 6.00"E Latitude:-19.3325; Longitude: 31.4350 and is 1 190 m above sea level. Buhera District is a rural district and the local economy depends mainly on farming. About 689 mm of precipitation falls annually. Buhera is a dry low lying area with low rainfall and exhausted infertile sandy soils contributing to poor harvests and food shortages. Main crops grown in the district are maize, sorghum, millet, groundnut, cowpeas and groundnuts. The rains are not very reliable, the area is fertile and a few irrigation schemes supplement the populace's meager harvests.

3.4: Data Analysis

Statistical analysis was performed using IBM SPSS Statistics Version 20 computer package. Tables, means, and percentages were used to summarise the data.

3.5: Results

A total of 100 farmers were sampled for a structured survey as presented in Table 3.1. The results show that women farmers dominated the survey with 52% of the surveyed population, while men occupied 48% of the total population. This confirms that cowpea production is predominantly performed by women in Buhera District. The level of education of the farmers varied with 58% having been to secondary school, 28% with a primary school education, and 14% having attained tertiary level qualifications. Only 27% of the farmers were trained in cowpea production by extension officers or during tertiary training, while 73% of farmers had not received any training.

Cowpea yield per season varied considerably among the farmers. Forty eight per cent of the farmers harvested 200 kg, 32% harvesting 150 kg, 12% harvesting 250 kg, 2% harvesting 300 kg, 2% harvesting 500 kg, and 4% harvesting 100 kg of cowpeas per season or less. Apart from cowpeas, 83% of the farmers interviewed grew maize, 16% grew sorghum, and 1% grew groundnuts 1%. Maize and sorghum are important staple crops for the district. Most farmers intercrop cowpeas with sorghum, millet, maize, finger millet, and sunflower. Forty-nine percent of farmers had a total plot size of 2 ha, 43% had 1 ha, 7% had 3 whereas 1% had 4 ha

and above. Twenty-seven percent of farmers had six household members, 19% had five, 13% had eight, 9% had four, 4% had nine, 3% had three, and 2% had 10.

Table 3.1: Number of farmers interviewed and distribution of respondents and cowpea farming systems.

	Males	Females	Total
Number of farmers interviewed	48	52	100
Level of education	Frequency	Percent	Cumulative
Primary	28	28	28
Secondary	58	58	86
Tertiary	14	14	100
Extension Services			
Training in cowpea production	27	27	27
No training in cowpea production	73	73	100
Cowpea yields	Frequency	Percent	Cumulative
100kg	4	4	4
150kg	32	32	36
200kg	48	48	84
250kg	12	12	96
300kg	2	2	98
500kg	2	2	100
Other Crops Grown	Frequency	Percent	Cumulative
Maize	83	83	83
Groundnuts	1	1	84
Sorghum	16	16	100
Farm Size	Frequency	Percent	Cumulative
1ha	43	43	34
2ha	49	49	92
3ha	7	7	99
4ha	1	1	100
No of Members per Household	Frequency	Percent	Cumulative
3	3	3	3
4	9	9	12
5	19	19	31
6	27	27	58
7	23	23	81
8	13	13	94
9	4	4	98
10	2	2	100

Farmers identified several constraints to cowpea production as shown in Table 3.2. Eighty-three percent of farmers cited the shortage, unavailability, and cost of fertiliser; 16% of farmers acknowledged that they do not have access to quality seed; and 1% cited labour as the major

constraint in cowpea production. On major field pests, 81% of farmers identified aphids as the major pests, while 3% identified thrips, 3% identified legume pod borers, and 2% identified stem and stalk borers. Only 11% were not able to positively identify any field pest. With regards to diseases, 91% of farmers' ranked rust as the major disease, 2% ranked storage rot, and 1% ranked anthracnose. Five percent of farmers were not able to identify problematic diseases in storage.

In cowpea production most smallholder farmers do not consult or attend field days with extension officers. Cowpeas are mostly grown in Buhera District through experience passed on from generation to generation. All farmers concurred that weevils are the major pest in storage. This was compounded by the high temperatures of above 45 °C (Katsande, 2016) in Buhera District, which caused weevils to breed and multiply faster. A total of 55% of farmers acknowledged the existence of parasitic weeds, such as *Striga* species, and the damage caused by weeds, while 45% did not experience any problematic weeds. Thirty-four percent of farmers concurred that at times, low market prices affect the selling of cowpeas, especially in years that there are good harvests. *Striga gesnerioides* causes yield losses ranging from 40 to 70%, and total crop losses of susceptible cultivars have been reported (Ayeni *et al.*, 2017). Most of the damage to the crop occurs even before the weed emerges from the soil, where it produces thousands of seeds, thereby making weeding an ineffective option in reducing yield losses.

On abiotic factors, the farmers interviewed ranked heat (86%), drought (10%), and soil fertility (4%) as the most important abiotic factors. Most farmers grow cowpeas without use of inorganic fertilisers as they are perceived as expensive and unaffordable in this drought-prone region. Most farmers would prefer using fertilisers on other staple crops, such as maize and sorghum.

Fifty percent of farmers had no specific colour preference, while 32% preferred white, 14% preferred brown, 3% preferred red, and 1% preferred tan. Ninety-four percent of interviewed farmers were not concerned about the pod shape, while 3% preferred the kidney, 2% preferred the spherical and 1% preferred the globular shapes.

On variety preference, 99% of farmers agreed that they needed high-yielding varieties per unit area and only 1% were unsure. Fifty-two percent of farmers preferred varieties that are resistant to diseases such as rust, whereas 48% were not concerned about diseases. Farmers acknowledged that rust was a major problem when cowpeas were planted with cereals such as

millet and sorghum. Farmers intercropped cowpea with other crops because of limited hectareage and high cost of inputs such as fertiliser and seeds. Ninety-nine percent of the farmers interviewed preferred cowpea varieties that are bred for drought tolerance, as the Buhera District is frequented by intermittent droughts. In 2015, Buhera District suffered one of its worst farming seasons because of an *El Niño*-induced drought (Mutizwa, 2016). It is against this background that smallholder farmers in the district prefer growing crops that are drought tolerant as they are the hardest hit by changing weather patterns especially erratic rainfall.

For post-harvest insect pests such as weevils, 94% of the farmers preferred varieties that are resistant while 6% did not consider it a priority. For maturity period, 59% preferred early maturing varieties while 36% had medium to late maturing varieties as preferences. Others (5%) preferred the late maturing varieties as their leaves can be cooked and sundried as a vegetable for later use. This forms an important component of farmers' diets when green vegetables are scarce as the region is prone to severe and frequent droughts.

Thirty-four percent of farmers concurred that low market prices were a major problem especially when selling cowpeas after harvest whereas 66% of farmers were not concerned about the market price as the crop was saved mainly for home consumption.

For quantitative traits such as grain size, pod size, plant height, and head size, the preferences of farmers varied. Forty-four percent of farmer respondents preferred larger cowpea grains while 56% were not concerned about the size of the grain. A paltry 2% of farmers were interested in pod size while 98% did not regard it as important. Thirteen percent of the farmers were interested in climbing varieties while 87% considered high grain yield as of the utmost importance. Three percent of farmers preferred cowpeas with plenty of biomass for livestock feed, while 97% were for grain yield as the most preferred trait.

Table 3.2: Production constraints and farmers seed trait preferences.

Category	Item	Frequency	Percent	Cumulative
Inputs	Fertiliser	83	83	83
	Seed	16	16	99
	Labour	1	1	100
Field pests	Aphids	81	81	81
	Thrips	3	3	84
	Pod borer	3	3	87
	Stem borer	2	2	89
	None	11	11	100
Diseases	Downey mildew	1	1	1
	Rust	91	91	92
	Anthraco nose	1	1	93
	Storage rots	2	2	95
	None	5	5	100
Post-harvest pests	Weevils	100	100	100
Weeds	Parasitic weeds			
	Yes	55	55	55
	No	45	45	100
Abiotic factors	Drought	10	10	10
	Soil fertility	4	4	14

	Heat	86	86	100
Qualitative traits	Grain colour			
	White	32	32	32
	Tan	1	1	33
	Brown	14	14	47
	Red	3	3	50
	None	50	50	100
	Pod shape			
	Spherical	2	2	2
	Kidney	3	3	5
	Globular	1	1	6
	None	94	94	100
Yield	High yield			
	High	99	99	99
	Not sure	1	1	100
Resistance/tolerance	Resistance to diseases			
	Resistant/tolerant	52	52	52
	None	48	48	100
	Tolerance to drought			

	Tolerant	99	99	99
	Not sure	1	1	100
	Resistance to storage Pests			
	Resistant	94	94	94
	None	6	6	100
Maturity	Maturity period			
	Early	59	59	59
	Medium	36	36	95
	Late	5	5	100
Other	Low market prices			
	Low prices	34	34	34
	Not sure	66	66	100
Quantitative traits	Grain size			
	Large grains	44	44	44
	Any types	56	56	100
	Head size			
	Large	2	2	2
	Not interested	98	98	100

	Plant height			
	Climbing types	13	13	13
	Not interested	87	87	100
	Biomass			
	Large biomass	3	3	3
	Not interested	97	97	100

Some cowpea landraces grown include *Barapara*, *Chibundi*, *Ziso*, *Kangorongondo*, *Mutonono*, *Dahwa*, *Zvenyika*, and *Mupengo*, while other cowpea hybrids grown include IT18, CBC1, and CBC2 (Table 3.3). The top ranked accessions were CBC1, IT18, and *Chibundi Chitsvuku*, while the least ranked was *Kangorongondo*.

Table 3.3: Cowpea varieties grown by farmers in Buhera District and their characteristics.

Variety	Characteristic and colour	Growth habit	Advantages	Maturity	Purpose	Rank
<i>Barapara purple</i>	Purple, landrace	Upright and bushy	Locally available	Late	Grain/Leaves	9
<i>Mupengo black</i>	Black, landrace	Upright and bushy	Locally available	Late	Grain/Leaves	13
<i>Chibundi chemavara</i>	Speckled/brown, landrace	Upright and bushy	Locally available	Late	Grain/Leaves	11
<i>Dahwa</i>	Brown, landrace	Upright and bushy	Locally available, sweet, good cooking qualities	Late	Grain/Leaves	10
<i>Chibundi chitsvuku</i>	Brown, landrace	Upright and bushy	Locally available	Late	Grain/Leaves	3
<i>Barapara jena</i>	White, landrace	Upright and bushy	Locally available	Late	Grain/Leaves	6
<i>Mupengo mavara</i>	Brown, landrace	Upright and bushy	Locally available	Late	Grain/Leaves	7
<i>Ziso dema</i>	Black and white, landrace	Upright and bushy	Locally available	Late	Grain/Leaves	12
<i>Barapara mavara</i>		Upright and bushy	Locally available	Late	Grain/Leaves	4
<i>Kangorongondo</i>	White, landrace	Climber	Locally available	Early	Grain	15
<i>Mutonono</i>	Brown, landrace	Upright and bushy	Locally available	Late	Grain/Leaves	14
<i>Zvenyika</i>	Brown, landrace	Upright and bushy	Locally available	Late	Grain/Leaves	5
CBC 1	Brown, hybrid	Upright and bushy	Good emergence, high yield	Early	Grain/Leaves	1
CBC 2	Brown, hybrid	Upright and bushy	Good emergence, high yield	Early	Grain	8
IT 18	Brown, hybrid	Upright and semi-bushy	Good emergence, high yield	Early	Grain/Leaves	2

Farmers' experience in growing cowpeas varied from 5 to 30 years (Table 3.4). From the survey, farmers with 15 and 20 years of experience dominated the group, both at 11%. Nine percent of the farmers had 10 years of experience in growing cowpeas, while those with 8 and 11 years of experience were both at 8% each. The lowest number of farmers were those with 22, 27, and 30 years of experience, who totalled 1% of the population each.

Table 3.4: Years of experience in growing cowpeas.

Years	Frequency	Percent	Cumulative percent
5	3	3	3
7	6	6	9
8	8	8	17
9	4	4	21
10	9	9	30
11	8	8	38
12	5	5	43
13	3	3	46
14	5	5	51
15	11	11	62
17	4	4	66
18	3	3	69
19	4	4	73
20	11	11	84
21	3	3	87
22	1	1	88
23	4	4	92
24	2	2	94
25	2	2	96
26	2	2	98
27	1	1	99
30	1	1	100

3.6: Discussion

The survey indicated that cowpea is a women-grown crop, as 52% of the participants were women and 48% were men, which concurs with the studies by ACB (2015) and Sirinathsinghji (2015). Women play a pivotal role in food security as they tend to feed children while most men opt to look for work in towns. Farmers highlighted the importance of drought tolerance, high yield potential, early maturity, pest and disease resistance, and sweet taste as the most important traits in cowpea varieties. Salifou *et al.* (2017) also observed the same preferred traits

such as earliness, high yield potential, sweet taste, and tolerance to insect pests and diseases in cowpea production in Niger. In Ghana, Egbadzor *et al.* (2013) observed that farmers preferred cowpeas with large, cream-coloured seeds, which are sweet and easy to cook.

From the survey, all participants concurred that the majority of cowpeas were to be used for domestic consumption. This is probably because Buhera District is generally dry and receives less than 650 mm of annual average rainfall; hence is an important food crop for the district (Chinokwetu and Togo, 2018). Farmers grow cowpea for food security and, in years of surplus, mostly for cash. Most farmers in the district preferred early cowpeas as a supplement to major staple food crops such as maize, sorghum, and pearl millet. Cowpea is mostly grown for its protein content and as a buffer crop against total crop failure due to recurrent droughts in the district. Some farmers indicated that financial needs were the reason for growing cowpea as a cash crop.

The survey also underscored the need for extension services to be up-scaled. Most farmers in the district rely on knowledge passed on from generation to generation, while it is necessary to fuse the knowledge gained with modern farming techniques. Farming requires improved decision-making in the use of resources, and linking farmers to markets and extensionists can aptly fill this void. To be successful, farmers need the skills to produce what the market wants and what satisfies consumers. Extension workers may also help farmers in dissemination information on controlling weevils especially in storage and the use of resistant cowpea cultivars. Weevils cause significant damage in storage and are a menace in storage. Other low cost traditional controlling measures can be applied to control weevil scourge in the district.

Small-scale farmers mostly grow cowpea intercropped with maize, millet, sorghum, finger millet and cotton. This is highly beneficial through nitrogen fixation as cowpea is a legume and animal fodder, and can be used for soil erosion protection. Buhera District is characterised by its loose sandy soils prone to erosion. Intercropping is highly beneficial in controlling high rates of soil erosion. Muzezewa and Gwata (2015) observed that when cowpea is intercropped with sunflower, it reduces the evaporation of soil moisture by increasing the soil cover. Several interventions are needed to enhance the uptake and growth of cowpea in the district so as to meet the food security and income needs of smallholder farmers. Yields are generally low in Buhera District among all crops grown; they are severely affected by intra- and inter-seasonal rainfall variability and recurrent droughts. Drought is a perennial problem in Buhera; over the

years, *El El-Niño* induced drought has ravaged the district, leaving dry land patches and massive livestock deaths (Moyo, 2016). Therefore, stable and, high-yield, early maturing varieties are needed that can withstand drought stress. There is a need to upscale extension services to farmers and integrate this with locally available techniques so as to increase cowpea production and mitigate against the effects of recurrent droughts in the district. Cowpea can be regarded as the backbone of sustainable farming in semi-arid lands (DAFF, 2014).

During the 2016/2017 farming season, Manicaland had the lowest proportion of planted legumes at 26%, of which cowpea was included (ZimVAC, 2016). In the 2015/2016 farming season, most of the cowpea seed planted was from retained stock and 25% was bought from retail outlets (ZimVAC, 2016). However in southern Africa in 2016/2017, more than 60% of cowpea seed used by farmers was recycled or retained seeds (Mazvimavi *et al.*, 2016). From the survey, it was concluded that the majority of the seeds planted by farmers were sourced from on-farm seed saved from past harvests. This might have contributed to the poor harvest; thus, there is a need for the provision of hybrid early maturing seeds at affordable prices for farmers to purchase. Farmers highlighted several concerns with retained seeds, such as poor emergence, weevil attacks, loss of vigour, and poor yields. There is thus a need to make sure that quality high-yield seeds are available for farmers so that they plant on time. This can be aptly achieved through the conveyance of improved quality seeds through formal channels such as agro-dealers and farmer groups.

3.7: Conclusion

Farmers noted the absence of new cowpea varieties and generally low cowpea yields. They also recognised and highlighted declining soil fertility and increasing effects of drought. Farmers' knowledge of plant, soil and water interactions is lacking and thus they have formed misconceptions about their current farming practices. Rapidly changing climatic conditions especially with respect to high temperatures and reduced rainfall have played havoc on cropping systems in the district. However from the survey farmers showed willingness to try new cowpea varieties as well as get consistent agronomic information from extension workers.

The identified constraints to cowpea farming included insect pests, diseases, drought, *Striga* spp. weeds, and harvesting difficulties. This shows that there is a great need to breed for both

biotic and abiotic factors, such as drought and moisture stress, as well as against diseases and pests.

References

- ACB, 2015. GM and Seed Industry Eye Africa's Lucrative Cowpea Seed Markets: The Political Economy of Cowpea in Nigeria, Burkina Faso, Ghana and Malawi. African Centre for Biodiversity. <https://acbio.org.za/wp-content/uploads/2015/07/GM-Cowpea-report.pdf>.
- Asiwe, J.A.N., 2009. Needs Assessment of Cowpea Production Practices, Constraints and Utilization in South Africa. *African Journal of Biotechnology* 820: 5383-5388.
- Ayeni, D.F., Omoigui, L. O.N., and Ikwebe, J., 2017. Response of Cowpea Genotypes to Striga Gesnerioides Infection under Varied Phosphorus Levels. *AASCIT Journal of Bioscience* 5: 40-46.
- Chathuni, J., Rizliya, V., Afka, D., Ruksheela, B., Barana, C.J., Srinivas, N., and Ruvini, L., 2018. Cowpea: An Overview on Its Nutritional Facts and Health Benefits. Review. *Journal of the Science of Food and Agriculture* 13:4793-4806. <https://doi.org/10.1002/jsfa.9074>.
- Chinokwetu, V., and Togo, M., 2018. Examining Barriers and Opportunities for Sustainable Adaptation to Climate Change for Smallholder Farmers in Semi-Arid Buhera District, Zimbabwe. Conference Proceedings of Adaptation Futures 2018. <https://adaptationfutures2018.capetown/wp-content/uploads/2019/01/AF18-Conference-Proceedings.pdf>.
- DAFF, 2014. Production Guidelines for Cowpea. Department of Agriculture Forestry and Fisheries. [https://www.daff.gov.za/daffweb3/PortalsBrochures and Production Guidelines / Cowpea Production Guidelines.2014.pdf](https://www.daff.gov.za/daffweb3/PortalsBrochures%20and%20Production%20Guidelines%20for%20Cowpea%20Production%20Guidelines.2014.pdf).
- Egbadzor, K.F., Yeboah, M., Offei, S.K., Ofori, K., and Danquah, E.Y., 2013. Farmers' Key Production Constraints and Traits Desired In Cowpea in Ghana. *Journal of Agricultural Extension and Rural Development* 5: 14-20. [http:// academicjournals.org/jaerd](http://academicjournals.org/jaerd) doi: 10.5897/jaerd12.118.
- FAOSTAT, 2017. FAO Statistics available at: <http://faostat3.fao.org/download/Q/QC/E>.
- FAOSTAT, 2018. Pulse Crops for Sustainable Farms in Sub-Saharan Africa. <http://www.fao.org/3/I8300EN/i8300en.pdf>.

Johnson, N.L., Lilja, N. and Ashby, J.A., 2003. Measuring the Impact of User Participation in Agricultural and Natural Resource Management Research. *Agricultural Systems* 78: 287–306.

Katsande, I., 2016. Climate Change Adaptation Success in Buhera. [http:// www.sustainzim.org/climate-change-adaptation-success-buhera/](http://www.sustainzim.org/climate-change-adaptation-success-buhera/).

Mazvimavi, K., Murendo, C., and Chivenge, P., 2016. The Impacts of the El Niño-Induced Drought on Seed Security in Southern Africa: Implications for Humanitarian Response and Food Security. [http://oar.icrisat.org/10279/1/Final Consolidated Seed Security Assessment Report.pdf](http://oar.icrisat.org/10279/1/Final%20Consolidated%20Seed%20Security%20Assessment%20Report.pdf).

Mfeka, N., 2017. Morphology and Mineral Content of Cowpea Lines in Response to Planting Date and Zinc Application Rate. Masters of Technology Thesis. Cape Peninsula University of Technology: 25-68.

Moyo, J., 2016. El Nino-Induced Drought in Zimbabwe. <http://www.ipsnews.net/2016/04/El-Nino-induced-drought-in-Zimbabwe/>.

Mutizwa, G., 2016. Zimbabwe's Poorest Feel El Nino Pain as Drought Withers Harvests, Kills Livestock. https://www.unicef.org/zimbabwe/media_18098.html.

Muzezewa, J., and Gwata, E.T., 2015. Analysis of Soil Water Storage under Sunflower x Cowpea Intercrop in the Limpopo Province of South Africa. *Alternative Crops and Cropping Systems*. Petr Konvalina. IntechOpen. doi: 10.5772/62764.

Nkongolo, K.K., Chinthu, L., Malusi, M., and Vokhiwa, Z., 2008. Participatory Variety Selection and Characterization of Sorghum (*Sorghum Bicolor* (L.) Moench) Elite Accessions from Malawian Genepool Using Farmer and Breeder Knowledge. *African Journal of Agricultural Research* 3: 273-283.

Olalekan, A.J., and Bosede, B.F., 2010. Comparative Study on Chemical Composition and Functional Properties of Three Nigerian Legumes (Jack beans, pigeon pea and cowpea). *Journal of Emerging Trends in Engineering and Applied Sciences* 1: 89–95.

Salifou, M., Tignegre, J.B.L.S., Tongoona, P., Ofei, S., Ofori, K., and Danquah, E., 2017. Farmers' Preferred Traits and Perceptions of Cowpea Production and Constraints in Niger. *Journal of Agriculture and Food Technology* 7:1-11.

Sibiya, J., Tongoona, P., Derera, J., and Makanda, I., 2013. Farmers' Desired Traits and Selection Criteria for Maize Varieties and Their Implications for Maize Breeding: A Case Study from Kwazulu-Natal Province, South Africa. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* 114: 39–49.

Sirinathsinghji, E., 2015. GM African Cowpea to Enter African Markets. http://www.i-sis.org.uk/GM_African_Cowpea_to_Enter_African_Markets.php.

Witcombe, J.R., Joshi, K. D., Gyawali, S., Musa, A.M., Johansen, C., Virk, D.S., and Sthapit, B.R., 2005. Participatory Plant Breeding Is Better Described As Highly Client-Oriented Plant Breeding. Four Indicators of Client-Orientation in Plant Breeding. *Experimental Agriculture* 41: 299–319.

ZimStat, 2015. Statistics for Poverty Eradication. www.zimstat.co.zw.

ZimVac, 2016. Rural Livelihood Assessment. cluster.org/sites/default/files/documents/zimvac_2016_rural_livelihoods_assessment_pdf.

CHAPTER 4

GENETIC DIVERSITY ANALYSIS OF COWPEA [*Vigna unguiculata* (L.) Walp.] ACCESSIONS USING DIVERSITY ARRAYS TECHNOLOGY SEQUENCING (DArTSeq) TECHNIQUE.

ABSTRACT

Cowpea is a legume crop that is used for various purposes, ranging from food, feed and green manure. Assessment of its genetic diversity is of paramount importance for selections and successful breeding programmes. The objective of this study were to assess the genetic diversity and examine the population structure among cowpea accessions using genotype by sequencing. A total of 85 cowpea accessions were investigated in this study, using 18,284 SNPs. In this study, only 51% of the SNPs were polymorphic across the 85 accessions and fulfilled the selection criteria. The genetic distance, estimated based on Nei's index among genotypes, ranged from 0.14 to 0.44, with a mean value of 0.35. The PIC ranged from 0.024 to 0.50, with a mean value of 0.25. Twenty-six percent of the SNPs had genetic diversity values of greater than 0.40, suggesting that the genotypes were highly genetically diversified. A high gene flow (N_m) of 4.89 was observed between Zimbabwean and South African accessions, indicating a high germplasm exchange among these neighbouring countries. The analysis of molecular variance revealed highly significant variation among individual accessions and low variation within individuals. The accessions showed significant ($p < 0.001$) but low levels of differentiation among geographic regions. Cluster analysis of the 85 accessions generated by the unweighted pair group method with arithmetic mean (UPGMA) procedure clustered the accessions into three distinct groups. The clustering patterns showed that accessions collected from the same geographic regions tended to cluster in the same group. This technique revealed the existence of high levels of genetic diversity among cowpea accessions collected from Southern Africa and Nigeria. However, the low variation within individuals suggested the need to further widen the genetic base of the crop. The molecular data should be backed by morphological data to link the SNP markers that are associated with desirable agronomic attributes, such as high tolerance to biotic and abiotic stress factors.

Keywords: DArTSeq, genetic diversity, single nucleotide polymorphism, *Vigna unguiculata* (L.) Walp.

4.1: Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.], native to Africa and is a primary source of protein in sub-Saharan Africa. It is grown and used as fresh or dry grains and foliage, for food and forage (Muñoz-Amatriaín *et al.*, 2016). The ability of cowpea to produce stable yields under abiotic stress conditions (drought, heat, low soil fertility) and to replenish nitrogen back into the soil (Muchero *et al.*, 2009) makes it an important crop in low-input farming systems. The yield of cowpea is generally low in developing countries because of the lack of improved cultivars, low input use, and poor management practices (Boahen *et al.*, 2017).

The recent advances in molecular biology have made molecular characterisation of the germplasm, genetic mapping and development of quantitative trait loci (QTL) easier (Boukar *et al.*, 2018) in different cowpea research programmes. In cowpeas, different marker technologies have been used, such as restriction fragment length polymorphism (RFLP) (Boukar *et al.*, 2016), amplified fragment length polymorphism (AFLP) (Kolade *et al.*, 2016), single sequence repeat (SSR) (Wamalwa *et al.*, 2016), random amplified polymorphic DNA (RAPD) (Damarany *et al.*, 2018), and single nucleotide polymorphisms (SNPs) (Chidebe *et al.*, 2018). Boukar *et al.* (2018) indicated that molecular marker techniques are now being widely used in genetic diversity analysis, variety identification, phylogenetic analysis, gene mapping, and resource classification. Damarany *et al.* (2018), used RAPD assays to identify DNA markers in seven cultivars of cowpea and also to evaluate the proportions of genetic similarities among the cultivars. Positive and negative markers can serve as both a positive and a negative marker by conferring an advantage to the host under one condition, but inhibiting growth under a different condition (Damarany *et al.*, 2018). In cowpeas, a total of 25 simple sequence repeat (SSR) primers were identified and used to differentiate a new cowpea variety, VBN 3, from other cowpea varieties, such as Vamban 1 and CO (CP) 7 (Ragul *et al.*, 2018).

In molecular biology, next generation sequencing (NGS) technologies have a great impact on crops regarding the analyses of genetic diversity in population, gene, and quantitative trait locus (QTL) mapping. Genetic maps are a foundation for QTL and gene mapping, marker-assisted selection, and the assembly of genome sequences (Huang *et al.*, 2018). The first genetic map of cowpeas was constructed using RFLP markers based on individuals derived from a cross between IT 2246-4 and TVNI 963 (Fatokun *et al.*, 1992). Since then, many genetic maps have been developed using various molecular marker techniques.

Diversity Arrays Technology (DArTSeq) is a new hybridisation method that combines DArT complexity reduction methods with a next-generation sequencing platform (Nemli *et al.*, 2017). Further, a DArTSeq complexity reduction approach in combination with Illumina short-read sequencing (HiSeq 2000) can be applied to enable rapid and accurate sequencing (Kilian *et al.*, 2016). DArTSeq has been developed to generate genome-wide SNPs without prior knowledge of a plant genome and the capacity to produce high-density data, scoring thousands of unique genomic-wide DNA fragments in a single low-cost experiment (Kilian *et al.*, 2016). Nemli *et al.* (2017), identified 43,018 SNPs from 173 common bean accessions of Andean and Mesoamerican origin using SNPs detected by a DArTSeq approach.

DArTSeq-derived SNPs have emerged as a powerful tool in genetic diversity studies compared to other markers such as AFLP and SSR (Varshney *et al.*, 2007) because SNPs are abundant in the genomes of plants and other organisms (Deuvolt *et al.*, 2010). DArTSeq is a high-throughput, highly reproducible, and low-cost microarray hybridisation technology, with no previous sequence information for the detection of loci for a trait of interest (Nadeem *et al.*, 2017). A single reaction assay of as little as 50–100 ng of genomic DNA can genotype several thousand genomic loci (Nadeem *et al.*, 2017). A subset of 298 lines from a mini core collection of 370 landraces were genotyped based on a genotyping-by-sequencing (GBS) assay to assess the genetic diversity of the lines using three different methods of cluster analyses (Fatokun *et al.*, 2018). Xiong *et al.* (2016), analysed the genetic diversity of cowpea and estimated the population structure of 768 cultivated cowpea genotype collections obtained from the Germplasm Resource Information Network (GRIN), which were originally collected from 56 countries. Three well-differentiated genetic populations were postulated from 768 worldwide cowpea collections based on the model-based ancestry, phylogenetic tree, and principal component analyses. Muñoz-Amatriaín *et al.* (2016), developed bacterial artificial chromosome (BAC) libraries and a BAC-based physical map, assembled sequences from 4,355 BACs, as well as a whole-genome shotgun (WGS) assembly using the African cultivar IT97K-499-35. The WGS sequences of a further 36 different cowpea accessions developed a genotyping assay for over 50,000 SNPs, which was then applied to five bi-parental recombinant inbred line (RIL) populations to produce a consensus genetic map containing 37,372 SNPs (Muñoz-Amatriaín *et al.*, 2016). The objective of this study were to assess the genetic diversity and to examine the population structure of 85 cowpea genotypes collected from different geographic origins using DArTSeq genotype by sequencing techniques. These cowpea accessions are commonly grown in Africa hence there were chosen for the study.

4.2: Materials and Methods

4.2.1: Plant material

A total of 85 cowpea accessions collected from three geographic regions were used in this study, of which 45 accessions were obtained from the International Institute of Tropical Agriculture (IITA) in Nigeria, 25 accessions were from the Agricultural Research Council – Grain Crops in South Africa, and 15 were from smallholder farmers in Buhera District in Zimbabwe (Table 4.1). Genotype GH33 was omitted from the analysis since the majority of the SNPs failed to generate fragments.

4.2.2: DNA extraction, sequencing, and SNP calling

Seeds of cowpea accessions were planted in 20 cm diameter pots in topsoil mixed with compost (3:1) in a greenhouse at the Agriculture Research Council – Grain Crops, Potchefstroom, South Africa. At the three-leaf stage, young, fresh, and succulent leaves were harvested from each accession. The leaf samples were excised and freeze dried for three days using a VirTis freeze dryer (SP Scientific, Warminster, Pennsylvania, USA). Leaf samples were sealed in a zip-lock bag labelled with the corresponding genotypic code and sent to the Integrated Genotyping Service and Support (IGSS), Biosciences eastern and central Africa Hub – International Livestock Research Institute (BecA-ILRI), Nairobi, Kenya, for sequencing and SNP analysis.

The DArTSeq technique was used to evaluate the genetic diversity of 85 cowpea accessions collected from Nigeria, South Africa, and Zimbabwe. DNA extractions and sequencing were performed using the DArTseq protocol (Diversity Arrays Technology Pty Ltd., Canberra, Australia). About a gram of young leaf tissue from each accession was used for genomic DNA extraction. Genomic DNA was isolated from the frozen leaves using a modified cetyltrimethyl ammonium bromide (CTAB)/chloroform/ isoamyl alcohol method (Doyle and Doyle, 1987). The frozen leaf tissue was ground and mixed with 2% pre-warmed (60 °C) CTAB isolation buffer of 1.4 M NaCl, 100 mM Tris (pH 8.0), and 20 mM EDTA (Sigma, Saint Louis, USA). The mixture was then transferred to a 2 ml microcentrifuge tube and incubated at 60 °C for 1 h. DNA was extracted once with chloroform--isoamyl alcohol (ChI/IAA; 24:1) (Sigma, Saint Louis, USA) and precipitated with two volumes of isopropanol. The obtained pellet was washed with 70% EtOH, dried, and dissolved in 100 µl of TE buffer with 50 µg/ml RNase A

(Sigma, Saint Louis, USA). The extracted DNA was quantified by 0.8% agarose gel electrophoresis, and was adjusted to 50 ng/μl for DArT and SNP genotyping.

Table 4.1: List of cowpea accessions used in this study obtained from three geographic origins.

No	Name	Code	Source	Origin	No	Name	Code	Source	Origin
1	IT99K-573-2-1	GH01	IITA	Nigeria	44	RV503	GH44	ARC GC	South Africa
2	VAM14-143-4-1	GH02	IITA	Nigeria	45	98K-503-1	GH45	IITA	Nigeria
3	IT07K-292-10	GH03	IITA	Nigeria	46	RV551	GH46	ARC GC	South Africa
4	Dahwa	GH04	Buhera	Zimbabwe	47	RV553	GH47	ARC GC	South Africa
5	Chibundi chitsvuku	GH05	Buhera	Zimbabwe	48	RV554	GH48	ARC GC	South Africa
6	CBC1	GH06	Buhera	Zimbabwe	49	TV9620	GH49	IITA	Nigeria
7	RV 344	GH07	ARC GC	South Africa	50	TVU13004	GH50	IITA	Nigeria
8	TVU 13932	GH08	IITA	Nigeria	51	97K-499-35	GH51	IITA	Nigeria
9	RV207	GH09	ARC GC	South Africa	52	TVU12637	GH52	IITA	Nigeria
10	RV555	GH10	ARC GC	South Africa	53	IT93K-129-4	GH53	IITA	Nigeria
11	Bechuana White	GH11	ARC GC	South Africa	54	IT96D-610	GH54	IITA	Nigeria
12	RV568	GH12	ARC GC	South Africa	55	CH47	GH55	ARC GC	South Africa
13	Barapara purple	GH13	Buhera	Zimbabwe	56	RV343	GH57	ARC GC	South Africa
14	Mupengo dema	GH14	Buhera	Zimbabwe	57	TVU12746	GH58	IITA	Nigeria
15	Chibundi chemavara	GH15	Buhera	Zimbabwe	58	TVU9596	GH60	IITA	Nigeria
16	IT07-318-33	GH16	IITA	Nigeria	59	TVU2095	GH61	IITA	Nigeria
17	IT97-499-35	GH17	IITA	Nigeria	60	TVU3416	GH62	IITA	Nigeria
18	TVU3000	GH18	IITA	Nigeria	61	98D-1399	GH64	IITA	Nigeria
19	IT07K-274-2-9	GH19	IITA	Nigeria	62	RV574	GH65	ARC GC	South Africa

No	Name	Code	Source	Origin	No	Name	Code	Source	Origin
20	IT90K-59	GH20	IITA	Nigeria	63	86D-1010	GH66	IITA	Nigeria
21	IT89KD-288	GH21	IITA	Nigeria	64	90K-284-2	GH67	IITA	Nigeria
22	Barapara jena	GH22	Buhera	Zimbabwe	65	RV500	GH68	ARC GC	South Africa
23	CBC2	GH23	Buhera	Zimbabwe	66	RV351	GH69	ARC GC	South Africa
24	Mupengo wemavara	GH24	Buhera	Zimbabwe	67	99K-494-6	GH70	IITA	Nigeria
25	98K-476-8	GH25	IITA	Nigeria	68	Encore	GH71	ARC GC	South Africa
26	RV194	GH26	ARC GC	South Africa	69	TVU13778	GH72	IITA	Nigeria
27	Dr Saunders	GH27	ARC GC	South Africa	70	83S-911	GH73	IITA	Nigeria
28	TVU9443	GH28	IITA	Nigeria	71	TVU9671	GH74	IITA	Nigeria
29	Oleyin	GH29	IITA	Nigeria	72	RV558	GH75	ARC GC	South Africa
30	TVU11986	GH30	IITA	Nigeria	73	Orelo	GH76	IITA	Nigeria
31	Ziso dema	GH31	Buhera	Zimbabwe	74	RV202	GH77	ARC GC	South Africa
32	Barapara remavara	GH32	Buhera	Zimbabwe	75	95K-589-2	GH79	IITA	Nigeria
33	Kangorongondo	GH33	Buhera	Nigeria	76	TVU 13998	GH80	IITA	Nigeria
34	IT90K-76	GH34	IITA	Nigeria	77	RV342	GH81	ARC GC	South Africa
35	IT93K-452-1	GH35	IITA	Nigeria	78	RV204	GH82	ARC GC	South Africa
36	IT90K-277-2	GH36	IITA	Nigeria	79	IT98K-506-1	GH83	IITA	Nigeria
37	IT08K-150-27	GH37	IITA	Nigeria	80	IT82E-18	GH84	IITA	Nigeria
38	IT96D-610	GH38	IITA	Nigeria	81	RV213	GH85	ARC GC	South Africa
39	IT90K-207-15	GH39	IITA	Zimbabwe	82	PAN311	GH86	ARC GC	South Africa

No	Name	Code	Source	Origin	No	Name	Code	Source	Origin
40	IT18	GH40	Buhera	Zimbabwe	83	RV221	GH87	ARC GC	South Africa
41	Mutonono	GH41	Buhera	Zimbabwe	84	98D1399	GH88	IITA	Nigeria
42	Zvenyika	GH42	Buhera	Nigeria	85	99K-494-6	GH89	IITA	Nigeria
43	TVU14190	GH43	IITA						

IITA=International Institute of Tropical Agriculture
ARC GC=Agriculture Research Council-Grain Crops

4.2.3: DArT analysis

DNA was processed in digestion/ligation reactions, as reported by Kilian *et al.* (2012) by replacing a single *PstI*-compatible adaptor with two different adaptors corresponding to two different restriction enzyme (RE; *PstI* and *SphI*)-compatible adaptors. The *PstI*-compatible adapter was designed to incorporate an Illumina flow cell attachment sequence with staggered sequences of varying length barcode region, similar to the sequence reported by Elshire *et al.* (2011). The reverse adapter contained a flow cell attachment region with *SphI*-compatible overhang sequence. Only “mixed fragments” (*PstI*–*SphI*) were effectively amplified in 30 rounds of PCR using the following reaction conditions: 94°C for 1 min, then 30 cycles of 94 °C for 20 s, 58 °C for 30 s, 72 °C for 45 s, and 72 °C for 7 min. After PCR, equimolar amounts of amplification products from each sample of the 96-well microtiter plate were bulked and applied to c Bot (Illumina) bridge PCR followed by sequencing on the Illumina HiSeq 2500 system (Illumina, San Diego, California, USA). The sequencing (single read) was run for 77 cycles.

Sequences generated from every lane were processed using proprietary DArT analytical pipelines (PLs). In the primary pipeline analysis, fragments of poor-quality sequences with reproducibility below 90% and read depths lower than 3.5 for SNPs or 5 for presence–absence markers were filtered out. More stringent selection criteria were applied to the barcode region compared to the rest of the sequences. The assignments of the sequences to specific samples carried within the barcode splitting step were very reliable. No samples were dropped because of low coverage across loci; however, individual sequences were removed if they did not meet the above criteria. Approximately 2.5 million sequences per barcode/sample were identified and used in marker calling. The average browsing depth across loci was 9.2 reads per individual per locus for reference alleles and 6.5 for SNP alleles. Finally, identical sequences were collapsed into “fastqcoll” files. The fastqcoll files were groomed using DArT PL’s proprietary algorithm, which corrects low-quality bases from singleton tags into correct bases using collapsed tags with multiple members as a template. The groomed fastqcoll files were utilised in the secondary pipeline for DArT PL’s proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation; PA markers) calling algorithms using DArTsoft14 (Diversity Arrays Technology Pty Ltd., Canberra, Australia).

SNP calling were performed for all tags from all libraries enclosed within the DArTsoft14 analysis clustered using DArT PL’s C⁺⁺ algorithm program at a brink distance of three. Parsing

of the clusters into separate SNP loci was performed using a technique called balance of read counts for the allelic pairs. Additional choice criteria were further added to the algorithm program supported by an analysis of roughly 1,000 controlled cross populations. Testing for deviations from the Hardy–Weinberg equilibrium of alleles in these populations was conducted to facilitate the selection of technical parameters to effectively discriminate true allelic variants from paralogous sequences. In addition, multiple samples were processed from DNA to allelic calls as technical replicates, and scoring consistency was used as the main selection criteria for high-quality/low-error rate markers. Calling quality was assured by a high average browse depth per locus (average across all markers was over 30 reads/locus). DNA was diluted to 50 ng/μl for GBS analysis.

4.3: Data analysis

Null alleles (those that failed to amplify any fragment), monomorphic SNPs, SNPs with minor allele frequencies of less than 2%, and SNPs that had missing alleles for more than 20% of the genotypes were filtered out. Genotypic data were subjected to analyses with various measures of genetic diversity within and among genotypes using GenAlex software version 6.5 (Peakall and Smouse, 2012). Genetic diversity parameters, such as observed heterozygosity (H_o), Shannon information Index, gene diversity (H_e), and polymorphic information content (PIC) were determined using the protocol of Nei and Li (1979). Based on the geographic stratification, genetic diversity analysis within and among populations and the analysis of molecular variance (AMOVA) were performed using GenAlex.

Cluster analysis of the 85 cowpea genotypes was conducted using the Jaccard dissimilarity matrix index. Cluster analysis was performed based on a neighbour-joining algorithm using the unweighted pair group method using arithmetic average (UPGMA) in DARwin 5.0 software (Perrier and Jacquemoud-Collet, 2006). A dendrogram was then generated on the dissimilarity matrix. To investigate the genetic relationships among accessions, genetic distances among all pairs of individual accessions were estimated to draw dendrogram. Bootstrap analysis was performed for node construction using 10,000 bootstrap values.

4.4: Results

4.4.1: Genetic diversity and SNP characterisation

The SNPs were filtered by removing the rare alleles (less than 2%), high-missing ratios (more than 20%), and monomorphic alleles. Out of a total of 18,284 SNPs tested, only 7,799 (51.15%) were found to be polymorphic across the 85 accessions and fulfilled the selection criteria. The 7,799 selected SNPs were subjected to genetic analyses, and Table 4.2 presents the genetic diversity parameters measured from 85 cowpea genotypes. The number of polymorphic SNPs per chromosome varied from 448 on chromosome 5 to 940 on chromosome 3, with an overall mean of 650 SNPs per chromosome. Chromosome 2 had the highest polymorphic loci content (56.51%), while chromosome 5 had the lowest with 39.12%. Observed heterozygosity (H_o) ranged from 0.034 to 0.069 per chromosome. Similarly, gene diversity (H_e) ranged from 0.222 to 0.286, with a mean of 0.255. Chromosome 10 and chromosome 2 revealed the highest and lowest H_o and H_e values, respectively. The observed mean fixation rate (F_{IS}) was 79.8%. Chromosome 2 had the highest F_{IS} value at 0.867, while chromosome 9 had the lowest F_{IS} value at 0.770. Markers on chromosome 10 had the highest PIC value of 0.284, while on chromosome 2 had the lowest value of 0.217, with a mean PIC of 0.254.

Table 4.2: Genetic diversity within and among 85 cowpea genotypes based on 7799 SNPs markers.

Chromosome	No SNPs used	Polymorphic SNPs	% P	H_o	H_e	F_{IS}	PIC
1	1155	465	40.26	0.050	0.260	0.807	0.259
2	1145	647	56.51	0.034	0.222	0.867	0.217
3	2169	940	43.34	0.048	0.268	0.808	0.267
4	1606	731	45.52	0.049	0.252	0.804	0.251
5	1452	568	39.12	0.052	0.235	0.781	0.233
6	1425	669	46.95	0.043	0.261	0.823	0.259
7	1760	852	48.41	0.045	0.265	0.808	0.264
8	1326	602	45.40	0.056	0.253	0.781	0.251
9	1367	566	41.40	0.049	0.234	0.770	0.233
10	1531	671	43.83	0.061	0.286	0.789	0.284
11	1542	640	41.50	0.055	0.243	0.778	0.242
Unknown	1770	448	25.25	0.069	0.273	0.730	0.272
All chromosomes	1520	650	42.73	0.050	0.255	0.798	0.254
SE	-	-	-	0.001	0.002	0.003	0.002

%P = percentage polymorphic markers per chromosome; H_o = observed gene diversity within genotypes; H_e = average gene diversity within genotypes; F_{IS} = inbreeding coefficient; PIC = polymorphic information content; SE = standard error.

The distribution and genetic diversity parameter estimates of the 7,799 SNPs used in this study are presented in Figure 4.1. Observed heterozygosity (H_o) values ranged from 0.00 to 0.914 with a mean of 0.05 (Figure 4.1B). The majority of the SNPs (49%) had H_o values ranging from 1.1% to 5%, indicating that the alleles of these SNPs were fixed among the cowpea genotypes, while only 67 SNPs had H_o values greater than 5%. The SNP diversity ranged from 0.024 to 0.505, in which 26.6% had H_e values greater than 0.40, suggesting that the genotypes were highly genetically diversified (Figure 4.1a). Generally, the PIC value ranged from 0.024 to 0.50, with an average value of 0.25. Approximately 40% of the SNPs used in this study had PIC values exceeding the mean value 0.30 and 27% SNPs had PIC values between 0.40 and 0.50, demonstrating the high discriminatory power of the markers (Figure 4.1c). The

inbreeding coefficient, in contrast, displayed contrasting values ranging from -0.83 to 1.00 , with a mean of 0.798 . Of the $7,799$ SNPs tested, 319 SNPs showed negative F_{IS} values, indicating that these markers were highly heterozygous among genotypes. More than 92% of the SNPs had F_{IS} values exceeding 0.50 and 17% of the SNPs had PIC values of 1.00 , demonstrating that the majority of the SNPs were fixed (Figure 4.1d).

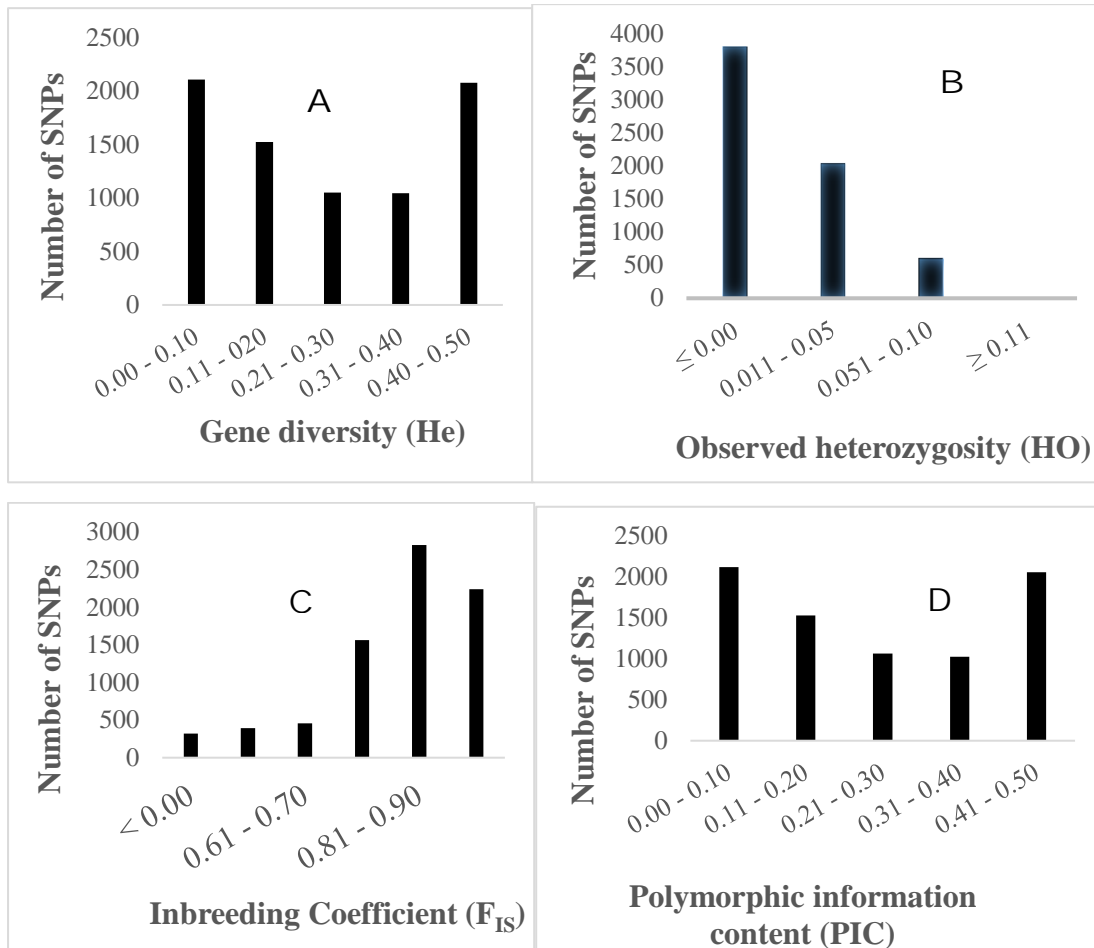


Figure 4.1: Distribution of the 7,799 SNPs estimated on 85 cowpea genotypes (A) Gene diversity (H_e); (B) Observed heterozygosity (H_o); (C) Inbreeding coefficient (F_{IS}); and (D) Polymorphic information content (PIC).

4.4.2: Genetic diversity of inter- and intra-populations

The genetic diversity was further analysed by geographic origin as a stratification criterion. The average observed gene diversity within genotypes per population (H_o) ranged from 0.047 in the Zimbabwe accessions to 0.052 for accessions from Nigeria, with an overall mean value of 0.050 (Table 4.3). The mean values of the total Shannon information index ranged from 0.325 to 0.381 for accessions from Nigeria and Zimbabwe respectively, with an overall mean value of 0.355, while the average gene diversity within genotypes per population (H_e) ranged from 0.216 to 0.247 with an overall mean value of 0.232. The inbreeding coefficient (F_{IS}) ranged from 0.717 for South African accessions to 0.770 for accessions from Nigeria, with an overall mean value of 0.740, while the percentage of polymorphic loci (%P) ranged from 73.59 for accessions from Zimbabwe to 92.31 for accessions from Nigeria, with an overall mean value of 83.68 (Table 4.3). Diversity indices observed among the three geographic origins revealed that the Nigerian accessions had the highest number of private alleles (475), Shannon index (0.381), expected gene diversity (0.247) and percentage of polymorphic loci (92.3%) (Table 4.3).

Table 4.3: Genetic diversity within and among the cowpea populations stratified based on geographic origin.

Origin	N	P _A	I	H _o	H _e	F _{IS}	%P
Nigeria	45	475	0.381	0.050	0.247	0.770	92.31%
South Africa	23	222	0.358	0.052	0.233	0.717	85.14%
Zimbabwe	15	147	0.325	0.047	0.216	0.731	73.59%
Overall mean	-	-	0.355	0.050	0.232	0.740	83.68%
SE	-	-	0.002	0.001	0.001	0.002	5.45%

N = number of genotypes tested per population; P_A = private allele; I = Shannon information index; H_o = average observed gene diversity within genotypes per population; H_e = average gene diversity within genotypes per population; F_{IS} = inbreeding coefficient; %P = Percentage of polymorphic loci; SE = Standard error.

According to the standard guidelines for the interpretation of genetic differentiation (Wright, 1978), the range 0–0.005 indicates little, 0.05–0.15 indicates moderate, 0.15–0.25 indicates great, and above 0.25 indicates very great genetic differentiations. Genetic differentiation (F_{ST})

revealed moderate genetic differentiation among the accessions ranging from 0.04 to 0.07 in all regions (Table 4.4). Similarly, Slatkin (1989) and Morjan and Rieseberg (2004) indicated that gene flow (N_m) < 1 is considered to be low, while $N_m = 1$ is considered to be moderate and $N_m > 1$ is considered to be high. In this study, a very high gene flow (4.89) was observed between Zimbabwean and South African accessions, indicating that there was germplasm exchange between the neighbouring countries.

Table 4.4: Pair-wise estimates of genetic differentiation (F_{ST}) (above diagonal off brackets), gene flow (N_m) (above diagonal within brackets); genetic distance GD (lower diagonal off brackets) and genetic identity (GI) (lower diagonal within brackets).

Population	Nigeria	South Africa	Zimbabwe
Nigeria		0.049 (4.89)	0.065 (3.57)
South Africa	0.037 (0.965)		0.048 (4.92)
Zimbabwe	0.050 (0.951)	0.027 (0.974)	

Analysis of molecular variance (AMOVA) was carried out on the three regions, and revealed substantial geographic differentiation in cowpea accessions (Table 4.5). A highly significant differentiation was observed among populations, among individuals, and within individuals ($p < 0.001$). The variation between individuals (78%) was higher than those between populations (8%) and within individual varieties (15%). There is a moderate amount of differentiation between the three regions, indicating that the accession in the three regions are relatively genetically distinct. The significant F_{IS} values observed indicates that the cowpea lines within the regions were inbred lines.

Table 4.5: Analysis of molecular variance (AMOVA) among 85 cowpea accessions classified based geographic origin using 7799 SNPs markers.

Source	df	SS	MS	Est. Var.	Per. Var.	F-Statistics
Among populations	2	14175.1	7087.5	98.2	8%	$F_{ST} (P \leq 0.001)$
Among individual	81	177612.7	2192.7	1002.7	78%	$F_{IS} (P \leq 0.001)$
Within individual	84	15730.0	187.3	187.3	15%	$F_{IT} (P \leq 0.001)$
Total	167	207517.8	-	1288.2	100%	

df= degree of freedom, SS= sum of squares, MS= mean sum of squares, Est. var. = estimated variance, Per. Var. = Percentage variation

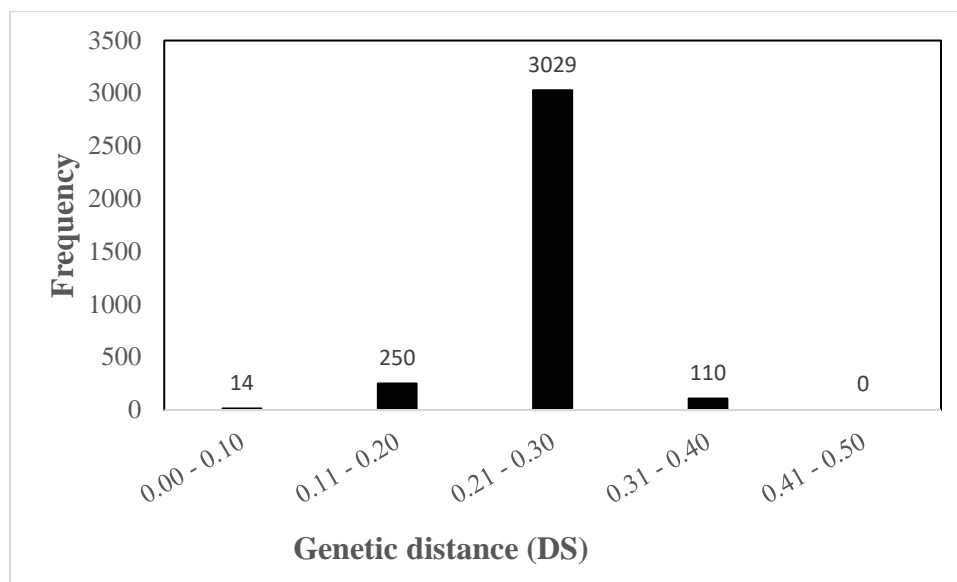


Figure 4. 2: Genetic distance estimate among 85 cowpea genotypes.

The genetic distance value estimated on the basis of SNP markers ranged from 0.14 to 0.44 with a mean value of 0.35 (Figure 4.2). The majority (89%) of the genetic distance based on differences at marker loci between pairs of accessions ranged from 0.20 to 0.30. Neighbour-joined cluster analysis generated by UPGMA clearly divided the 85 cowpea accessions into three distinct clusters (Figure 4.3). Cluster one indicated by black colour was made up of 49 accessions (59%) admixture from the three geographic origin with most accessions from Zimbabwe and South Africa. Cluster 2 designated by blue colour had 30 cowpea accessions with majority of the accessions (27) from Nigeria. Cluster 3 with a red colour designation contained only four accessions, three (GH43, GH45 and GH50) from Nigeria and GH 47 from South Africa.

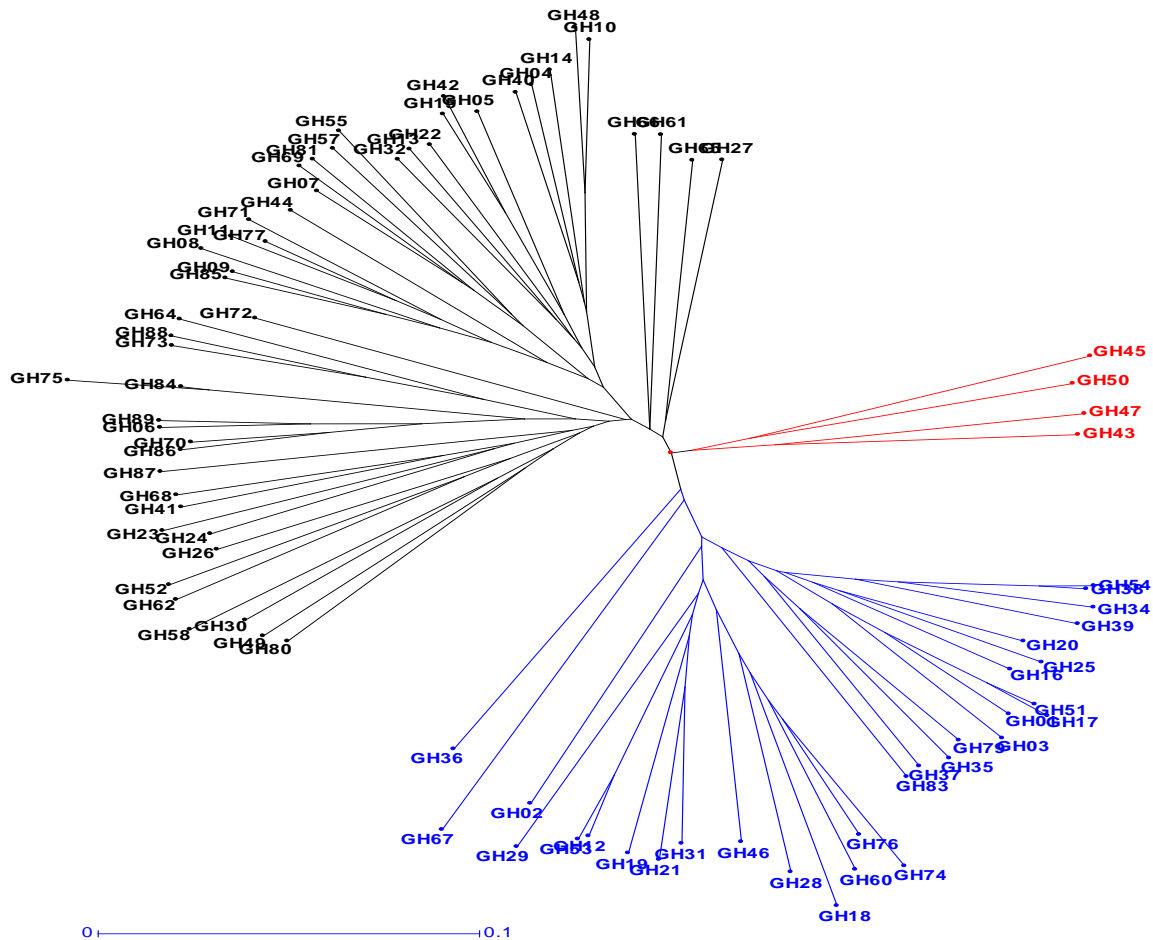


Figure 4.3: Clustering patterns of the 85 cowpea accessions constructed based on neighbour-joining algorithm using unweighted pair group method (UPGMA)

4.4.3: Discussion

The assessment of the genetic diversity of *Vigna unguiculata* (L.) Walp. accessions using informative molecular markers is important for the management, genetic improvement, and conservation. From such studies, accessions with great potential can be selected and further improved for cultivation as it is an important crop in the smallholder farming sector of sub-Saharan Africa.

In this study, DArTSeq was used to analyse the genomes of 85 cowpea accessions. It is a cheap and efficient platform that allows genome-wide marker discovery through restriction enzyme-

mediated genome complexity reduction and sequencing of the restriction fragments (Melville *et al.*, 2017). Although, DArTSeq yields a lower density of markers from 10,000 to 35,000 loci compared to the GBS approach, which yields over 800,000 loci, DArTSeq has substantially higher genome wide coverage and lower missing data (Barilli *et al.*, 2018; Lambert *et al.*, 2016). In addition, DArTSeq provide a means to directly score samples as heterozygous/homozygous at each locus with the lower density approach, and provide thousands of short primers with polymorphic loci. Restriction site-associated DNA sequencing (RADseq) typically yields markers of 85 bp or longer, while DArTSeq criteria produce sequences 69 bp or shorter in length (Lambert *et al.*, 2016). It was suggested that GBS markers had low redundancy, and it was the best technique for further diversity analyses and genomic selection.

In this study, over 18, 000 SNPs were used to assess the level and pattern of genetic variation among cowpea genotypes collected from three geographic origins. However, only 51% of the SNPs were polymorphic and the remaining SNPs, were either monomorphic or contained rare alleles of less than 2% allele frequency or null alleles for more than 20% of the genotypes, were eliminated from the analysis. Dias *et al.* (2015), found 76% polymorphism using ISSR markers in their study on cowpea genotypes from Brazil and Nigeria, while Ghalmi *et al.* (2010), in their diversity analysis of local cowpea varieties from Africa, reported 63% polymorphism using ISSR markers. The high level of polymorphism provided by ISSR markers might be attributed to their high number of alleles and their abundance in the genome (Mahfouz, 2015). In this study, the PIC value ranged from 0.024 to 0.50, with an average value of 0.25. In an assessment of genetic diversity in the Ethiopian cowpea germplasm using SSR markers, Desalegne *et al.* (2016), observed relatively high mean PIC values ranging from 0.23 to 0.68. The low level of PIC values in this study is due to the different marker systems used. However, the low level of polymorphism observed in their study was because of the self-pollinated reproduction mechanism of cowpea and a bottleneck induced by a single domestication event in this crop.

From the results, the observed genetic distance between pairs of cowpea genotypes based on 7,799 SNP markers ranged from 0.14 to 0.44, with a mean value of 0.35. Similar findings were reported by Fatokun *et al.* (2018), using 370 accessions sampled from world cowpea collections. However, Huynh *et al.* (2013), reported a wider range of genetic distances (0.01 to 0.72) based on shared alleles among cowpea landraces collected from 56 countries. Wang *et*

al. (2008), in their study of genetic diversity using gene-derived markers and sequencing on the USDA *Vigna* germplasm collection, reported low genetic diversity and minimal genetic distance among cowpea accessions. The low level of polymorphism detected in the present study and in other previous studies may be attributed to the self-pollinated reproduction mechanism of cowpea, and the restriction induced by a single domestication event (Diouf and Hilu, 2005; Badiane *et al.*, 2004; Wamalwa *et al.*, 2016). Xiong *et al.* (2016) ,observed that the degree of genetic diversity has a positive correlation with the number of countries from which the accessions were collected. Kambua *et al.* (2019) observed that when there are more accessions from different places of origin, a higher genetic diversity will be detected. In this study, only accessions from three countries, Nigeria, South Africa, and Zimbabwe, were used; hence, the genetic diversity was low. Populations from different areas with similar genetic structures always have a smaller distance and similar genetic diversity.

AMOVA analysis revealed a moderate but significant differentiation in the cowpea accessions collected from three geographic regions. The AMOVA indicated significant differences among populations and individuals, and within individuals ($p < 0.001$). The variance among populations was significantly low (8% of the total variation), while the variance among individuals was significantly high (78% of the total variation). Similar results have been reported by Fatokun *et al.* (2018) and Chen *et al.* (2017), in which the highest variations were observed among accessions compared to those within accessions and among populations. Thus most of the genetic variation observed in cowpea is attributed to that among individuals rather than geographic alignment. The relatively low variation within accessions can be explained by the low outcrossing rate of cowpea, as its floral structure only promotes inbreeding (Lush, 1979). However, the high individual variation, could probably be attributed to the high level of germplasm exchange by smallholder farmers across geographic regions. Additionally, the low level of variation observed among regions could be the result of high gene flow within regions with little time for genetic differentiation along geographical lines (Wamalwa *et al.*, 2016). The low levels of differentiation among geographic regions and the high levels of variation within regions suggests that a large random collection would capture most of the genetic variation within cowpea accessions in each region (Xiong *et al.*, 2016).

The clustering patterns of the 85 cowpea accessions collected from different geographic regions revealed the presence of three distinct groups. The observed clustering pattern was, to some extent, consistent with the geographic origins of the accessions. The study showed a

similar genetic makeup among some of the accessions this was exhibited by the closeness of the accessions in the UPGMA analysis. Cluster 1 had the most diverse accessions, GH43, GH45, and GH50 from Nigeria and GH47 from South Africa. Menssen *et al.* (2017) reported that the clustering of genotypes originating from different countries on the same cluster could be due to the fact that cowpea played a significant role in human history. Furthermore, these four accessions in Cluster 1 could be used to infuse new genetic diversity into cowpea breeding programmes, as they are not closely related to either of the two clusters. Mafakheri *et al.* (2019), assessed 32 cowpea genotypes for 17 morphological traits. The study confirmed the existence of a high morphological variation in cowpea genotypes, which is an important aspect for plant breeding programmes to introduce new and hybrid varieties.

The UPGMA analysis showed three clusters, with Cluster II dominated by accessions from Zimbabwe and South Africa, with a few from Nigeria. This implies that there is a high level of import, export, and exchange of accessions through human activity between South Africa and Zimbabwe; they are neighbouring countries that share the same geographic and political boundaries. The fewer Nigerian accessions in this cluster could have happened because of human migration ages ago. However, Cluster III contained mostly Nigerian accessions, which implies that these could have originated from West Africa. Udensi *et al.* (2016), found six clusters from 20 cowpea accessions. The UPGMA-based cluster analysis revealed that cowpea accessions obtained from the same geographical locations were found on the same cluster. This geographically based clustering of the accessions was affirmed by the genetic distances results. This implies that cowpea accessions found on the same cluster were genetically similar, while those found on different clusters were genetically diverse.

The study revealed great diversity within individuals among the 85 cowpea accessions. Some accessions, according to the UPGMA analysis, were closely related, especially in Cluster II and Cluster III, even though they were from different geographic regions. The differences and similarities of accessions in some clusters as a result of their locations indicate the extent of accession exchange among farmers from different regions (Al-Saady *et al.*, 2018). However, Cluster II contains mostly Nigerian accessions, which implies that these accessions evolved in specific environments and shared a similar environmental bottleneck. Cluster III had four unique genotypes from Nigeria and South Africa. Menssen *et al.* (2017), reported that the clustering of genotypes originating from different countries on the same cluster could be due to the fact that cowpea played a significant role in human history. Furthermore, these four

accessions in Cluster III may be used to infuse new genetic diversity into cowpea breeding programmes, as they are not closely related to either of the two clusters. It is also imperative to widen the genetic base of cowpea accessions, which could be achieved through the use of alleles either from IITA or from closely related wild relatives of cowpea, such as *Vigna triphylla* and *Vigna reticulata* (Ali *et al.*, 2015).

4.4.5: Conclusion

In this study, SNP data analysis indicated the existence of high levels of genetic diversity among cowpea accession collected from southern Africa and Nigeria. The current study also found that the DArTSeq-derived SNP markers were efficient in genetic diversity analysis and relatedness in cowpeas. Genotypes closely related in Cluster 1 include GH43, GH45, GH47, and GH50. In Cluster II, GH30, GH58 and GH80 closely resembled each other. GH60, GH76, and GH74 in Cluster III were also closely related. However, there is a need to further improve the genetic base of the crop by further probing accessions GH18, GH74, and GH75 and using them as stock for introgression. GH8, GH45, GH47, GH48, GH75 could also be selected for hybridisation or for use as parents. In order to provide biological meaning to the clusters, molecular data should be backed by morphological data. These can be used in the identification of SNP markers that are associated with desirable agronomic attributes, such as high tolerance to biotic and abiotic stress factors.

References

- Ali, Z. B., Yao, K. N., Odeny, D. A., Kyalo, M., Skilton, R., and Eltahir, I.M., 2015. Assessing the Genetic Diversity of Cowpea [*Vigna unguiculata* (L.) Walp.] Accessions from Sudan Using Simple Sequence Repeat (SSR) Markers. *African Journal of Plant Science* 9: 293-304. doi: 10.5897/AJPS2015.1313.
- Al-Saady, N.A., Nadaf, S.K., Al-Lawati, A.H., and Al-Hinai, S.A., 2018. Germplasm Collection and Seed Diversity of Cowpea (*Vigna unguiculata* (L.) Walp.) *Asian Journal of Agriculture and Food Sciences* 6 .Issue 04:129-139.doi:10.24203/ajafs.v6i4.5349.
- Badiane, F.A, Diouf, D., Sané D., Diouf, O., Goudiaby, V., and Diallo, N., 2004. Screening Cowpea [*Vigna unguiculata* (L.) Walp.] Varieties by Inducing Water Deficit and RAPD Analyses. *African Journal of Biotechnology* 3:174–178.
- Barilli, E., Cobos, M.J., Carrillo, E., Kilian, A., Carling, J., and Rubiales, D., 2018. A High-Density Integrated Dartseq SNP-Based Genetic Map of *Pisum Fulvum* and Identification of Qtls Controlling Rust Resistance. *Frontiers in Plant Science* 9: 167.
- Boahen, S.K., Savala, C.E.N., Chikoye, D., and Abaidoo, R., 2017. Growth and Yield Responses of Cowpea to Inoculation and Phosphorus Fertilization in Different Environments. *Frontiers in Plant Science* 8:646. <https://doi.org/10.3389/fpls.2017.00646>.
- Boukar, O., Fatokun, C.A., Huynh, B.L., Roberts, P.A., and Close, T.J., 2016. Genomic Tools in Cowpea Breeding Programs. Status and Perspectives. *Frontiers in Plant Science* 7:757.<https://doi.org/10.3389/fpls.2016.00757>. Accessed on 24/04/2019.
- Boukar O., Belko, N., Chamarthi S., Togola, A., Batiemo, J., Owusu, E., Haruna, M., Diallo, S., Umar, M.L., Olufajo, O., and Fatokun, C., 2018. Cowpea (*Vigna unguiculata*): Genetics, Genomics and Breeding. *Plant Breeding* 2018:1-10. <https://doi.org/10.1111/pbr.12589>.
- Chen, H., Chen, H., Hu, L., Wang, L., Wang, S., Wang, M.L., and Chen, X., 2017. Genetic Diversity and a Population Structure Analysis of Accessions in the Chinese Cowpea [*Vigna unguiculata* (L.) Walp.] Germplasm Collection. *The Crop Journal* 5.363–372, <https://doi.org/10.1016/j.cj.2017.04.002>.

Chidebe, I.N., Jaiswal, S.K., and Dakora, F.D., 2018. Distribution and Phylogeny of Microsymbionts Associated with Cowpea (*Vigna unguiculata*) Nodulation in Three Agroecological Regions of Mozambique. *Applied Environmental Microbiology* 84:1–25. <https://doi.org/10.1128/AEM.01712-17>.

Damarany, A., El-Dkeshy, M.H.Z., Attallah, S.Y., and Haridy, A.G., 2018. Molecular Markers and Genetic Variation Revealed by RAPD-PCR in Seven Cowpea (*Vigna unguiculata* (L.) Walp.) Cultivars. *International Journal of Biosciences* 13: 66-74.

Desalegne, B.A., Mohammed, S., Dagne, K., and Timko, M.P., 2016. Assessment of Genetic Diversity in Ethiopian Cowpea [*Vigna unguiculata* (L.) Walp.] Germplasm Using Simple Sequence Repeat Markers. *Plant Molecular Biology Reporter* 34:978. <https://doi.org/10.1007/s11105-016-0979-x>.

Deulvot, C., Charrel, H., Marty, A., Jacquin, F., Donnadieu, C., Lejeune-Hénaut, I., Burstin, J., and Aubert, G., 2010. Highly-Multiplexed SNP Genotyping for Genetic Mapping and Germplasm Diversity Studies in Pea. *BMC Genomics* 11: 1:468.

Dias, F.T.C., Bertini, C.H.C.M., Silva, A.P.M., and Cavalcanti, J.J.V., 2015. Genetic Variability in Early-Cycle Erect Cowpea Analysed with RAPD and ISSR Markers. *Revista Ciência Agronômica* 46: 563–572.

Diouf, D., and Hilu, K.W., 2005. Microsatellites, RAPD Markers to Study Genetic Relationships among Cowpea Breeding Lines and Local Varieties in Senegal. *Genetic Resources and Crop Evolution* 52:1057–1067.

Doyle, J. J., and Doyle, J. L. 1987. A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochemical Bulletin* 19: 1–15.

Elshire R.J., Glaubitz J.C., Sun Q., Poland, J.A., Kawamoto, K., Buckler, E.S., and Mitchel, S.E., 2011. A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. *PLoS One* 6: e19379.

Fatokun, C.A., Menancio-Hautea, D.I., Danesh, D., and Young, N.D., 1992. Evidence for Orthologous Seed Weight Genes in Cowpea and Mung Bean Based On RFLP Mapping. *Genetics* 1132:841–846.

Fatokun, C., Girma, G., Abberton, M., Gedil, M., Unachukwu, N., Oyatomi, O., Yusuf, M., Rabbi, I., and Boukar, O., 2018. Genetic Diversity and Population Structure of a Mini-Core Subset from The World Cowpea (*Vigna unguiculata* (L.) Walp.) Germplasm Collection. Scientific Reports 8:16035. doi 10.1038/41598-018-34555-9.

Huang, H., Tan, H., Dongmei Xu, D., Tang, Y., Niu, Y., Lai, Y., Tie, M., and Huanxiu, L., 2018. High-Density Genetic Map Construction and Comparative Genome Analysis in Asparagus Bean. Scientific Reports 8:4836. doi.org/10.1038/s41598-018-23873-7.

Huynh, B.L., Close, T.J., Roberts, P.A., Hu, Z., Wanamaker, S., Mitchell R. Lucas, M.R., Chiulele, R., Cissé, N., David, A., Hearn, S., Fatokun, C., Diop, N., and Ehlers, J.D., 2013. Genepools and the Genetic Architecture of Domesticated Cowpea. Plant Genome 6:1-8. <https://doi.org/10.3835/plantgenome2013.03.0005>.

Ghalmi, N., Malice, M., Jacquemin, J.M., Ounane, S.M., Mekliche, L., and Baudoin, J.P., 2010. Morphological and Molecular Diversity within Algerian Cowpea (*Vigna unguiculata* (L.) Walp.) Landraces. Genetic Resources and Crop Evolution 57: 371–386.

Kambua, M.R., George, M.E., Mwangi, G.S., and Miinda, A.E., 2019. Genotypic Diversity of Cowpea from Lower Eastern Kenya. International Journal of Agronomy and Agricultural Research 14 (5): 9-19.

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., Caig, V., Heller-Uszynska, K., Jaccoud, D., Hopper, C., Aschenbrenner-Kilian, M., Evers, M., Peng, K., Cayla, C., Hok, P., and Uszynski, G., 2012. Diversity Arrays Technology: A Generic Genome Profiling Technology on Open Platforms. Methods Molecular Biology 888:67–89. doi: 10.1007/978-1-61779-870-2_5.

Kilian, A., Sanewski, G., and Ko, L., 2016. The Application of DArTseq Technology to Pineapple. Acta Horticulture 1111: 181-188.

Kolade, O.A., Olowolafe, M.O., and Fawole, L., 2016. Characterisation of Mutant Cowpea (*Vigna unguiculata*) Lines Using Random Amplified Polymorphic DNAs (RAPDS) and Amplified Fragment Length Polymorphism (AFLP) Markers. African Journal of Biotechnology 15:2530-2537.

Lambert, M. R., Skelly, D. K., and Ezaz, T., 2016. Sex-Linked Markers in the North American Green Frog (*Rana clamitans*) Developed Using DArTseq Provide Early Insight into Sex Chromosome Evolution. *BMC Genomics* 17: 844. doi: 10.1186/s12864-016-3209-x.

Lush, W.M., 1979. Floral Morphology of Wild and Cultivated Cowpeas. *Economic Botany* 33:442–447.

Mafakheri, K., Bihamta, M.R., and Abbasi, A.R., 2017. Assessment of Genetic Diversity in Cowpea (*Vigna unguiculata* L.) using Morphological and Molecular Characterisation. *Cogent Food and Agriculture* 3:1.doi:10.1080/23311932.1327092.

Mahfouz, H.M., 2015. Assessment of Genetic Diversity in Cowpea (*Vigna unguiculata*) Using SDS-PAGE, Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) Markers. *Egyptian Journal of Genetics and Cytology* 44: 75–91.

Melville, .J, Haines, M.L., Boysen, K., Hodkinson, L., Kilian, A., Date, K.L.S., Potvin, D.A., and Parris, K.M., 2017. Identifying Hybridization and Admixture Using SNPs: Application of the Dartseq Platform in Phylogeographic Research on Vertebrates. *Roy Society Open Science* 4:1-14. <https://doi.org/10.1098/rsos.161061>.

Menssen, M., Linde, M., Omondia, E.O., Abukutsa-Onyango, M., Dinssad, F.F., and Winkelmann, T., 2017. Genetic and Morphological Diversity of Cowpea (*Vigna unguiculata* (L.) Walp.) Entries from East Africa. *Scientia Horticulturae* 226: 268-276. <https://doi.org/10.1016/j.scienta.2017.08.003>.

Morjan C.L. and Rieseberg L.H., 2004. How Species Evolve Collectively: Implications of Gene Flow and Selection for the Spread of Advantageous Alleles. *Molecular Ecology* 13: 1341–1356.

Muchero, W., Diop, N.N., Bhat, P.R., Fenton, R.D., Wanamaker, S., Pottorff, M., Hearne, S., Cisse, N., Fatokun, C., Ehlers, J.D., Roberts, P.A., and Close, T.J., 2009. A Consensus Genetic Map of Cowpea [*Vigna unguiculata* (L) Walp.] and Synteny Based on EST-Derived SNPs. *Proceedings of the National Academy of Sciences of the United States of America* 106:18159-64.<https://doi.org/10.1073/pnas.0905886106>.

Muñoz-Amatriaín, M., Mirebrahim, H., Xu, P., Wanamaker, S., Luo, M.C., Alhakami, H., Alpert, M., Atokple, I., Batiemo, B.J., Boukar, O., Bozdog, S., Cisse, N., Drabo, I., Ehlers, J.D., Farmer, A., Fatokun, C., Gu, Y.Q., Guo, Y.N., Huynh, B.L., Jackson, S.A., Kusi, F., Lawley, C.T., Lucas, M.R., Ma, Y., Timko, M.P., Wu, J., You, F., Barkley, N.A., Philip A. Roberts, P.A., Lonardi, S., and Timothy J. Close, T.J., 2016. Genome Resources for Climate-Resilient Cowpea, an Essential Crop for Food Security. *The Plant Journal* 89:1042-1054. doi.org/10.1111/tpj.13404.

Nadeem, M.A., Nawaz, M.A., Shahid, M.Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G., and Baloch, F.S., 2017. DNA Molecular Markers in Plant Breeding: Current Status and Recent Advancements in Genomic Selection and Genome Editing: *Biotechnology and Biotechnological Equipment* 32: 261-285. doi: 10.1080/13102818.2017.1400401.

Nei, M., and Li W., 1979. Mathematical Method for Studying Genetic Variation in Terms of Restriction Endonucleases. *Proceedings of National Academy of Sciences* 76:5269-5273.

Nemli, S., Ascioğul, T.K., Ates, D., Esiyok, D., and Tanyolac, M.B., 2017. Diversity and Genetic Analysis through DArTseq in Common Bean (*Phaseolus vulgaris* L.) Germplasm from Turkey. *Turkish Journal of Agriculture and Forestry* 41: 389-404. doi: 10.3906/tar-1707-89.

Peakall, R., and Smouse, P.E., 2007. GENEALEX 6. Genetic Analysis in Excel. Population Genetic Software for Teaching and Research. *Molecular Ecology Notes* 6: 288-295.

Perrier, X., and Jacquemoud-Collet, J.P., 2006. DARwin Software. Dissimilarity Analysis and Representation for Windows. Available from <http://www.darwin.cirad.fr/darwin.html>.

Ragul, S., Manivannan, N., Mahalingam, A, Prasad, V.B.R., and Narayanan, S.L., 2018. SSR Marker Based DNA Fingerprinting for Cowpea Varieties of Tamil Nadu [*Vigna unguiculata* (L.) Walp.]. *International Journal of Current Microbiology and Applied Sciences* 7:641-647. <https://doi.org/10.20546/ijcmas.2018.704.072>.

Slatkin, M., 1989. Population Structure and Evolutionary Progress. *Genome* 31:196–202.

Udensi, O.U., Okon, E.A., Ikpeme, E.V., Onung, O.O., and Ogban, F.U., 2016. Assessing the Genetic Diversity in Cowpea (*Vigna unguiculata* L. Walp) Accessions Obtained from IITA, Nigeria Using Random Amplified Polymorphic DNA (RAPD). *International Journal of Plant Breeding and Genetics* 10: 12-22.

Varshney, R.K., Chabane, K., Hendre, P.S., Aggarwal, R.K., and Graner, A., 2007. A Comparative Assessment of ESTSSR, EST-SNP and AFLP Markers for Evaluation of Genetic Diversity and Conservation of Genetic Resources Using Wild, Cultivated and Elite Barleys. *Plant Science* 173:638–49.

Wamalwa, E. N., Muoma, J., and Wekesa, C., 2016. Genetic Diversity of Cowpea (*Vigna unguiculata* (L.) Walp.) Accession in Kenya Gene Bank Based on Simple Sequence Repeat Markers. *International Journal of Genomics* 2016: 8956412. doi:10.1155/2016/8956412.

Wang, M.L., Barkley, N.A., Gillaspie, G.A., and Pederson, G.A., 2008. Phylogenetic Relationships and Genetic Diversity of the USDA *Vigna* Germplasm Collection Revealed by Gene-Derived Markers and Sequencing. *Genetic Resources* 90: 467–480.

Wright, S., 1978. *Evolution and the Genetics of Populations. Volume 4. Variability Within and Among Natural Populations.* University of Chicago Press, Chicago.

Xiong, H., Shi, A., Mou, B., Qin, J., Motes, D., Lu, W., Ma, J., Weng, Y., Wei Yang, W., and Wu, D., 2016. Genetic Diversity and Population Structure of Cowpea (*Vigna unguiculata* L. Walp). *PLoS ONE* 11: e0160941. doi: 10.1371/journal.pone.016094.

CHAPTER 5

PHENOTYPING COWPEA ACCESSIONS AT THE SEEDLING STAGE FOR DROUGHT TOLERANCE USING THE POT METHOD IN A CONTROLLED ENVIRONMENT.

ABSTRACT

One of the most important screening techniques used in cowpea selection for drought tolerance is screening at the seedling stage. The objective of this study were to phenotype 60 cowpea genotypes for seedling drought tolerance in screen houses (glasshouse and greenhouse). The accessions were planted in pots in the screen houses and a triplicated 6×10 alpha lattice design was used for the experiment. After planting, pots were watered to field capacity for their establishment, thereafter which watering was completely withheld for 4 weeks after planting (WAP), when plants were at the three-leaf stage. Principal component analysis revealed that of the 14 variables, the first four expressed more than 1 eigenvalue. Data showed that PC1, PC2, and PC3 contributed 39.3%, 15.2%, and 10% respectively, and 64.68% cumulative variation. Bartlett's test of sphericity was significant at $p < 0.05$, while the Kaiser-Meyer-Olkin measure of sampling adequacy was 77, indicating sufficient items for each factor. A PCA plot and biplot showed that the number of pods (NP), seeds per pod (SP), survival count (SC), pod weight (PWT), and stem wilting in week one (WWK1) had the most significant contributions to genetic variability in the drought tolerance of cowpea accessions, as well as to the yield after stress imposition. In both the PCA plot and biplot, accessions placed far from each other were more diverse. Based on the PCA, biplot, and scatter plot, the accessions IT 07-292-10, RV 343, and IT 95K-2017-15 had the maximum variability in terms of NP, SP, SC, PWT, and WWK1 after drought imposition. Cowpea accessions 835-911, IT 07-292-10, RV 344, *Kangorongondo*, and IT 90K-59 were the major individuals that contributed to both domain information model (DIM) 1 and 2. The accessions that contributed the least were IT 89KD288, *Chibundi mavara*, and TVU12746. There were significant differences among most drought-related traits at the seedling stage, with the exception of environment \times genotype on days to emergence (DTE), environment \times genotype on stem greenness at week 1 after imposition of water stress (SGWK1) and among replicates in terms of DTE. Thirty-six cowpea accessions from both screen houses were tolerant to drought, 15 were moderately tolerant, while 23 were susceptible. The findings of the study provided a useful tool for screening and determining drought-tolerant and -susceptible accessions at the seedling stage.

Keywords: Phenotype, accessions, biplot, sphericity, Kaiser-Meyer-Olkin, Eigenvalue, Dim

5.1: Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.], Fabaceae, ($2n = 2x = 22$) is an important leguminous crop in developing countries, especially in sub-Saharan Africa, Asia, and Latin America, with a genome size of about 620 million base pairs (Boukar *et al.*, 2018). The improvement of cowpea is mainly dependent on breeding and selection from existing landraces according to the existence of phenotypic variability, which is largely influenced by environmental conditions.

According to the Food and Agriculture Organization of the United Nations (FAO), cowpea was grown on 1 million ha in Africa in 2014, with the bulk of production occurring in West Africa, particularly in Niger, Nigeria, Burkina Faso, Mali and Senegal (FAOSTAT, 2017). The global cowpea production was 5.59 million and the average yield 443.20 kg/ha (Gull *et al.*, 2018). Africa leads in both area and production, accounting for about 95% of each. Niger and Nigeria are the leading producers of cowpea, together accounting for about 70% of the area and 67% of production worldwide. Most cowpea cultivars have relatively short growth and maturation cycles of 60 to 80 days, which makes them suitable for drought-prone regions (Kyei-Boahen *et al.*, 2017).

Drought is one of the most serious environmental stresses, and it has a significant negative impact on crop yield. Gomes *et al.* (2019), recommend the use of water-efficient varieties in combination with good crop husbandry practices. Cowpea plants exposed to temperatures of 30 to 38 °C from eight days after emergence to maturity had very limited vegetative growth and reproductive potential (Singh *et al.*, 2010). Hall *et al.* (2003), observed that there is a great need to screen and breed for drought-tolerant and water-efficient varieties in Africa, as cowpea is grown mostly under rain-fed conditions, with frequent exposure to intermittent droughts. Gomes *et al.* (2019), recommends the use of well-adapted, early maturing cultivars in the smallholder farming sector to escape losses from late season droughts. In an experiment by Araujo *et al.* (2018), to ascertain the growth of cowpea cultivars under osmotic stress, seeds of three cowpea cultivars (BRS Tumucumaque, BRS Aracê, and BRS Guariba) were germinated at five osmotic potentials after three pretreatments: presoaking in deionised water, presoaking in salicylic acid, and without presoaking. It was observed that salicylic acid promoted a reduction in abiotic stress, and BRS Guariba was more tolerant to water deficits and adjusted its cellular electrolyte leakage to increase its proline content under induced water stress.

In a wooden box experiment to screen cowpea recombinant inbred lines (RILs) for seedling drought tolerance, Alidu *et al.* (2018), used 200 inbred lines. It was observed that 12 RILs performed well for recovery, 13 RILs were susceptible to drought stress, and 11 RILs had higher relative water and chlorophyll contents. Ajayi *et al.* (2018), analysed 10 cowpea accessions under greenhouse conditions and observed significant differences among accessions for percentage plant recovery, stem regrowth, and stem greenness. For the evaluation of four Mozambican cowpea landraces for drought tolerance, Martins *et al.* (2014) determined that variability exists among the landraces in terms of growth under drought conditions, with *Timbawene moteado* having considerably higher leaf dry biomass, leaf and nodule protein content, and symbiotic nitrogen fixation compared to those of other landraces, as well as the lowest increase in proteolytic activity.

In a screenhouse experiment to select drought-tolerant cowpea seedlings, Ismai'la *et al.* (2015), evaluated 23 cowpea accessions at the seedling stage in the 2013 and 2014 growing seasons. They observed that the plant height, number of leaves, and stem greenness were all affected by drought stress. It was found that five varieties, Kanannado, Danila, IT07K-297-13, IT03K-378-4, and Aloka local, were highly tolerant to drought. In addition, six varieties IT07K-322-40, IT07K-313-41, IT07K-291-92, IT06K-270, IT07K-244-1-1, and IT06K-275, were classified as highly susceptible to drought and the remaining 12 varieties were found to be neither tolerant nor susceptible to drought. Ismai'la *et al.* (2015), recommends the use of early maturing cowpea cultivars in order for farmers to escape the effects of a late season drought. Most cowpea plants exposed to moisture variation during the vegetative or reproductive stages perform poorly; hence, seedling-stage screening is ideal in this scenario. The objective of this study were to phenotype 60 cowpea genotypes for seedling drought tolerance in screen houses.

5.2: Materials and Methods

5.2.1: Plant material

A total of 60 cowpea accessions collected from three geographic origins were used in this study (Table 5.1). Out of these, 33 accessions were from the International Institute of Tropical Agriculture (IITA) in Nigeria, 19 accessions were from the Agricultural Research Council – Grain Crops in South Africa, and eight accessions were from smallholder farmers in Buhera District in Zimbabwe.

5.2.2: Planting and data collection

Seeds of cowpea accessions were planted in 20 cm diameter pots in topsoil mixed with compost (3:1) in a greenhouse at the Agriculture Research Council – Grain Crops in Potchefstroom, South Africa, in January 2019. The experiment was repeated in a glasshouse in February 2019. An alpha lattice design with four blocks was used for both experiments. A total of 60 accessions were carefully selected for drought tolerance at the seedling stage and were used in the experiments. A triplicated 10×6 alpha lattice design was used for the experiments. After planting, pots were watered to field capacity for their establishment, thereafter which watering was completely withheld for three weeks after planting (WAP), when plants were at the three-leaf stage. Thereafter, wilted plants of each variety were counted daily until all the plants of the susceptible lines had wilted. Stress was measured by observing all dead plants in the susceptible group. Watering resumed at three weeks after stressing in both the greenhouse and glasshouse experiments until harvest. After the resumption of watering, numbers of recovered seedlings were rated for recovery. Based on the days to wilting and percentage recovery, the accessions were rated as either drought-tolerant or –susceptible.

Table 5.1: List of cowpea accessions used in this study obtained from three geographic origin.

Entry	Name	Source	Origin
1	Dr Saunders	ARC-GC	South Africa
2	IT96D-610	IITA	Nigeria
3	RV 574	ARC-GC	South Africa
4	RV 342	ARC-GC	South Africa
5	Pan 311	ARC-GC	South Africa
6	Bechuana white	ARC-GC	South Africa
7	Barapara jena	Buhera	Zimbabwe
8	TVU 9443	IITA	Nigeria
9	95K-589-2	IITA	Nigeria
10	RV 344	ARC-GC	South Africa
11	Agriawa	ARC-GC	South Africa
12	IT 95K-207-15	IITA	Nigeria
13	Orelo	IITA	Nigeria
14	TVU 9671	IITA	Nigeria
15	Mutonono	Buhera	Zimbabwe
16	UAM-14-143-4-1	IITA	Nigeria
17	98K-503-1	IITA	Nigeria
18	RV 503	ARC-GC	South Africa
19	86 D 1010	IITA	Nigeria
20	TVU 9620	IITA	Nigeria
21	RV 202	ARC-GC	South Africa
22	RV 351	ARC-GC	South Africa
23	Encore	ARC-GC	South Africa
24	TVU 14190	IITA	Nigeria
25	IT 89KD-288	IITA	Nigeria
26	RV 551	ARC-GC	South Africa
27	IT 82E-18	IITA	Nigeria
28	Barapara purple	Buhera	Zimbabwe
29	Kangorongondo	Buhera	Zimbabwe
30	835-911	IITA	Nigeria
31	ITOOK 76	IITA	Nigeria
32	98K-476-8	IITA	Nigeria
33	Ziso dema	Buhera	Zimbabwe
34	Chibundi mavara	Buhera	Zimbabwe
35	90K-284-2	IITA	Nigeria
36	RV 221	ARC-GC	South Africa
37	RV 343	ARC-GC	South Africa
38	IT 98K-506-1	IITA	Nigeria
39	Oleyin	IITA	Nigeria
40	IT 07-292-10	IITA	Nigeria
41	IT 08K-150-27	IITA	Nigeria
42	RV500	ARC-GC	South Africa
43	IT 90K-277-2	IITA	Nigeria
44	98D-1399	IITA	Nigeria
45	ITOOK 1263	IITA	Nigeria
46	RV 563	ARC-GC	South Africa
47	IT 18	Buhera	Zimbabwe
48	RV 194	ARC-GC	South Africa
49	335-95	IITA	Nigeria
50	TVU 12746	IITA	Nigeria
51	IT 07-274-2-9	IITA	Nigeria
52	97K-499-35	IITA	Nigeria
53	IT 07-318-33	IITA	Nigeria
54	IT89-KD-288	IITA	Nigeria
55	RV558	ARC-GC	South Africa
56	IT 99K-573-2-1	IITA	Nigeria
57	Mupengo dema	Buhera	Zimbabwe
58	CH47	ARC-GC	South Africa
59	TVU 13004	IITA	Nigeria
60	IT 90K-59	IITA	Nigeria

IITA-International Institute of Tropical Agriculture; ARC GC-Agriculture Research Council Grain Crops.

5.2.3: Data collection

i. Temperature conditions of the screen houses

The daily minimum and maximum temperatures of the screen houses were captured using temperature loggers. The loggers were placed in the screen houses and set to record the temperature at hourly intervals for the whole period of the experiment. The highest and lowest day temperatures recorded in the greenhouse (environment one) were 35.75 °C and 27.67 °C, respectively. The highest and lowest night temperatures recorded in the greenhouse were 26.87 °C and 19.99°C respectively. The highest and lowest daytime temperatures recorded in the glasshouse (environment 2) were 36.4 °C and 19 °C respectively. The highest and lowest temperatures recorded in the glasshouse was 23.64 °C and 18.5 °C respectively.

ii. Agronomic traits

Drought tolerance was estimated using the wilting score (WS) as the degree of wilting severity, based on the 0–4 score scale as described by Singh *et al.* (2013). Data were collected on number of days to seedling emergence, stem greenness, and wilting at 14, 21, and 30 days after planting (DAP), and rated on a scale of 0–4 (Muchero *et al.*, 2008).

Stem greenness

0 = leaves and stem completely yellow

1 = 75% of the leaves yellow, brown either from the base or tip of the stem

2 = 50% yellow or pale green, stem not turgid

3 = 25% yellow, 75% green, stem less turgid

4 = completely green, stem turgid

Wilting

0 = no sign of wilting

1 = 25% wilting

2 = moderate wilting, 50%

3 = yellow and brown leaves with 75% wilting

4 = completely wilted

After rewatering, data were collected on the survival count (SC): the number of surviving plants per genotype.

Recovery type

0 = no recovery

0.5 = recovery from the basal meristem

1 = recovery from the apical meristem

Recovery rate (RR)

The RR is computed as follows: (No. of dead plants/No. of emerged plants) × 100

5.3: Data Analysis

A two-way analysis of variance (ANOVA) was used to determine significant differences in days to emergence (DTE), wilting scores, survival count, and yield-related traits. GenStat (version 19) software (www.genstat.kb.vsnl.co.uk) was used for the statistical analysis of data. Statistical analysis was performed using IBM SPSS (version 20) (www.ibm.com/support/pages/spss-statistics-20-available-download) statistical computer package for principal component analysis, scree plot, and rotated component plot.

5.4: Results

There were significant differences among most drought-related traits at the seedling stage, with the exception of environment × genotype on DTE, stem greenness at week 1 after the imposition of water stress (SGWK1) (Table 5.2).

Table 5.2. Mean square of traits from the analysis of variance combined from two- screen houses for 60 cowpea accessions under drought stress conditions.

Source	DF	Trait								
		DTE	RR	SC	SGWK1	SGWK2	SGWK3	WWK1	WWK2	WWK3
Envt	1	36.74**	9343.2**	137.52**	52.90**	62.5**	46.94**	154.71**	18.68**	122.50**
Envt x Genotype	118	0.46ns	1012.2**	0.30ns	0.44ns	0.78**	0.72**	0.52**	1.29**	0.57**
Rep	2	0.81ns	3432.5**	84.94**	9.29**	4.84**	0.58**	3.10**	3.94**	34.70**
Residual	238	0.34	317.4	0.81	0.31	0.38	0.39	0.2292	0.39	0.31

** : significant at $p < 0.01$, ns: not significant. DTE=date to emergence, RR=recovery rate, SC=survival count, SGWK1=stem greenness in week 1 after imposition of water stress, SGWK2=stem greenness in week 2 after imposition of water stress, SGWK3=stem greenness in week 3 after imposition of water stress, WWK1=level of wilting in week 1 imposition of water stress, WWK2=level of wilting in week 2 after imposition of water stress and WWK3= level of wilting in week 3 after imposition of water stress

There were significant differences among most yield-related traits, with the exception of the environment on the number of pods (NP), and number of seeds (NS) (Table 5.3).

Table 5.3. Mean square of yield-related traits from the analysis of variance combined from two-screen houses for 60 cowpea accessions after drought stress.

Source	DF	Trait					
		AVSD	NP	NSDS	PL	PWDTH	PWT
Envt	1	59.211**	30.044ns	20.07ns	226.768**	1.22267**	512.298**
Envt x Gen	118	28.831**	17.574**	1006.99**	51.074**	0.33401**	215.105**
Rep	2	14.925**	10.703ns	899.76**	12.388ns	0.04170ns	59.292**
Residual	238	1.936	3.462	62.35	2.453	0.01265	7.068

** : significant at $p < 0.01$, ns: not significant. AVSD = average seeds, NP = number of pods, NSDSS = number of seeds, PL = pod length, PWDTH = pod width and PWT = pod weight.

5.4.1: Principal component analysis

Principal component analysis (PCA) revealed that of the 14 component variables (PCs) only four PCs with eigenvalue greater than 1 were maintained (Table 5.4). The proportion of variance among the four principal components (PCs) was 39.4% for PC 1, 15.2% for PC2, 10.1% for PC3, and 7.4% for PC4. The cumulative variance was 39.38% for PC1, 54.6% for PC2, 64.7% for PC3, and 72.1% for PC4. The first principal component (PC) was positively influenced by PWT, with a value of 0.358, as well as by pod length (PL) (0.286), seeds per pod (SP) (0.263), seed weight (SWT) (0.255), and NP (0.181). PC2 was influenced by stem greenness at three weeks after planting (SGWK3), with a value measuring 0.332, and survival count (SC), with a value of 0.232. In PC3, stem greenness at week one (SGWK1) had the highest value (0.384), followed by SGWK2 (0.295) and PWT (0.109). In PC4, the DTE had a positive influence (0.926), as did SGWK2 (0.194).

Table 5.4: Eigen-values, proportions of variability and morphological traits that contributed to the first four PCs of cowpeas.

	PC1	PC2	PC3	PC4
Eigen values	5.51	2.13	1.41	1.04
Proportion of variance (%)	39.4	15.2	10.1	7.4
Cumulative variance (%)	39.38	54.6	64.7	72.1
Day to emergence	0.034	0	-0.008	0.926
SGWK1	-0.05	-0.101	0.384	-0.169
SGWK2	-0.045	0.032	0.295	0.194
SGWK3	-0.123	0.332	-0.004	0.088
WWK1	-0.07	0.13	-0.339	-0.039
WWK2	0.069	-0.117	-0.216	0.063
WWK3	0.111	-0.277	-0.052	0.004
SC	0.004	0.232	-0.178	0.025
Recovery Rate	0.095	-0.322	0.055	0.095
NP	0.181	0.063	-0.036	-0.047
SP	0.263	-0.012	-0.074	0.033
PL	0.286	-0.048	-0.074	0.035
PWT	0.358	-0.283	0.109	0.022
SWT	0.255	-0.034	0.027	0.012

SGK1 = stem greenness in week1; SGWK2 = stem greenness in week2; SGWK3 = stem greenness in week3; WWK1 = wilting in week1; WWK2 = wilting in week2; WWK3 = wilting in week3; SC= survival count; RR = recovery rate; NP = number of pods; SP = seeds per pod; PL = pod length; PWT = pod weight; SWT = seed weight.

A scree plot to show the relationship between eigenvalues and principal components was constructed to summarise the contribution of PCs (Figure 5.1). The plot showed that maximum variation was present in variable 1 with the highest eigenvalue of 5.8 followed by variable 2 (2.1), variable 3 (1.4), and variable 4 (1). Variable 14 had the lowest eigenvalue (0).

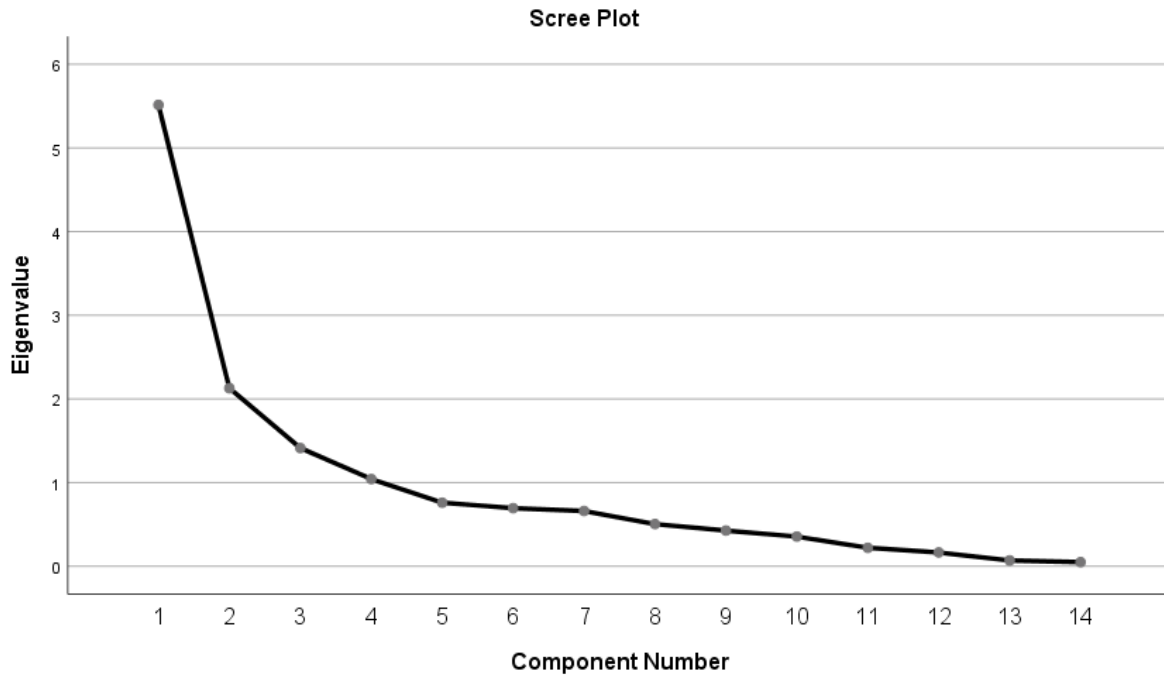


Figure 5.1: Scree plot showing contributions of PCs to variability.

A further PCA with VARIMAX rotation was conducted to assess how the variables were clustered. (Figure 5.2). The component plot in rotated space (Figure 5.2) highlights the important variables in order when all three components are compared. WWK1, WWK2 and SC are the most important variables of the three components respectively. Bartlett’s test of sphericity was significant at $p < 0.05$ while the Kaiser-Meyer-Olkin measure of sampling of adequacy was 77, indicating sufficient items for each factor.

Component Plot in Rotated Space

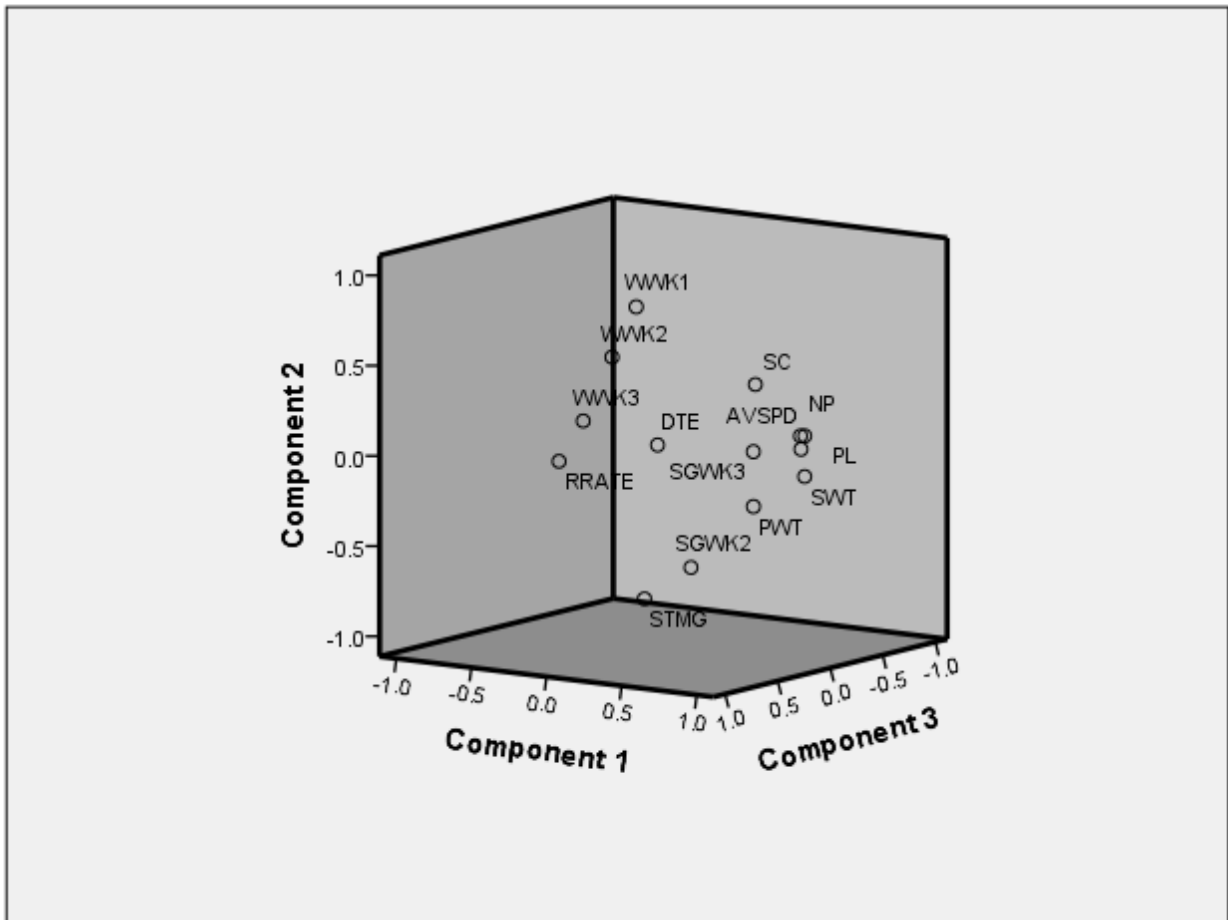


Figure 5.2: Component plot in rotated space showing contributions of principal component variables.

The day and night temperature ranges in both screen houses varied during the three-week period when the plants were stressed. The highest and lowest daytime temperatures recorded in the greenhouse (environment one) were 35.75 °C and 27.67 °C, respectively, with a mean daytime temperature of 32.24 °C (Figure 5.3). The highest and lowest night-time temperatures recorded in the greenhouse were 26.87 °C and 19.99 °C, with a mean night-time temperature of 23.98 °C respectively. The highest and lowest daytime temperatures recorded in the glasshouse (environment 2) was 36.4 °C and 19 °C, with a mean daytime temperature of 26.06 °C respectively. The highest and lowest temperatures recorded in the glasshouse was 23.64 °C and 18.5 °C, with a mean night-time temperature of 21.42 °C respectively.

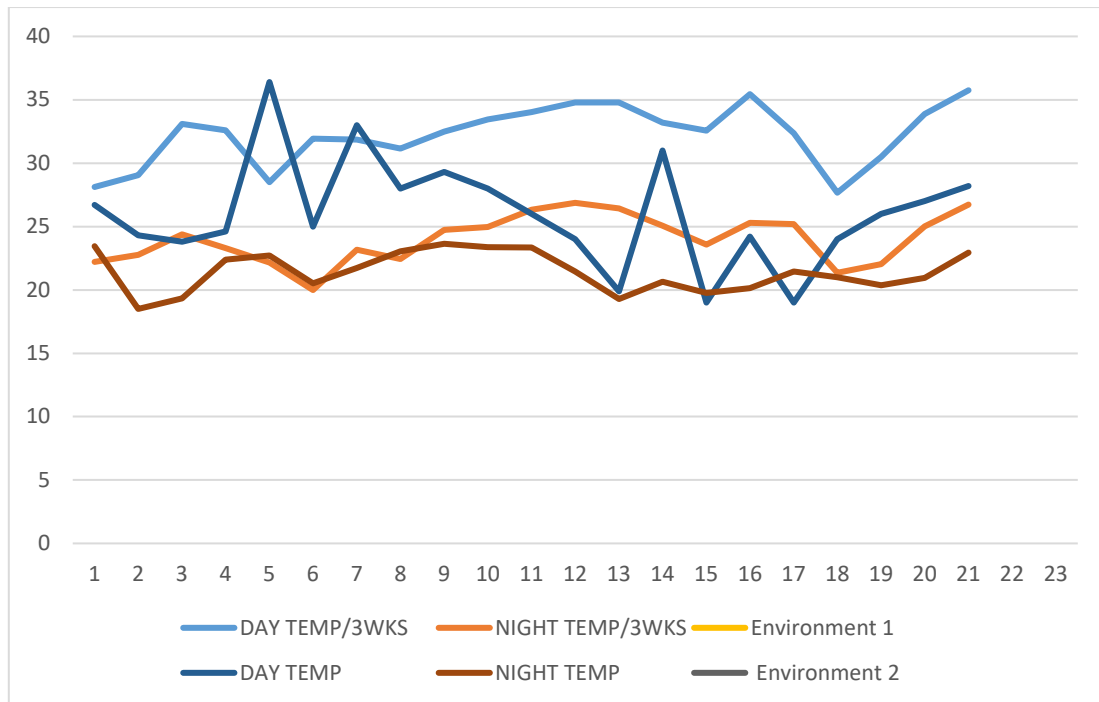


Figure 5.3: Graph showing day and night temperature ranges for 3 weeks.

In the PCA plot, NP, SP, SC, PW and WWK1 had the most significant contributions to genetic variability in the drought tolerance cowpea accessions, as well as to yield after stress imposition (Figure 5.4).

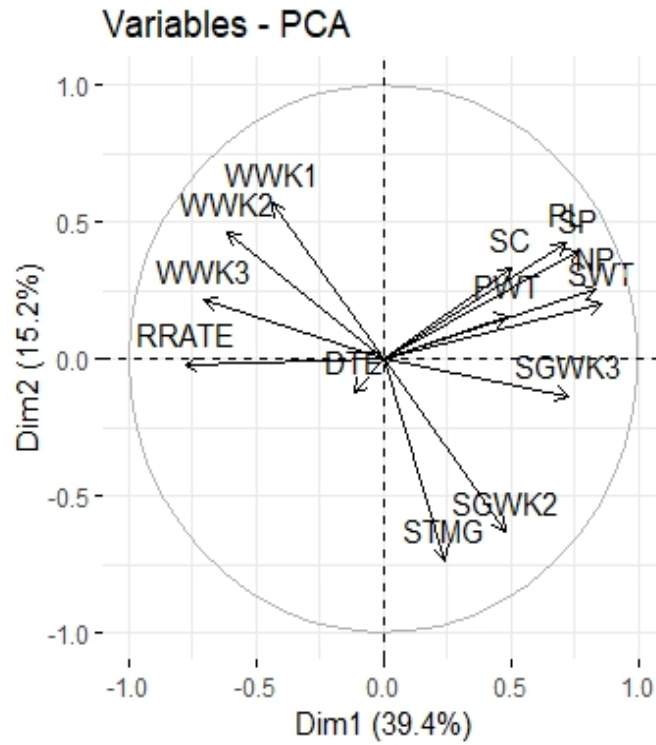


Figure 5.4: The contribution of various variables among 60 cowpea accessions screened for drought tolerance.

Cowpea accessions 835-911, IT 07-292-10, RV 344, *Kangorongondo*, and IT 90K-59 contributed the most to both domain information model (DIM) 1 and DIM 2 (Figure 5.5). The accessions IT 89KD288, *Chibundi mavara*, and TVU12746 contributed the least.

The main contributors to DIM 3 were PWT), SGWK3, and RR (Figure 5.6). The variables that contributed the least were SWT, PL, and SPD.

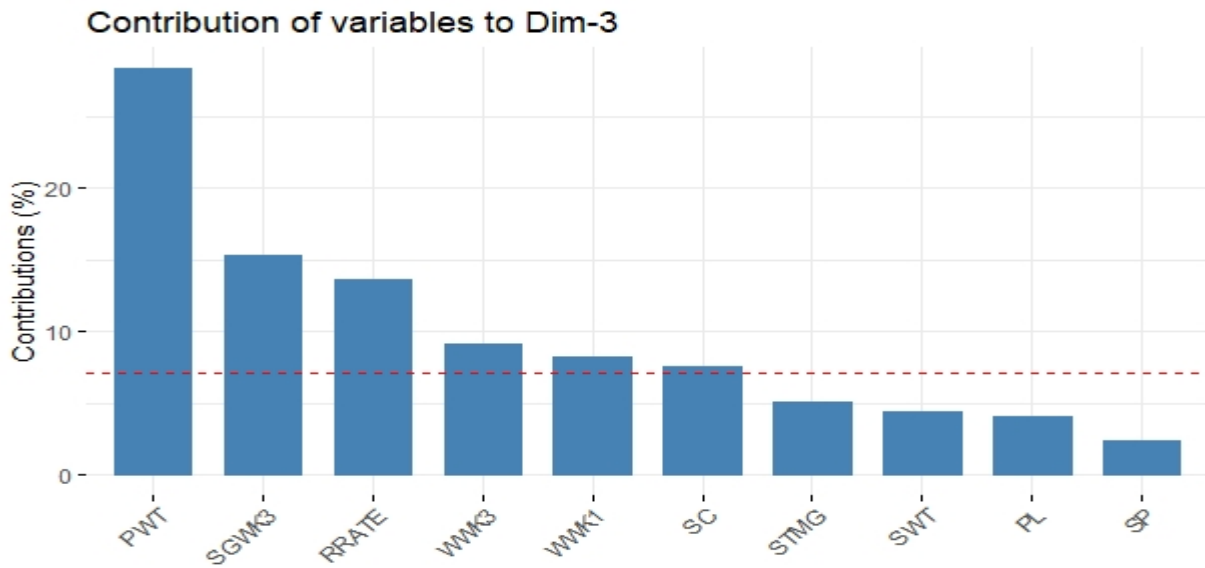


Figure 5.6: The contribution of various variables among 60 cowpea accessions screened for drought tolerance.

Figure 5.7 shows the relationships among traits in DIM 1 to DIM 5. Dim 1 was dominated by SWT, NP, and SGWK3. DIM 2 was dominated by SGWK1 and stem greenness at two weeks after drought imposition and SGWK2. PWT was the dominant trait in DIM 3, while that in DIM 4 was DTE

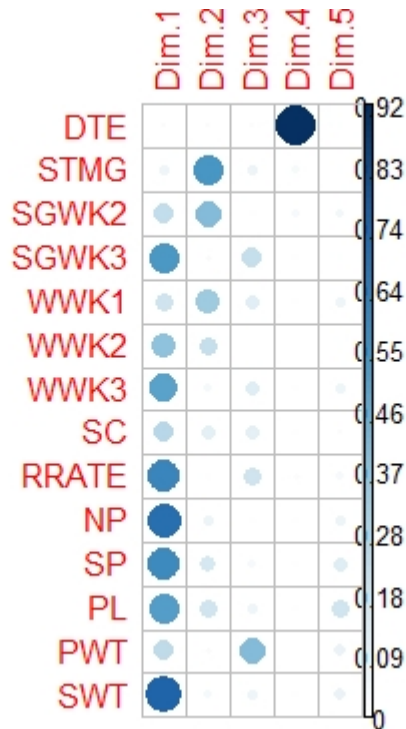


Figure 5.7: The contribution of various variables to Dim 1 to Dim 5.

The cluster plot analysis showed that the cowpea accessions can be grouped into three distinct clusters; red, blue, and green (Figure 5.8). Most accessions were grouped into the red and blue clusters. However, there was an overlap of accessions in the green and red clusters. As such, some accessions (TVU 13004, ITOOK 1263, IT89 KD 288, RV 588, Bechuana White, TVU 12746, IT07-318-33, and TVU 9671) managed to withstand water stress and went on to flower and produce pods when irrigation was resumed after three weeks of water stress.

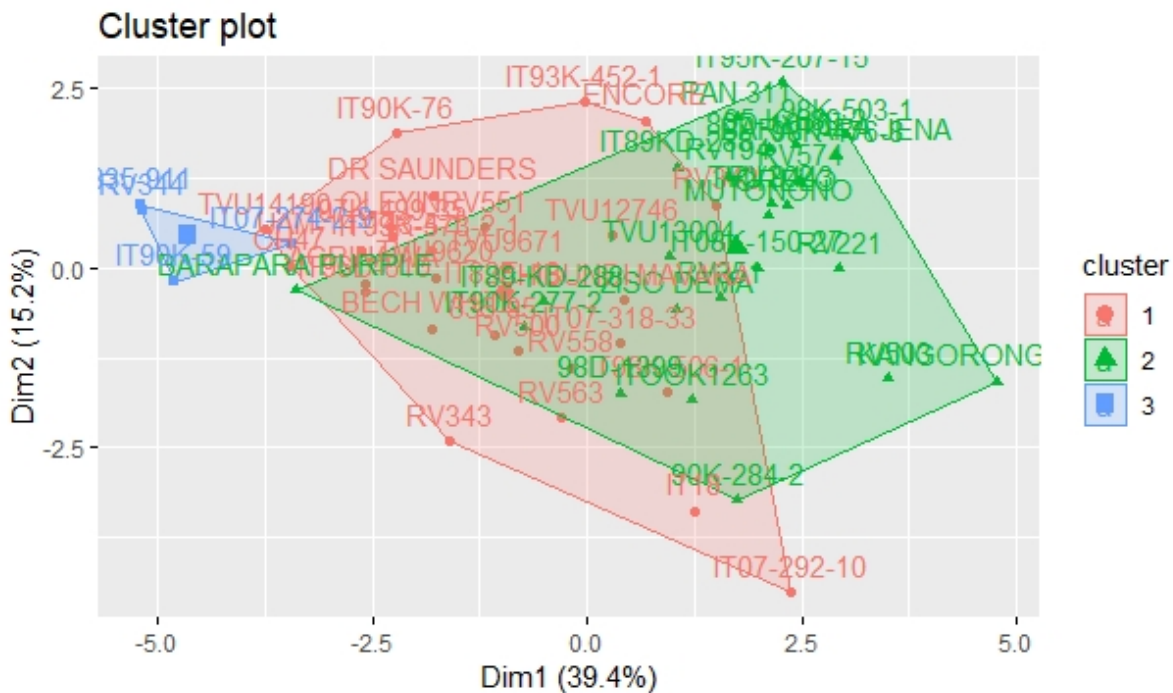


Figure 5.8: Cluster Plot showing the three groups of cowpea accessions grouped according to their levels of drought tolerance. Cluster 1 Moderately tolerant, Cluster 2 Susceptible and Cluster 3 Tolerant.

The relationship of cowpea traits was studied using correlation coefficients. The correlation coefficient was weak and not statistically significant for SWT. However, the correlation coefficient was statistically significant in SGWK2 compared to those of other traits. This means that there was an inverse relationship between two stem greenness and weeks. All of the significant correlation coefficients were positive and were mainly between DTE, SGWK1, SGWK3, SC, NP, SP, PL, PWT, and SWT (Table 5.4). Pearson correlation analysis showed that the most significant relationships were observed from SGWK2 up to SWT. In addition, DTE had mostly weak negative correlations with most of the measured attributes. Most positive correlations were observed in SWT, PWT, and PL.

Table 5.5: Pearson correlation coefficients of traits assessed for 60 cowpea accessions evaluated under two moisture regimes.

	DTE	STMG	SGWK2	SGWK3	WWK1	WWK2	WWK3	SC	RRATE	NP	SP	PL	PWT	SWT
DTE	1	-0.062	0.132	0.047	-0.012	0.042	0	-0.034	0.07	-0.204	-0.102	-0.118	-0.216	-0.179
STMG		1	0.460**	0.11	-0.476**	-0.362**	-0.256*	-0.104	-0.079	-0.008	0.003	-0.028	0.068	0.064
SGWK2			1	0.372**	-0.356**	-0.475**	-0.266*	0.091	-0.24	0.178	0.141	0.096	0.169	0.214
SGWK3				1	-0.255*	-0.360**	-0.729**	0.285*	-0.696**	0.516**	0.402**	0.349**	0.439**	0.472**
WWK1					1	.0371**	0.349**	0.108	0.162	-0.19	-0.184	-0.203	-0.246	-0.258*
WWK2						1	0.456**	-0.218	0.449**	-0.313*	-0.286*	-0.195	-0.324*	-0.378**
WWK3							1	-0.324*	0.667**	-.460**	-.396**	-0.358**	-.397**	0-.446**
SC								1	-0.506**	0.374**	0.382**	0.367**	0.421**	0.416**
RRATE									1	-0.687**	-0.550**	-0.456**	-0.602**	-0.640**
NP										1	0.781**	0.723**	0.912**	0.939**
SP											1	0.933**	0.813**	0.839**
PL												1	0.778**	0.795**
PWT													1	0.954**
SWT														1

significant at $p < 0.01$, *: not significant. DTE; date to emergence, SGWK1: stem greenness in week 1, SGWK2: stem greenness in week 2, SGWK3: stem greenness in week 3; WWK1: wilting in week1; WWK2: wilting in week2; WWK3: wilting in week3; SC: survival count; RR: recovery rate; NP: number of pods; SP: seeds per pod; PL: pod length; PWT: pod weight; SWT: seed weight

The 60 cowpea accessions used in this study varied in their response to drought imposition. Thirty-six cowpea accessions from both screen houses were tolerant to drought, 15 were moderately tolerant, while 23 were susceptible, based on the 14 traits measured (Table 5.5).

Table 5.6: The response to drought stress of 60 cowpea accessions based on 14 traits measured.

Entry	Name	Source	Origin	Response To Drought
1	Dr Saunders	ARC-GC	South Africa	Tolerant
2	IT96D-610	IITA	Nigeria	Tolerant
3	RV 574	ARC-GC	South Africa	Moderate
4	RV 342	ARC-GC	South Africa	Moderate
5	Pan 311	ARC-GC	South Africa	Moderate
6	Bechuana white	ARC-GC	South Africa	Tolerant
7	Barapara jena	Buhera	Zimbabwe	Susceptible
8	TVU 9443	IITA	Nigeria	Moderate
9	95K-589-2	IITA	Nigeria	Susceptible
10	RV 344	ARC-GC	South Africa	Susceptible
11	Agrinawa	ARC-GC	South Africa	Tolerant
12	IT 95K-207-15	IITA	Nigeria	Susceptible
13	Orelo	IITA	Nigeria	Moderate
14	TVU 9671	IITA	Nigeria	Tolerant
15	Mutonono	Buhera	Zimbabwe	Moderate
16	UAM-14-143-4-1	IITA	Nigeria	Tolerant
17	98K-503-1	IITA	Nigeria	Susceptible
18	RV 503	ARC-GC	South Africa	Moderate
19	86 D 1010	IITA	Nigeria	Moderate
20	TVU 9620	IITA	Nigeria	Tolerant
21	RV 202	ARC-GC	South Africa	Moderate
22	RV 351	ARC-GC	South Africa	Moderate
23	Encore	ARC-GC	South Africa	Moderate
24	TVU 14190	IITA	Nigeria	Tolerant
25	IT 89KD-288	IITA	Nigeria	Moderate
26	RV 551	ARC-GC	South Africa	Tolerant
27	IT 82E-18	IITA	Nigeria	Tolerant
28	Barapara purple	Buhera	Zimbabwe	Susceptible
29	Kangorongondo	Buhera	Zimbabwe	Susceptible
30	835-911	IITA	Nigeria	Tolerant
31	ITOOK 76	IITA	Nigeria	Tolerant
32	98K-476-8	IITA	Nigeria	Susceptible
33	Ziso dema	Buhera	Zimbabwe	Tolerant
34	Chibundi mavara	Buhera	Zimbabwe	Tolerant
35	90K-284-2	IITA	Nigeria	Tolerant
36	RV 221	ARC-GC	South Africa	Susceptible
37	RV 343	ARC-GC	South Africa	Tolerant
38	IT 98K-506-1	IITA	Nigeria	Tolerant
39	Oleyin	IITA	Nigeria	Tolerant
40	IT 07-292-10	IITA	Nigeria	Tolerant
41	IT 08K-150-27	IITA	Nigeria	Moderate
42	RV500	ARC-GC	South Africa	Tolerant
43	IT 90K-277-2	IITA	Nigeria	Tolerant
44	98D-1399	IITA	Nigeria	Tolerant
45	ITOOK 1263	IITA	Nigeria	Tolerant
46	RV 563	ARC-GC	South Africa	Tolerant
47	IT 18	Buhera	Zimbabwe	Tolerant
48	RV 194	ARC-GC	South Africa	Moderate
49	335-95	IITA	Nigeria	Tolerant
50	TVU 12746	IITA	Nigeria	Tolerant
51	IT 07-274-2-9	IITA	Nigeria	Tolerant
52	97K-499-35	IITA	Nigeria	Tolerant
53	IT 07-318-33	IITA	Nigeria	Tolerant
54	IT89-KD-288	IITA	Nigeria	Tolerant
55	RV558	ARC-GC	South Africa	Tolerant
56	IT 99K-573-2-1	IITA	Nigeria	Tolerant
57	Mupengo dema	Buhera	Zimbabwe	Moderate
58	CH47	ARC-GC	South Africa	Tolerant
59	TVU 13004	IITA	Nigeria	Tolerant
60	IT 90K-59	IITA	Nigeria	Tolerant

ARC-GC: Agriculture Research Council-Grain Crops; IITA: International Institute of Tropical Agriculture.

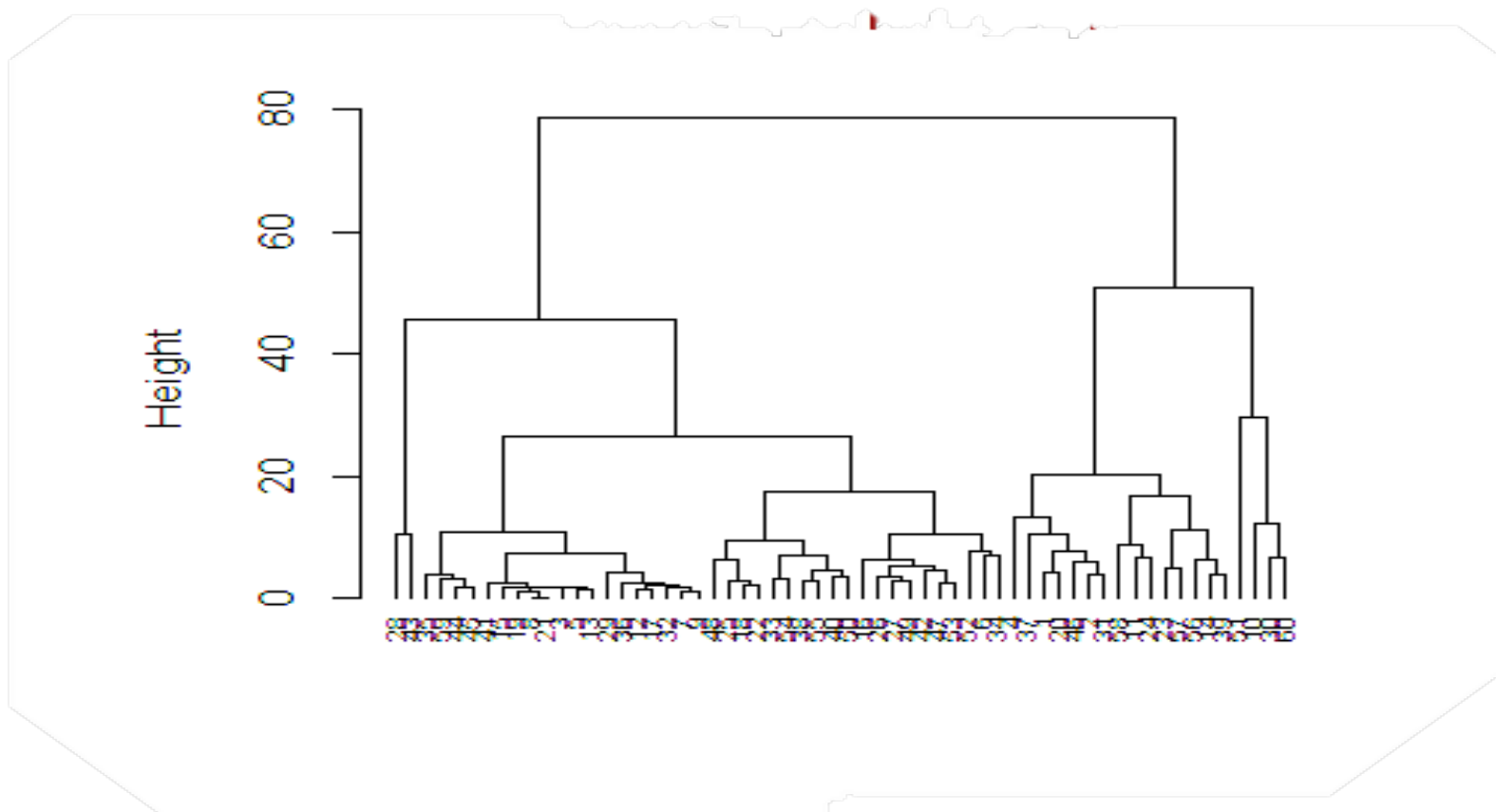


Figure 5.10: Clustering patterns of the 60 cowpea accessions constructed based on the neighbour-joining algorithm using the unweighted pair group method (UPGMA) according to drought tolerance and yield- related traits.

5.4.2: Discussion

This study revealed that moisture is a very important component in plant growth and reproduction. According to Padi (2004), when moisture stress is imposed during the vegetative stage, it has the most effect on shoot and dry weight reduction in cowpeas. It is also during the vegetative stage that plants set up their architecture for reproduction. Alidu (2018) observed that moisture stress imposed after the pod-filling stage in determinate accessions has a limited reduction on the shoot and root biomass.

Most of the cowpea accessions showed differences in their response to drought imposition in their stem greenness from week 1 to week 3 after drought imposition. A similar variation was also observed when wilting was recorded from week 1 to week 3 after drought imposition. In both environments, the temperature had a significant effect on the performance of the accessions. In the greenhouse experiment, the average day and night temperatures were 34.24 °C and 23.98 °C, respectively. In the glasshouse experiment, the mean day and night temperatures were 26.06 °C and 21.42°C respectively. According to DAFF (2011), the optimum temperature for growth and development of crops is around 30 °C; hence, 27 out of the 37 tolerant accessions were located in the glasshouse. Alidu *et al.* (2019), also confirmed that temperatures above 30 °C increases the intensity of stress levels in cowpeas thus fewer accessions were found in the greenhouse.

Both the PC plot and biplot highlight the importance of the distance of variables to PCs and their ultimate contributions to the drought tolerance of accessions, as well as to the yield after stress imposition. The PC plot and biplot showed that the NP, SP, SC, PWT, and WWK1 had the most significant contributions to genetic variability in drought tolerance in cowpea accessions, as well as to the yield after stress imposition. In both the PC plot and biplot, accessions placed far from each other were more diverse. Based on the PC, biplot, and scatter plot, the accessions IT 07-292-10, RV 343, and IT 95K-2017-15 had the maximum variability for the NP, SP, SC, PWT, and WWK1, and could be used in future breeding programmes. Al-Saady *et al.* (2018) used the PC plot and biplot to reveal the large variation among 64 cowpea accessions in terms of seed length and width, 100-seed weight, and seed colour.

In DIM 1 and DIM 2, SWT, NP, and SG were the major determinants. Both groups had accessions 835-911, IT07-292-10, IT90-59, IT89KD288, *Chibundi mavara*, and TVU12746,

which were tolerant to drought, while RV344 and *Kangorongondo* were susceptible to drought during the first week of drought imposition.

Walle *et al.* (2018), observed significant and positive correlations among the number of pods per peduncle and number of seeds per pod, PWT, seed length, seed thickness, SW, 100-seed weight, biomass, and harvest index at the genotypic and phenotypic levels. Diwaker *et al.* (2018), revealed that at the genotypic and phenotypic levels, a significant and positive correlation was shown by pod yield quintal per hectare with pod yield per plant and pod length. Ajayi *et al.* (2017), observed that the genotypic coefficient of variation (GCV) was lower than the phenotypic coefficient of variation (PCV) for all studied traits. They observed that both the GCV and PCV were reduced as drought stress went beyond 21 days among the wilting parameters and morphological traits, because of the influence of the environment on these traits.

The main traits that accounted for variability from PC1 to PC4 in the screenhouses were PWT, PL, SP, SWT, NP, SGWK, and DTE. This implies that accessions that emerged earlier and withstood the imposition of drought had higher chances of podding and producing seeds. Thus, it is imperative to consider these traits in further enhancing cowpea accessions' tolerance to drought at the seedling stage. Ajayi *et al.* (2018), recommend the drought susceptibility score, percentage of permanent wilting, stem greenness and regrowth, number of leaves, and stem girth as the most ideal traits for use in the study of drought tolerance in cowpea seedlings. However, Alidu (2018), recommends a wide collection of cowpea lines in order to select the most tolerant genotypes for various growth stages as parents in a hybridisation programme.

On the cluster plot analysis, accessions in cluster 1 had higher values compared to all other clusters for all traits investigated in this study except for SGWK1, 2 and 3 after drought imposition and wilting in WWK1 after drought imposition. In both greenhouse and glasshouse experiments, this cluster had early maturing and high yielding accessions that can be used in future cowpea breeding programmes for drought tolerance at the seedling stage. The accessions used in this study, however, showed very little variation as was highlighted by UPGMA. This supports the findings of the principal component analysis. The differences and similarities in accessions on some clusters as a result of their locations indicate the extent of accession exchange among farmers from different regions (Al-Saady *et al.*, 2018).

5.4.3: Conclusion

The findings of this study provided a useful tool for screening and determining drought-tolerant and -susceptible accessions at the seedling stage. The results of the investigation were also useful in selecting accessions especially for average seeds per pod (AVSPD), number of seeds (NS), pod length (PL), pod width (PWDTH), and pod weight (PWT) for further breeding programmes. Some accessions were able to perform well in both screenhouses, under different temperature conditions. This stability of accessions with minimal variation in any environment or location can serve as a genetic pool or germplasm collection for the breeding of drought-tolerant cowpea accessions.

References

- Ajayi, A.T., Olumekun, V.O., and Gbadamosi, A.E., 2017. Estimates of Genetic Variation among Drought Tolerant Traits of Cowpea at Seedling Stage. *International Journal of Plant Research* 7(2): 48-57. doi: 10.5923/j.plant.20170702.04.
- Ajayi, A.T., Olumekun, V.O., and Gbadamosi, A.E., 2018. Screening for Drought Tolerance in Cowpea (*Vigna unguiculata* L. Walp) at Seedling Stage under Screen House Condition. *International Journal of Biosciences and Technology* 11:1-19.
- Alidu, M.S., 2018. Evaluation of Cowpea Genotypes for Drought Tolerance Using the Pot Screening Approach. *Asian Research Journal of Agriculture*. Volume 10: 1-11. doi: 10.9734/ARJA/2018/45806.
- Alidu, M. S., Asante, I. K., Tongoona, P., Ofori, K., Danquah, A., and Padi, F.K., 2019. Development and Screening of Cowpea Recombinant Inbred Lines for Seedling Drought Tolerance. *Journal of Plant Breeding and Crop Science* 11 pp 1-10. doi:10.5897/JPBCS 2018.0768.
- Al-Saady, N.A., Nadaf, S.K., Al-Lawati, A.H., and Al-Hinai. S.A., 2018. Germplasm Collection and Seed Diversity of Cowpea (*Vigna unguiculata* (L.) Walp.) *Asian Journal of Agriculture and Food Science* 6:129-139.
- Araujo, E.D., Melo, A.S, and Rocha, M.S., 2018. Germination and Initial Growth of Cowpea Cultivars under Osmotic Stress and Salicylic Acid. *Revista Caatinga* 31:80-89. <https://dx.doi.org/10.1590/1983-21252018v31n110rc>.
- Boukar O., Belko, N., Chamarthi S., Togola, A., Batiemo, J., Owusu, E., Haruna, M., Diallo, S., Umar, M.L., Olufajo, O., and Fatokun, C., 2018. Cowpea (*Vigna unguiculata*): Genetics, Genomics and Breeding. *Plant Breeding* 2018; 00:1-10. <https://doi.org/10.1111/pbr.12589>.
- Diwaker, P., Sharma, M.K., Soni, A.K., Diwaker, A., and Singh, P., 2018. Character Association and Path Coefficient Analysis in Vegetable Cowpea [*Vigna unguiculata* (L.) Walp]. *Journal of Pharmacognosy and Photochemistry* 7: 2289-2293.

DAFF, 2011. Directorate of Plant Production. Production Guidelines for Cowpeas. [www.arc.agric.za/Cowpea Production Guidelines.pdf](http://www.arc.agric.za/Cowpea%20Production%20Guidelines.pdf).

FAOSTAT, 2017. Food and Agriculture Organization of the United Nations Statistics Division. Available at: <http://faostat3.fao.org/download/Q/QC/E>.

Genstat, 2019. GenStat version 19 software. Available at www.genstat.kb.vsn.co.uk.

Gomes, A.M.F., Ntantumbo, N., Ferreira-Pinto, M., Ferreira-Pinto, M., Massinga, R., Ramalho, J.C., and Ribeiro-Barros, A., 2019. Breeding Elite Cowpea [*Vigna unguiculata* (L.) Walp] Varieties for Improved Food Security and Income in Africa: Opportunities and Challenges. IntechOpen. doi: [dx.doi.org/10.5772/intechopen.84985](https://doi.org/10.5772/intechopen.84985).

Gull, M., Sofi, P.A., Mir, R.R., Ara, A., Dar, S.A., and Bhat, M.A., 2018. Maturity, Biomass Partitioning and Growth Response Indices in Cowpea (*Vigna unguiculata* L.) under Water Stress. International Journal of Agriculture, Environment and Biotechnology 11(6): 863-869. doi: [10.30954/0974-1712.12.2018.7](https://doi.org/10.30954/0974-1712.12.2018.7).

Hall, A.E., Cisse, N., Thiaw, S., Elawad, H.O.A., Ehlers, J.D., Ismail, A.M., Fery, R.L., Roberts, P.A., Kitch, L.W., Murdock, L.L., Boukar, O., Phillips, R.D., and McWatters, K.H., 2003. Development of Cowpea Cultivars and Germplasm by the Bean/Cowpea CRSP. Field Crops Research 82:103–134.

Isma'ila, M., Ramlatu, M.A., and Zakari, B.G., 2015. Screening of Selected Varieties of Cowpea Seedlings [*Vigna unguiculata* (L.) Walp.] for Drought Tolerance. Journal of Biology and Nature 5(1): 31-38.

Kyei-Boahen, S., Savala, C.E.N., Chikoye, D., and Abaidoo, R., 2017. Growth and Yield Responses of Cowpea to Inoculation and Phosphorus Fertilization in Different Environments. Frontiers in Plant Science 8:646. doi.org/[10.3389/fpls.2017.00646](https://doi.org/10.3389/fpls.2017.00646).

Martins, C.M., Lawlor, D.W., Quilambo, O.A., and Kunert, K.J., 2014. Evaluation of Four Mozambican Cowpea Landraces for Drought Tolerance. South African Journal of Plant and Soil.31:2, 87-91, doi: [10.1080/02571862.2014.907453](https://doi.org/10.1080/02571862.2014.907453).

Muchero, W., Ehlers, J.D., and Roberts, P.A., 2008. Seedling Stage Drought-Induced Phenotypes and Drought-Responsive Genes in Diverse Cowpea Genotypes. *Crop Science* 48:541-552.

Padi, F. K., 2004. Relationship between Stress Tolerance and Grain Yield Stability in Cowpea. *Journal of Agricultural Science* 142: 431–443.

Singh, S.K., Kakani, V.G., Surabhi, G.K., and Reddy, K.R., 2010. Cowpea (*Vigna unguiculata* [L.] Walp.) Genotypes Response to Multiple Abiotic Stresses. *Journal of Photochemistry and Photobiology* 100:135-146. doi: 10.1016/j.jphotobiol.2010.05.013.

Singh, D., Dikshit, H.K., and Singh, R., 2013. A New Phenotyping Technique for Screening for Drought Tolerance in Lentil (*Lens culinaris* Medik) Plant Breeding 132: 185–190. doi:10.1111/pbr.12033.

SPSS, 2020. IBM SPSS version 20. Available at www.ibm.com/support/pages/spss-statistics-20-available-download.

Walle, T., Mekbib, F., Amsalu, B. and Gedil, M., 2018. Correlation and Path Coefficient Analyses of Cowpea (*Vigna unguiculata* L.) Landraces in Ethiopia. *American Journal of Plant Sciences* 9:2794-2812. <https://doi.org/10.4236/ajps.2018.913202>.

CHAPTER 6

ASSOCIATION MAPPING FOR DROUGHT TOLERANCE AND YIELD-RELATED TRAITS IN COWPEA ACCESSIONS.

ABSTRACT

Cowpea is an important drought-tolerant crop that is grown mainly in Africa. The objective of this study were to conduct association mapping for drought tolerance at the seedling stage and yield-related traits in cowpeas. A total of 60 cowpea accessions were used in the study. Single-nucleotide polymorphisms (SNPs) discovered through genotyping by sequencing (GBS) were used for genotyping. Association mapping was conducted using single-marker regression (SMR) in Q Gene, and general linear model (GLM) and mixed linear model (MLM) built in TASSEL. The population of the cowpea accessions were analysed using STRUCTURE 2.3.4 and the peak of delta K in the greenhouse showed seven population types, whereas the peak of delta K in the glasshouse indicated the presence of six population types. One SNP marker, 14083649|F|0-9 was associated with NP with a p value <0.001 . Fifty SNP markers were associated with PWT at $p <0.001$. Four SNP markers, 14074781|F|0-16, 100047392|F|0-36, 14083801|F|0-28 and 100051488|F|0-49 were associated with AVSPD at $p <0.001$. SNP markers, 14074781|F|0-16, 14083801|F|0-28 and 100051488|F|0-49 were associated with PL at $P <0.001$. Five SNP markers, 100047392|F|0-36, 14083801|F|0-28, 100072738|F|0-34, 14076881|F|0-49 and 14076881|F|0-49 were associated with PWDTH at $p <0.001$. The major difference that was observed in association mapping in the two environments was due to temperature variations. The 65 SNP markers identified can be used in cowpea molecular breeding to select for AVSPD, NP, PL, PWDTH, PWT, and RR through marker assisted selection (MAS).

Key words: Association mapping, chromosomes, drought tolerance, markers, structure, traits.

6.1: Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is a food legume of the family Fabaceae/Papilionaceae (Jayathilake *et al.*, 2018). According to Sharma *et al.* (2016), all cultivated cowpeas are grouped under the species *Vigna unguiculata*, which is subdivided into four cultivar groups: *Unguiculata* (common cowpea for food and fodder), *Biflora* (catjang), *Sesquipedalis* (yard long or asparagus bean used as a vegetable), and *Textilis* (used for fibres). The crop is of major importance to many smallholder farmers in Africa and the developing world, as it serves as food, cash crop, animal feed, and manure (Agyeman *et al.*, 2014).

The major aims of cowpea breeding are high yield, early maturity for grain production, long vegetative period for vegetable production, high leaf and grain nutrient contents, high cooking quality, and high emergence rate. In order to provide farmers with quality seeds of improved cultivars, breeding programmes and seed systems should be based on information on the genetic diversity available in the germplasm (Menssen *et al.*, 2017). According to Wang *et al.* (2018), assessments of phenotypic or genotypic diversity in cultivated plants provide useful information for the improvement of germplasm collections, which provide material for genetic improvement and breeding.

Studies of association mapping for drought tolerance in cowpea using DArTSeq genotyping data are very limited. Drought is a major production constraint in the smallholder farming sector in Zimbabwe; thus, there is a need to develop drought-tolerant varieties, which in turn requires the identification of genotypes that carry genes associated with drought tolerance. Association mapping was used to investigate the associations among 76 SSR markers and six drought-related traits on a set of 107 barley accessions evaluated under well-watered and drought-stress conditions (Abou-Elwafa, 2016). A total of 36 significant marker–trait associations for drought-related traits were observed. Ballesta *et al.* (2019), used single nucleotide polymorphisms (SNP) associated with drought tolerance indices in 328 wheat lines using a genome-wide association study (GWAS) under fully irrigated and rain-fed conditions. Results showed that most associations were located on chromosome 4A, and that this chromosome is very important in drought tolerance and should be used in wheat improvement programmes.

In a study of correlation coefficient and path analysis in the cowpea germplasm line, Sharma *et al.* (2016), observed significant and positive correlations between the growth characters and seed yield of cowpea. Using a path analysis study, the experiment further concluded that seed yield in cowpea can be improved by focusing on the traits of biological yield per plant, harvest index, number of pods per plant, and plant height. Kouam *et al.* (2017), studied phenotypic and genotypic divergence for yield and related quantitative traits among 30 cowpea landraces in Cameroon. The study revealed strong correlations between seed length and grain yield, 100-seed weight and grain yield, 100-seed weight and seed length, number of seeds per pod and pod length, number of branches per plant and plant biomass, and grain yield and leaf width. Thus, characters such as seed length or 100-seed weight are very useful in early selection when improving yield.

Benjamin *et al.* (2018), evaluated the genetic variability among 20 wild cowpea accessions and observed high morphological variability among the accessions. The high variability observed among the wild cowpea accessions in terms of their agro-morphological and yield parameters provided useful traits in the crop that can be exploited for its improvement. Results obtained from Qin *et al.* (2016), on population structure analysis and association mapping of the seed antioxidant content in the 369-accession USDA cowpea [*Vigna unguiculata* (L.) Walp.] core collection using SNPs show that there were significant correlations between the seed antioxidant content and black seed colour. It was further observed and concluded that cowpea accessions with red and black seed coat colours were useful as parents in cowpea breeding programmes to provide new cowpea cultivars with high seed antioxidant contents.

Xu *et al.* (2017), analysed the genomic regions, cellular components, and gene regulatory basis underlying pod length variations in cowpeas, and found that cell proliferation, rather than cell elongation or enlargement, was the main reason for longer pods. Ravelombola *et al.* (2017a) analysed the salt tolerance index of 116 and 155 cowpea accessions at the emergence and seedling stages, respectively. A total of 1,049 single nucleotide polymorphisms (SNPs) were used for association analysis. Three SNPs, Scaffold 87490_622, Scaffold87490_630, and C35017374_128, were highly associated with salt tolerance at the germination stage. Seven SNPs, Scaffold93827_270, Scaffold68489_600, Scaffold87490_633, Scaffold87490_640, Scaffold82042_3387, C35069468_1916, and Scaffold93942_1089, were found to be related

to salt tolerance at the seedling stage. Thus, these SNP markers could be used as a tool to select salt-tolerant lines for breeding improved salt-tolerant cowpea cultivars.

The objective of this study were to conduct association mapping for drought tolerance at the seedling stage and yield-related traits in cowpea.

6.2: Materials and Methods

6.2.1: Phenotype data

A total of 60 cowpea accessions collected from three geographic origins were used in this study (Table 6.1). Of these, 33 accessions were from the International Institute of Tropical Agriculture (IITA) in Nigeria, 19 were from the Agricultural Research Council – Grain Crops in South Africa, and eight were from smallholder farmers in Buhera District in Zimbabwe.

The seeds used were grown under favourable conditions in two screen houses (glasshouse and greenhouse). The populations used are presented in Table 6.1. All of the populations phenotyped were grown in greenhouse and glasshouse trials. The cowpea accessions were planted in pots in topsoil mixed with compost (3:1) at the Agriculture Research Council – Grain Crops, Potchefstroom, South Africa in January 2019 for the greenhouse trial and February 2019 for the glasshouse trial. A triplicated 6× 10 alpha lattice design was used for the experiment. In all greenhouse and glasshouse trials, mature pods were harvested and dried for storage (<15% moisture) after screening for drought tolerance. Seeds were subsequently cleaned from the pods, counted, and weighed.

6.2.2: DNA extraction, sequencing, and SNP calling

The procedure is detailed in Chapter 4 section 4.2. The cowpea accessions used in the study are listed in Chapter 5 section 5.2.2.

6.3: Data Analysis

6.3.1: Population structure analysis

The population structure of the cowpea accessions evaluated for growth traits was inferred using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). Population structure (K) was assessed using

an admixture model with a correlated allele frequency model, which was independent for each run. Identification of the delta K values and optimal K, based on the formula established by Evanno *et al.* (2005), allowed a reliable screening of appropriate K values using Structure Harvester (<http://taylor0.biology.ucla.edu/structureHarvester/>; Earl and Von Holdt, 2011). A Q-matrix and K vectors were established shortly after the optimal K was computed. The Q-matrix was used for association analysis studies in TASSEL (Trait Analysis by Association Evolution and Linkage) (Bradbury *et al.*, 2007).

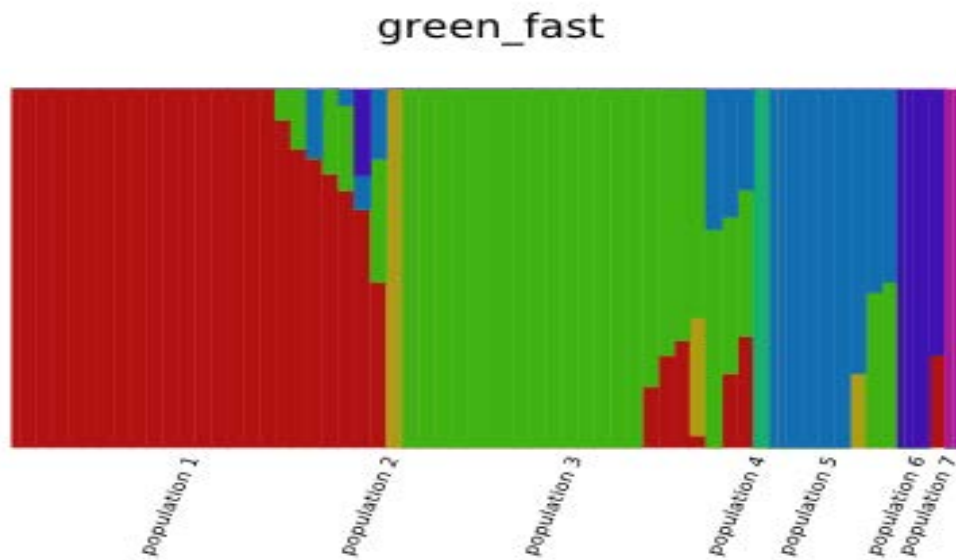
6.3.2: Association analysis

A statistical software package for R called “rrBLUP” (Endelman, 2011) and TASSEL were used to analyse association mapping. rrBLUP yields a quantile–quantile plot and a Manhattan plot with a significant threshold set at a 5% false discovery rate (FDR), calculated using the “qvalue” package. Marker–trait association analysis was evaluated using TASSEL v5.3.2. SNP genotype data generated was first filtered to remove the monomorphic SNP sites. The kinship matrix (K) was estimated from the genotype data in the TASSEL program. Both a general linear model (GLM) and mixed linear model (MLM) were used accordingly. Significantly associated SNP markers with traits were identified at $p < 0.001$ (Bradbury *et al.*, 2007).

6.4: Results

The population of the cowpea accessions were analysed using STRUCTURE 2.3.4 and the peak of delta K in the greenhouse (A) was $K = 7$, highlighting seven population types, whereas in the glasshouse (B), the peak of delta K was $K = 6$, indicating the presence of six population types (Figure 6.1).

A



B

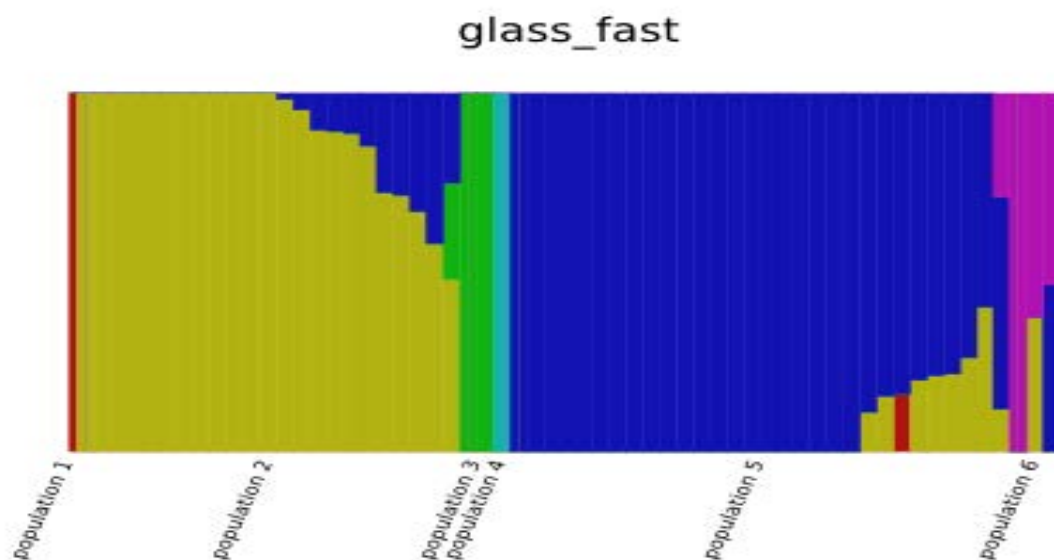


Figure 6.1: Classification of 60 cowpea accessions into seven populations in greenhouse (A) and six populations in glasshouse (B) using STRUCTURE Version 2.3.4

6.4.1: Association Analysis

Association analysis SNP markers were identified for number of pods, recovery rate, pod weight, average seeds per pod, pod length and pod width. Two SNP markers, 14083649|F|0-9 and 100100635|F|0-53 were associated with number of pods (NP) with a p value <0.001 (Table 6.1). The significant markers occurred on chromosome 10. SNP marker 100100635|F|0-53 contributed 43% of the phenotypic variation.

SNP marker 100084158|F|0-6 was associated with recovery rate (RR) at $p < 0.001$ and was positioned at chromosome 10 while R^2 was at 10%. (Table 6.1).

Fifty SNP markers were associated with pod weight (PWT) at $p < 0.001$ (Table 6.1). Out of these, 10 SNPs were on chromosome one, one SNP on chromosome two, six SNPs on chromosome three, four SNPs chromosome five, five SNPs on chromosome six, nine SNPs on chromosome seven, seven SNPs on chromosome eight, two SNPs on chromosome nine, six SNPs on chromosome 10 and four SNPs on chromosome 11. SNP marker 100051258|F|0-67 on chromosome nine contributed 53% of variation followed by chromosomes seven and nine which accounted for 52 % of the variation. SNPs on chromosome one accounted for 35% of phenotypic variation at PWT.

Four SNP markers, 14074781|F|0-16, 100047392|F|0-36, 14083801|F|0-28 and 100051488|F|0-49 were associated with average seeds per pod (AVSPD) at $p < 0.001$. All these SNPs were positioned at chromosome 3 (Table 6.1). SNP marker 14083801|F|0-28 had the highest R^2 at 35%. SNP markers, 14074781|F|0-16, 14083801|F|0-28 and 100051488|F|0-49 were associated with pod length (PL) at $p < 0.001$. These SNPs were all positioned on chromosome 3 while SNP marker 14083801|F|0-28 explained 34 % of phenotypic variation for PL. Four SNP markers, 100047392|F|0-36, 14083801|F|0-28, 100072738|F|0-34 and 14076881|F|0-49 were associated with pod width (PWDTH) at $p < 0.001$. These were all on chromosome 3 while marker 14083801|F|0-28 accounted for 32 % of phenotypic variation for PWDTH. Most of these markers were distributed on chromosomes 3 (17 markers), chromosome 1 (10 markers) and chromosome 7 (9 makers).

Table 6.1 List of markers associated with NP, RR, PWT, AVSPD, PL and PWDTH

Trait	CHR	SNP	BP	SE	R ²	P
PWT	1	100053502 F 0-62	1251569	12.4	0.3596	2.628e-06
PWT	1	14078204 F 0-43	1292063	11.75	0.3556	6.238e-07
PWT	1	100044718 F 0-57	1432640	12.36	0.3552	2.501e-06
PWT	1	100046227 F 0-46	1557122	11.75	0.3556	6.238e-07
PWT	1	100063465 F 0-35	1557915	11.85	0.3554	7.901e-07
PWT	1	100072600 F 0-25	1564066	11.75	0.3556	6.238e-07
PWT	1	100071153 F 0-66	1564132	12.16	0.3557	1.545e-06
PWT	1	100048141 F 0-63	1694886	11.75	0.3556	6.238e-07
PWT	1	100044957 F 0-63	3528678	12.06	0.3553	1.253e-06
PWT	1	100063872 F 0-10	28798986	11.75	0.3556	6.238e-07
PWT	2	100096611 F 0-40	15499573	11.75	0.3556	6.238e-07
PWT	3	100054066 F 0-68	14331695	12.38	0.3561	2.41e-06
PWT	3	100051321 F 0-47	53345546	7.327	0.2522	5.913e-05
PWT	5	100053097 F 0-7	16393146	12.04	0.3553	1.253e-06
PWT	5	100075598 F 0-66	16791034	9.149	0.2048	0.0003197
PWT	5	14058797 F 0-67	25444199	2.974	0.3953	1.63e-07
RR	5	100084158 F 0-6	4031158	5.219	0.2196	0.0004587
PWT	6	100070503 F 0-16	13371481	12.86	0.3553	6.216e-06
PWT	6	100045443 F 0-35	18431456	9.476	0.1759	0.0009464
PWT	6	100050903 F 0-29	18751348	11.75	0.3556	6.238e-07
PWT	6	100049686 F 0-66	18764966	11.97	0.3693	1.096e-06
PWT	6	100087696 F 0-41	26017102	9.135	0.2341	0.0001035
PWT	7	100047575 F 0-22	823054	10.04	0.5293	6.718e-11
PWT	7	100071982 F 0-68	3037310	10.3	0.5293	2.13e-10
PWT	7	14079990 F 0-24	31169450	5.395	0.2547	4.587e-05
PWT	7	100083914 F 0-58	32980963	10.04	0.5293	6.718e-11
PWT	7	100076974 F 0-16	32991155	10.46	0.5301	4.413e-10
PWT	7	100052969 F 0-35	33021922	10.27	0.5299	2.056e-10
PWT	7	14084025 F 0-9	33082576	6.857	0.1979	0.0009514
PWT	7	100084809 F 0-62	38432224	11.84	0.3556	7.851e-07
PWT	7	100073645 F 0-45	38481917	11.75	0.3556	6.238e-07
PWT	8	100052927 F 0-48	752816	10.19	0.5297	1.416e-10
PWT	8	100073137 F 0-62	29029951	9.106	0.3003	2.613e-05
PWT	8	14087242 F 0-6	34660664	5.361	0.3628	1.146e-06
PWT	8	14084814 F 0-10	34681513	2.758	0.3511	9.587e-07
PWT	8	100073582 F 0-29	35713044	10.44	0.5304	4.336e-10
PWT	8	100044312 F 0-24	35970502	10.19	0.5297	1.416e-10
PWT	8	100072423 F 0-62	36066768	10.04	0.5293	6.718e-11
PWT	9	100051258 F 0-67	7118132	10.35	0.5322	2.657e-10
PWT	9	100044652 F 0-17	12400903	4.727	0.1955	0.0008148
PWT	10	100045290 F 0-13	5588111	7.555	0.2036	0.0003779
PWT	10	100097542 F 0-53	15068089	10.21	0.5295	1.436e-10
PWT	10	100047842 F 0-31	34051300	7.633	0.1744	0.0009977
PWT	10	100049329 F 0-68	39976951	9.453	0.206	0.0005005
NP	10	100100635 F 0-53	27320523	2.221	0.4324	2.127e-07
NP	10	14083649 F 0-9	33863660	0.3972	0.1804	0.0008001
PWT	11	100087702 F 0-13	1176941	6.47	0.2047	0.0003207
PWT	11	100051586 F 0-57	1200317	8.605	0.2965	8.249e-06
PWT	11	100081173 F 0-34	40185748	9.476	0.1759	0.0009464
PWT	11	100049496 F 0-26	40225628	9.476	0.1759	0.0009464
AVPSD	3	14074781 F 0-16	56268741	0.7032	0.1906	0.0006128
AVPSD	3	100047392 F 0-36	59016170	0.6695	0.1902	0.0009797
AVPSD	3	14083801 F 0-28	59879328	0.5533	0.3582	5.547e-07
AVPSD	3	100051488 F 0-49	63223396	1.078	0.2163	0.0006666
PL	3	14074781 F 0-16	56268741	0.9575	0.2103	0.0002943
PL	3	14083801 F 0-28	59879328	0.7713	0.3438	1.061e-06
PL	3	100051488 F 0-49	63223396	1.438	0.2356	0.0003527
PWDTH	3	100047392 F 0-36	59016170	0.07287	0.2176	0.0003776
PWDTH	3	14083801 F 0-28	59879328	0.06198	0.3203	2.995e-06
PWDTH	3	100072738 F 0-34	60195503	0.07753	0.1939	0.0008615
PWDTH	3	14076881 F 0-49	60200022	0.07288	0.2043	0.0003264
PWT	3	100050332 F 0-42	6019724	4.405	0.1964	0.0004401
PWT	3	100071756 F 0-66	53358047	4.53	0.1974	0.0006774
PWT	3	14083801 F 0-28	59879328	0.9134	0.3088	4.899e-06
PWT	3	100047389 F 0-39	60895529	1.006	0.2022	0.0003529

Association mapping was performed using rrBLUP to identify loci linked to the evaluated traits. Significant SNPs were compared to those that passed a significance threshold of $\log_{10}(p) > 5$ in TASSEL 5.0 analysis. Figure 6.2 highlights the association in glasshouse experiment on the number of pods (NP), recovery rate (RR), and pod weight (PWT).

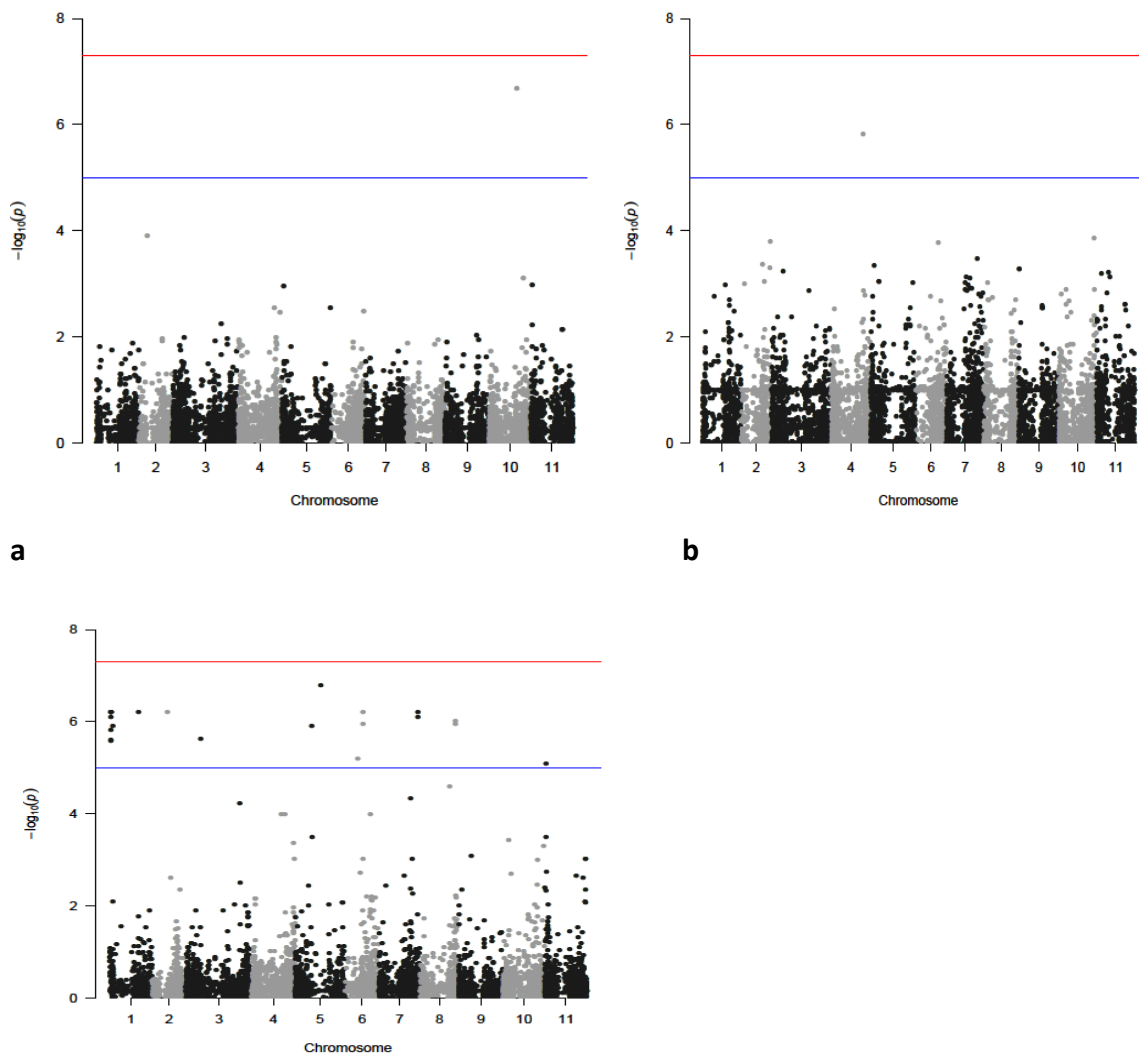


Figure 6.2: Association mapping results for wilting and yield related traits in 60 cowpea accession in glasshouse. Manhattan plots from association mapping using mixed linear model. X-axis shows the SNPs along the 11 chromosomes of cowpea accessions and Y-axis shows $-\log_{10}(p)$ value of association for each SNP. The solid horizontal blue line indicates the calculated threshold value for declaring a significant association. The red line indicates the significance threshold ($FDR < 0.005$). a. NP, number of pods; b. RR, recovery rate; c. PWT, pod weight

The average seeds per pod (AVSPD), pod length (PL), pod width (PWIDTH) and pod weight (PWT) had were significantly associated in the greenhouse experiment (Figure 6.3).

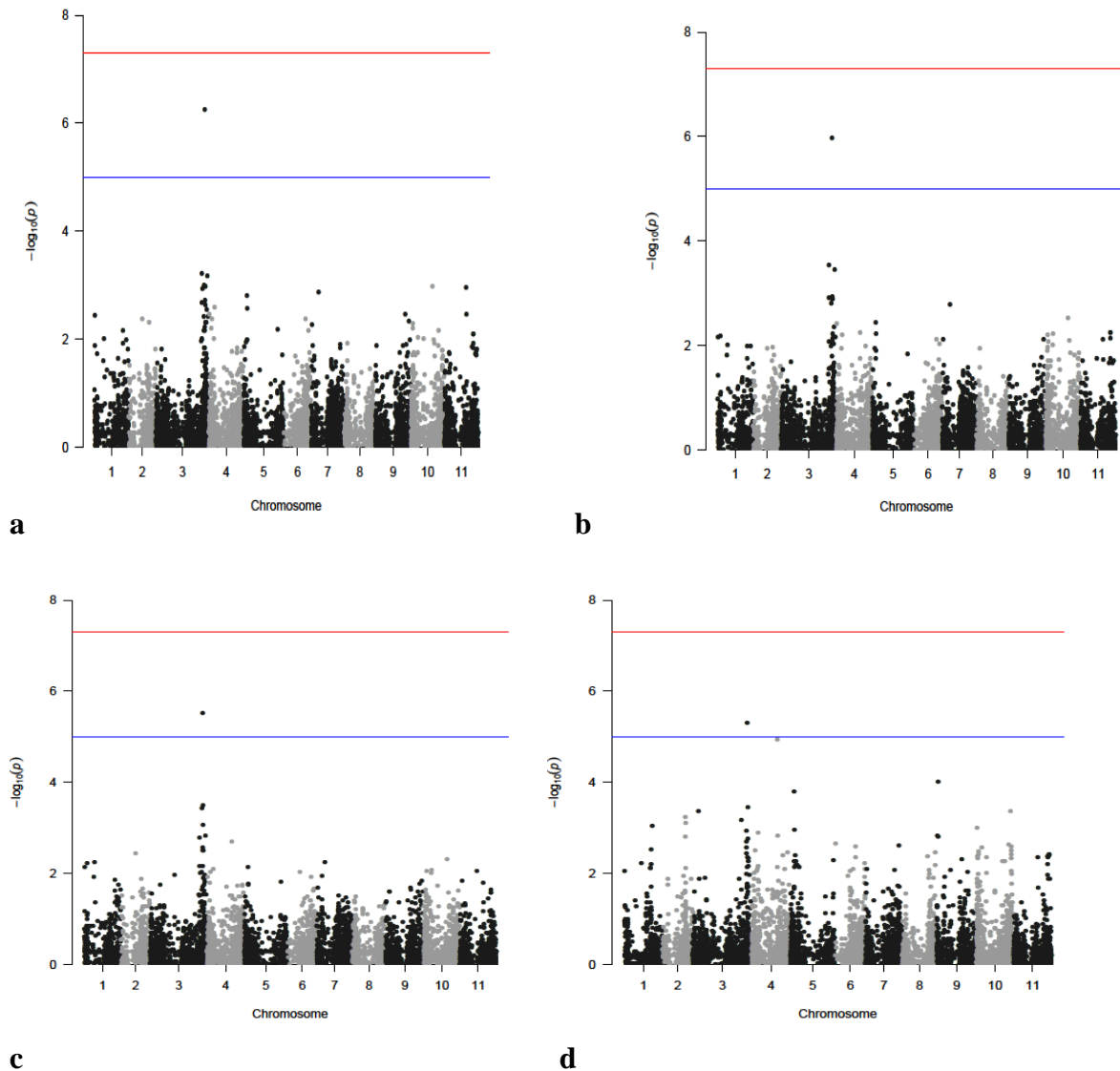
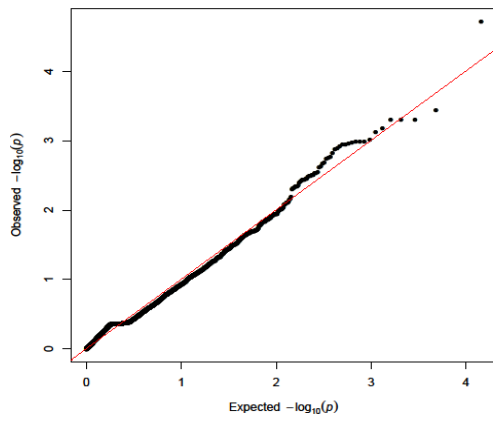


Figure 6.3: Association mapping results for wilting and yield related traits in 60 cowpea accession in greenhouse. Manhattan plots from association mapping using mixed linear model. X-axis shows the SNPs along the 11 chromosomes of cowpea accessions and Y-axis shows $-\log_{10}(p)$ value of association for each SNP. The solid horizontal blue line indicates the calculated threshold value for declaring a significant association.

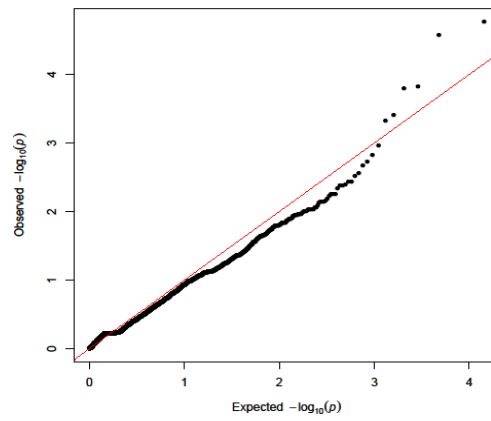
a. Average seeds per pod, AVSPD; b. pod length, PL; c. pod width, PWIDTH; d. pod weight, PWT

6.4.2: Population-trait associations

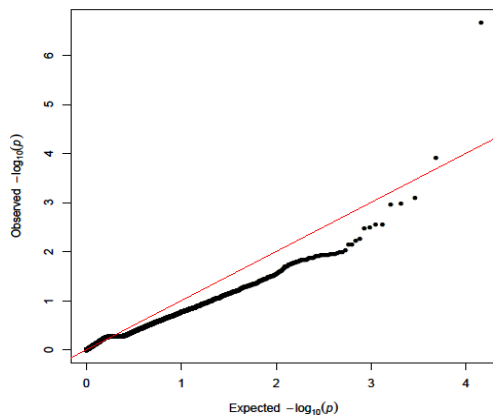
The following quantile–quantile plots in Figures 6.4 and 6.5 of association mapping in both the glasshouse and greenhouse experiments, respectively.



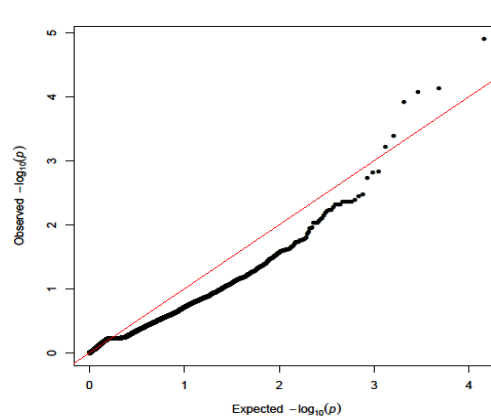
a



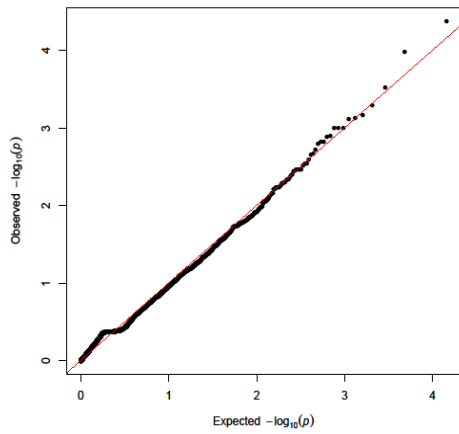
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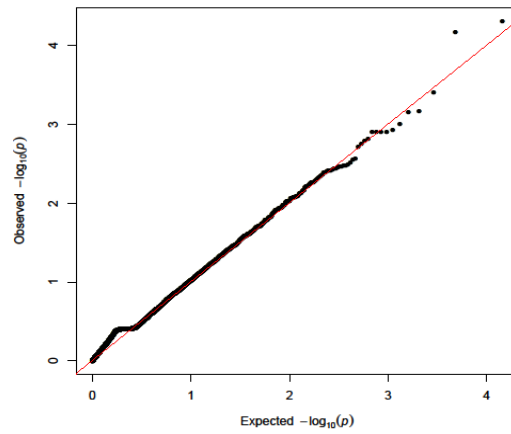
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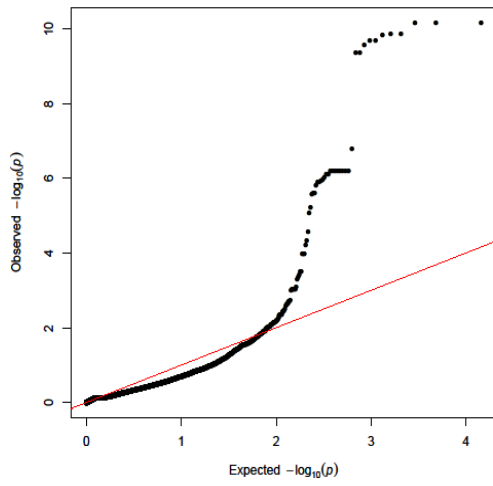
d



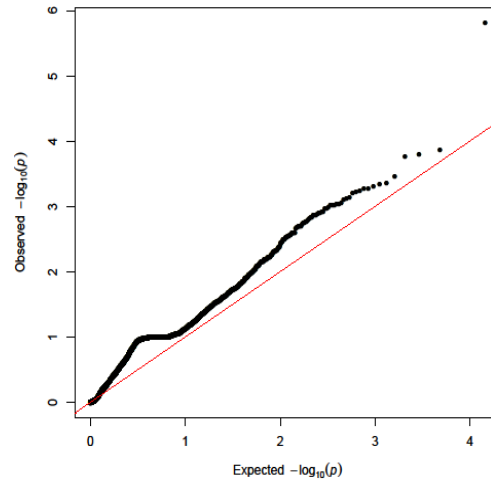
e



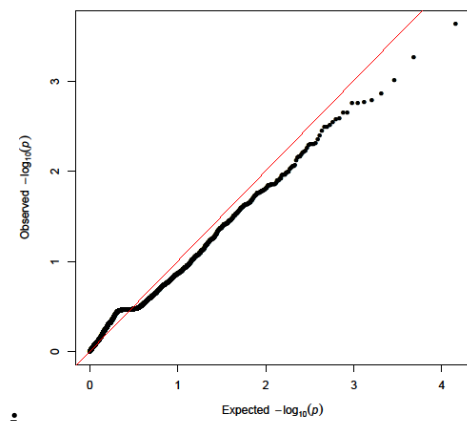
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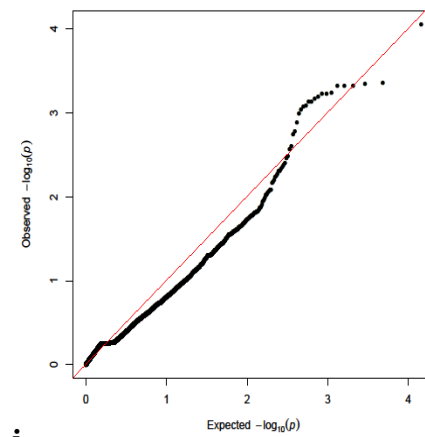
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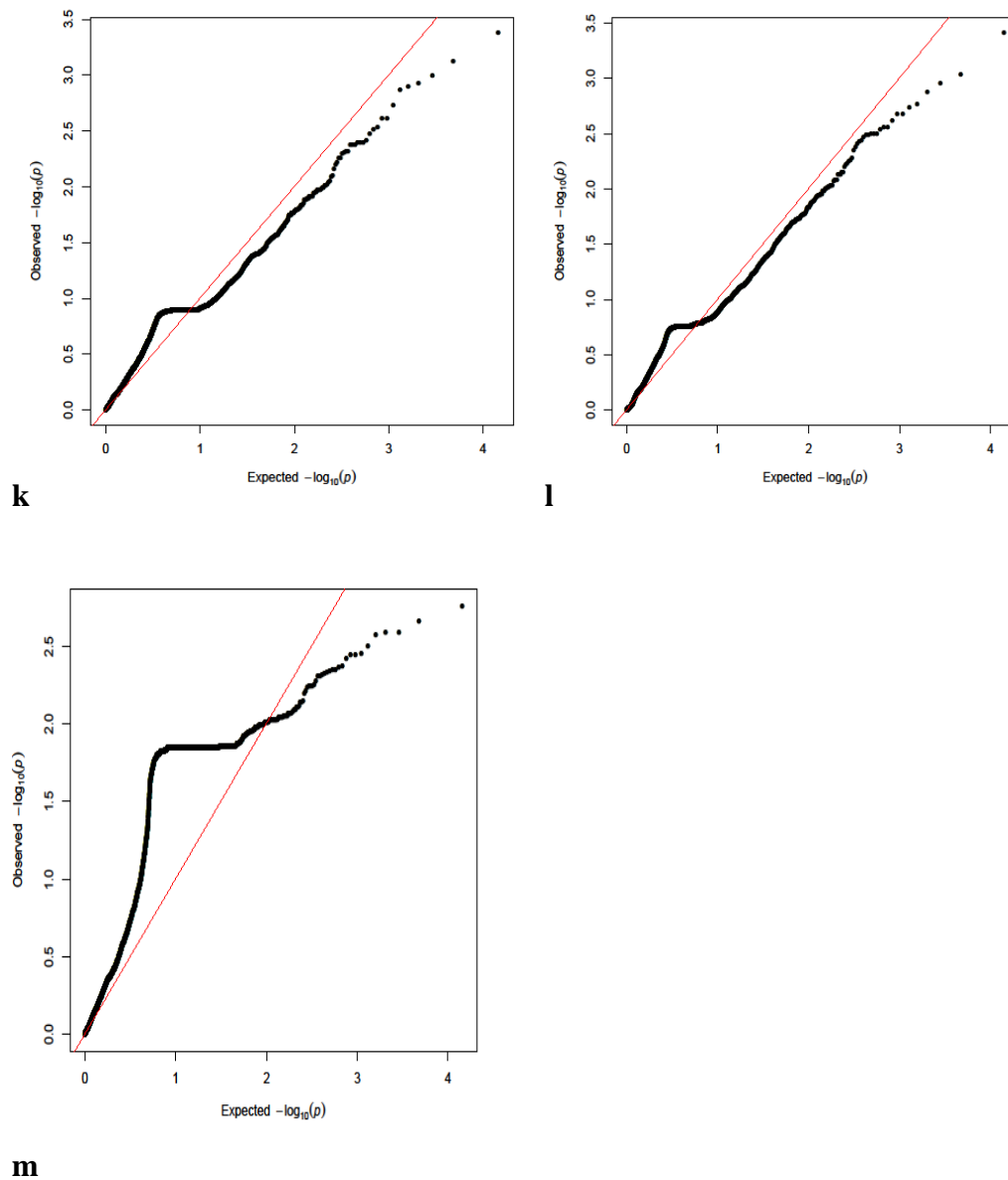
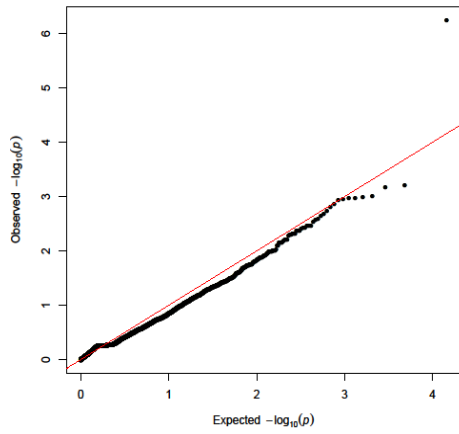
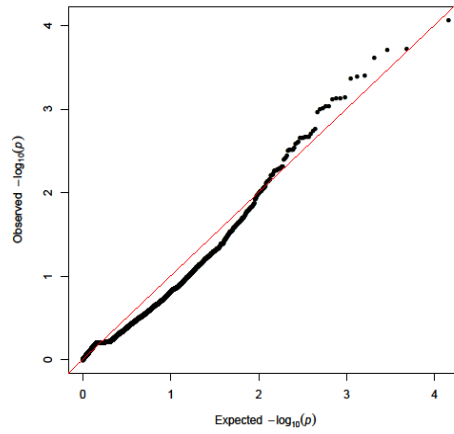


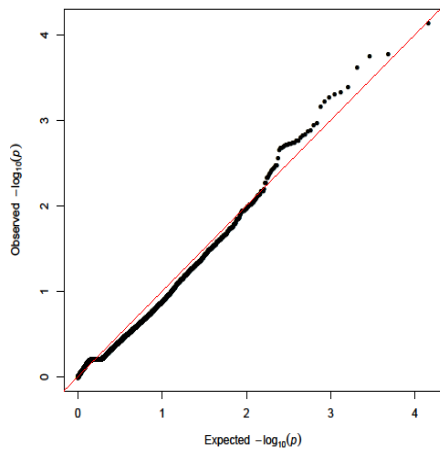
Figure 6.4: Q-Q plots showing the results of cowpea association mapping in glasshouse. The straight line in the Q-Q plot indicates the distribution of SNPs. **a**-AVSPD- average seeds per pod, **b**-DTE-days to emergence, **c**-NP-number of pods, **d**-number of seeds, **e**-PL-pod length, **f**-PWIDTH-pod width, **g**-PWT-pod weight, **h**-RR-recovery rate, **i**-SC-survival count, **j**-SWDT-seed weight, **k**-SGWK 3-stem greenness at 3 weeks after drought imposition, **l**-WWK2-wilting at 2 weeks after drought imposition, **m**- WWK3-wilting at 3 weeks after drought imposition



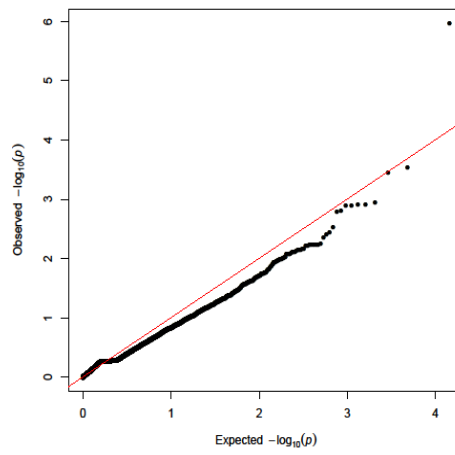
a



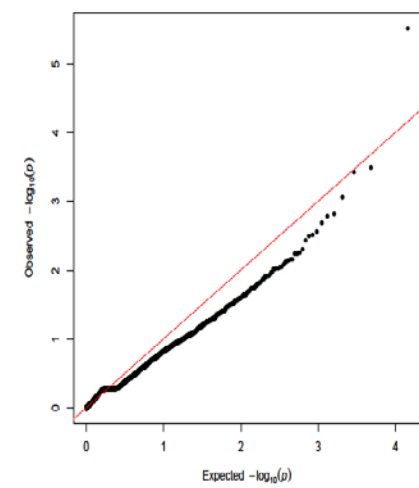
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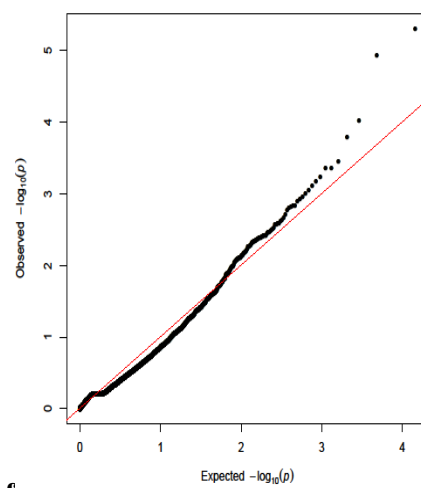
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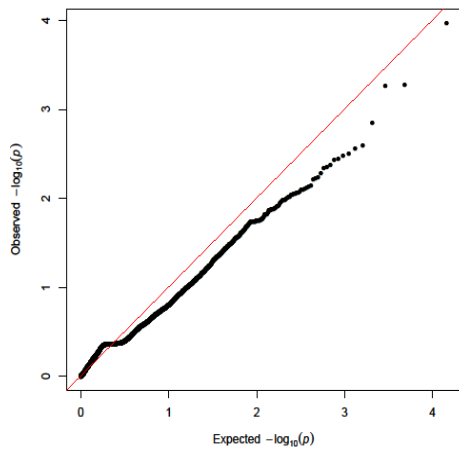
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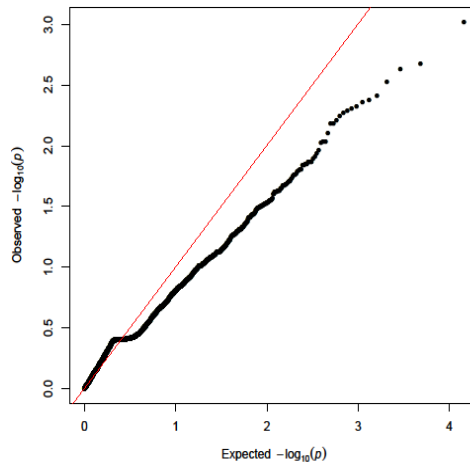
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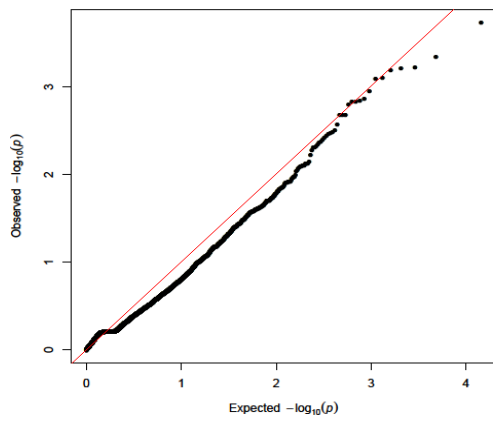
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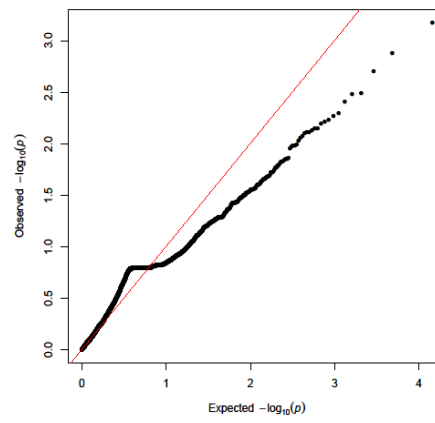
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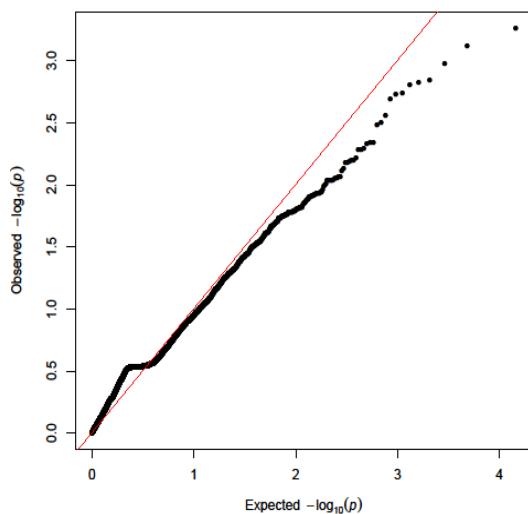
h



i



j



k

Figure 6.5: Q-Q plots showing the results of cowpea association mapping in greenhouse. The straight line in the Q-Q plot indicates the distribution of SNPs. **a**-AVSPD- average seeds per pod, **b**-NP-number of pods, **c**-NSDS-number of seeds, **d**-PL-pod length, **e**-PWIDTH-pod width, **f**-PWT-pod weight, **g**-RR-recovery rate, **h**-SC-survival count, **i**-SWDT-seed weight, **j**-WWK2-wilting at 2 weeks after drought imposition, **k**- WWK3-wilting at 3 weeks after drought imposition

6.5: Discussion

The association mapping study provided an insight on the importance of traits that were useful in screening cowpea accessions at seedling level in a controlled environment. In both greenhouse and glasshouse experiments, variations were observed with regards to traits that were important in screening cowpea accessions at seedling stage. All the two experiments, however, pointed out that yield related traits are of utmost importance when selecting cowpea accession to drought tolerance at seedling stage.

Population structure analysis revealed that 60 cowpea accessions can be divided into seven subpopulations, that is, population 1, population 2, population 3, population 4, population 5, population 6 and population 7, from both screen houses. The population analysis showed that each subgroup is diverse from others. Populations 1 and 3 under greenhouse experiment and populations 2 and 5 in glasshouse experiment. These results would facilitate choices of parental lines in cowpea-breeding programs.

From the study chromosomes 1, 2, 3, 5, 6, 7, 8, 9, 10 and 11 have the most important genetic information and traits with regards to drought tolerance at seedling stage in the screen houses. Important traits such as NP, RR, AVSPD, PL, PWDTH and PWT were associated with different chromosomes with regards to drought tolerance. From this research PWT was the most important trait represented by 50 SNPs on nine different chromosome positions. Most of these positions were based in the glasshouse experiment. Burrige *et al* (2016), observed that some marker performance indicators such as seed weight and seed number differ by environment. Pod formation and number of grains per pod depend on environmental factors before anthesis, while grain weight depend on environmental factors after anthesis (Freitas *et al.*, 2019). Thus the PWT trait was more pronounced in the glasshouse with more chromosomes exhibiting the trait than in the greenhouse. The study pointed out that these yield related traits are some of the important parameters to be used when selecting cowpea accessions for drought tolerance at seedling stage in screen houses. However, Muchero *et al.* (2008) observed that stem greenness, survival and recovery dry weights in greenhouse were the useful traits to screen cowpea genotypes for their ability to withstand drought stress at the seedling stage.

Under this study it was also observed that there was a strong co-location of SNP markers especially on chromosomes 1, 3, 5, 6, 7, 8, 9, 10 and 11. SNP markers 100051488|F|0-49 and 14083801|F|0-28 on chromosome 3 were associated with both AVSPD and PL. SNP marker 14083801|F|0-28 was also associated with PWDTH on chromosome 3. This suggests that drought tolerance traits are complex and these determines accurate measurements. Using restriction site polymorphisms to investigate co-location of candidate genes with QTL for seedling drought stress-induced premature senescence identified previously in cowpea, Muchero *et al* (2009), observed seven markers co-located with peaks of previously identified QTL. The co-location of these markers suggested that these markers were derived from genes which were involved in cowpea response to drought stress-induced premature senescence. Chitwood *et al* (2016), observed that when there is a smaller the p value, then that SNP marker is very ideal and should be validated for marker assisted selection (MAS).

Most of the trait-associated markers were different under the two screen houses, indicating the environmental effects in these associations (Baye *et al.*, 2011). These results showed that different genes might contribute to the same trait in several environments (Rumbaugh *et al.*, 1984) or there could be a change within the expression level of the same gene between two environments (House *et al.*, 2014). Associated markers repeatedly detected in two or more

different environments are considered more reliable than those present in just one environment (Diapari *et al.*, 2015). In this study, 2 markers showed stable association with different traits under both screen house conditions, notably markers 100051488|F|0-49 and 14083801|F|0-28. The detection of genomic regions associated with multiple traits across variable environments is essential in breeding crops for wide adaptation and yield stability (Saeed *et al.*, 2017).

Tomar *et al* (2016), observed that plants with good tolerance at early vegetative growth were able to withstand drought stress at a later stage of plant development. Some complex genetic architecture of drought tolerance demands embracing more efficient tools like genomic selection (GS) for accelerated trait improvement (Jha *et al.*, 2020). This is because during QTL analysis, Muchero *et al* (2009), observed that the tolerant genotypes also contributed alleles that negatively influenced drought tolerance, and that the susceptible parent contributed alleles that enhanced drought tolerance. The use of SNP markers at seedling stage for drought tolerance can be a fast and cheaper way than the use of conventional breeding methods in a field environment.

6.6: Conclusion

The screening of cowpea accessions in a controlled environment is a fast way of evaluation, especially where temperature regulation is needed. Some variability in drought tolerance-related traits among cowpea genotypes was observed in this study in both greenhouse and glasshouse experiments. The population structure analysis revealed that under that two screen houses there were seven subgroups although this was more pronounced in the greenhouse experiment. Drought tolerance in cowpea is controlled by multiple traits in cowpeas as was observed with SNPs 100051488|F|0-49 and 14083801|F|0-28. It is thus necessary to have accurate measurements of intended traits. In terms of drought tolerance at the seedling stage, various temperature regimes can be controlled; this can give desired results much quicker than field selection. The 65 SNP markers identified may be used in cowpea molecular breeding to select for AVSPD, NP, PL, PWDTH, PWT, and RR through MAS.

References

- Abou-Elwafa, S.F., 2016. Association Mapping For Drought Tolerance in Barley at the Reproductive Stage. *Comptes Rendus Biologies* 339:51-59.
- Agyeman, K., Berchie, J.N., Osei-Bonsu, I., Tetteh, E.N., and Fordjour, J.K., 2014. Seed Yield and Agronomic Importance of Seven Improved Cowpea (*Vigna unguiculata* L) Varieties in Ghana. *African Journal of Agricultural Research*. Volume 10 pp 215-221. doi:10.5897/AJAR2014.8835.
- Ballesta, P., Mora, F., and Del Pozo, A., 2019. Association Mapping of Drought Tolerance Indices in Wheat: QTL-Rich Regions on Chromosome 4A. *Scientia Agricola* 77(2) e20180153. <https://dx.doi.org/10.1590/1678-992x-2018-0153>.
- Baye, T.M., Abebe, T., and Wilke, R.A., 2011. Genotype-Environment Interactions and Their Translational Implications. *Personalised Medicine* 8(1):59–70. doi:10.2217/pme.10.75.
- Benjamin, U., Olamide, F., Yusuf, D.O.A., Abdulhakeem, A., Junior, N.D., David, T.S., and Muhammad, M.L., 2018. Phenotypic Variability Studies in Selected Accessions of Nigerian Wild Cowpea (*Vigna unguiculata* L. Walp) *GSC Biological and Pharmaceutical Sciences* 03: 019–027. <https://doi.org/10.30574/gscbps.2018.3.1.0015>.
- Bradbury, P. J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y. and Buckler, E.S., 2007. TASSEL: Software for Association Mapping of Complex Traits in Diverse Samples. *Bioinformatics* 23:2633-2635.
- Burridge, J.D., Schneider, H.M., Huynh, B.L., Roberts, P.A., Bucksch, A., and Lync, J.P., 2016. Genome-Wide Association Mapping and Agronomic Impact of Cowpea Root Architecture. *Theoretical and Applied Genetics*. doi 10.1007/s00122-016-2823-y.
- Chitwood, J., Shi, A., Evans, M., Clark, J., Motes, D., Chen, P., and Hensley, D., 2016. Population Structure and Association Analysis of Bolting, Plant Height, and Leaf Erectness in Spinach. *Hortscience* 51(5):481–486.

Diapari, M., Sindhu, A., Warkentin, T.D., Bett, K., and Tar'an, B., 2015. Population Structure and Marker-Trait Association Studies of Iron, Zinc and Selenium Concentrations in Seed of Field Pea (*Pisum sativum* L.). *Molecular Breeding* 35, 30. doi:10.1007/s11032-015-0252-2.

Earl, D.A., and Von Holdt, B.M., 2011. STRUCTURE HARVESTER: A website and Program for Visualizing STRUCTURE Output and Implementing the Evanno Method. *Conservation Genetic Resources* 4:359–361.

Evanno, G., Reganut, E., and Goudet J., 2005. Detecting the Number of Clusters of Individuals Using the Software STRUCTURE: A Simulation Study. *Molecular Ecology Notes* 14: 2611-2620.

Freitas, T.G.G., Silva, P.S.L., Dovale, J.C., Silva, I. N, and Silva, E.M., 2019. Grain Yield and Path Analysis in the Evaluation of Cowpea Landraces. *Revista Caatinga*, 32(2), 302-311. <https://doi.org/10.1590/1983-1252019v32n202rc>.

House, M.A., Griswold, C.K., and Lukens, L.N., 2014. Evidence for Selection on Gene Expression in Cultivated Rice (*Oryza sativa*). *Molecular Biology and Evolution* 31, 1514–1525. doi:10.1093/molbev/msu110.

Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B.C., Nammi, S., and Liyanage, R., 2018. Cowpea: An Overview on Its Nutritional Facts and Health Benefits. *Journal of the Science of Food and Agriculture*. Volume 98.pp 4793-4806. doi: 10.1002/jsfa.9074.

Jha, U.C., Bohra, A., and Nayyar, H., 2020. Advances in Omics Approaches to Tackle Drought Stress in Grain Legumes. *Plant Breeding* 139:1-27. <https://doi.org/10.1111.pbr.12761>.

Kouam, E.B., Ngompe-Deffo, T., Anoumaa, M., and Pasquet, R.S., 2018. Preliminary Study on Character Associations, Phenotypic and Genotypic Divergence for Yield and Related Quantitative Traits among Cowpea Landraces (*Vigna unguiculata*) from the Western Highland Region of Cameroon. *Open Agriculture* 3: 84–97. <https://doi.org/10.1515/opag-2018-0009>.

Menssena, M., Lindeb, M., Omondia, E.O., Abukutsa-Onyango, M., Dinssa, F.F., and Winkelmann, T., 2017. Genetic and Morphological Diversity of Cowpea (*Vigna unguiculata* (L.) Walp.) Entries from East Africa. *Scientia Horticulturae* 226 pp 268-276. <https://doi.org/10.1016/j.scienta.2017.08.003>.

Muchero, W., Ehlers, J.D., and Roberts, P.A., 2008. Seedling Stage Drought-Induced Phenotypes and Drought-Responsive Genes in Diverse Cowpea Genotypes. *Crop Science* 48:541–552.

Muchero, W., Ehlers, J.D., and Roberts, P.A., 2009. Restriction Site Polymorphism-Based Candidate Gene Mapping for Seedling Drought Tolerance in Cowpea [*Vigna unguiculata* (L.) Walp.]. *Theoretical and Applied Genetics* 120(3):509–518. doi: 10.1007/s00122-009-1171-6.

Pritchard, J.K., Stephens, M., and Donnelly, P., 2001. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* 155:945–959.

Qin, J., Shi, A., Xiong, H., Mou, B., Motes, D., Lu, W., Miller Jr, W.J.C., Scheuring, D.C., Nzaramba, M.N., Weng, Y., and Yang, W., 2016. Population Structure Analysis and Association Mapping of Seed Antioxidant Content in USDA Cowpea (*Vigna unguiculata* L. Walp.) Core Collection Using SNPs. *Canadian Journal of Plant Science* 96: 1026-1036. <https://doi.org/10.1139/cjps-2016-0090>.

Ravelombola, W., Shi, A., Weng, Y., Mou, B., Motes, D., Clark, J., Chen, P., Srivastava, V., Qin, J., Dong, L., Yang, W., Bhattarai, G., and Sugihara, Y., 2017a. Association Analysis of Salt Tolerance in Cowpea (*Vigna unguiculata* (L.) Walp) At Germination and Seedling Stages. *Theoretical and Applied Genetics*. Volume 131, Issue 1, pp 79–91. doi: 10.1007/s00122-017-2987-0.

Ravelombola, W., Shi, A., Weng, Y., Bhattarai, G., Dong, L., and Morris, J.B., 2017b. A SNP-Based Association Analysis for Plant Growth Habit in Worldwide Cowpea (*Vigna unguiculata* (L.) Walp) Germplasm. *Euphytica* 2017: 213: 284. <https://doi.org/10.1007/s10681-017-2077-z>.

Rumbaugh, M.D., Asay, K.H., and Johnson, D.A., 1984. Influence of Drought Stress on Genetic Variance of Alfalfa and Wheat Grass Seedling. *Crop Science* 24, 297–303. doi:10.2135/cropsci1984.0011183X0024000 20021x.

Saeed, I., Chen, X., Bachir, D.G., Chen, L., and Hu, Y.G., 2017. Association Mapping for Photosynthesis and Yield Traits under Two Moisture Conditions and Their Drought Indices in Winter Bread Wheat (*Triticum aestivum* L.) Using SSR Markers. Australian Journal of Crop Science 11: 248–257. doi:10.21475/ajcs.17.11.03.pne252.

Sharma, M., Sharma, P.P., Uphadhyay, B., and Bairwa, H.L., 2016. Study of Correlation Coefficient and Path Analysis in Cowpea [*Vigna unguiculata* (L.) Walp] Germplasm Line. International Journal of Development Research Volume 06 Issue, 08, pp. 9011-9016.

Tomar, R.S., Tiwari, S., Naik, B.K., Chand, S., Deshmukh, R., Mallick, N., Singh, S., Singh, N.K., and Tomar, S.M., 2016. Molecular and Morpho-Agronomical Characterization of Root Architecture at Seedling and Reproductive Stages for Drought Tolerance in Wheat. PLoS One 11(6):e0156528.

Wang, L., Peng Bai, P., Yuan, X., Chen, H., Wang, S., Chen, X., and Cheng, X., 2018. Genetic Diversity Assessment of a Set of Introduced Mung Bean Accessions (*Vigna radiata* L.) The Crop Journal 6:207 – 213. <https://doi.org/10.1016/J.CJ.2017.08.004>.

Xu, P., Wu, X., Muñoz-Amatriaín, M., Wang, B., Wu, X., Hu, Y., Huynh, B.L., Close, T.J., Roberts, P.A., Zhou, W., Lu, Z., and Guojing Li, G., 2017. Genomic Regions, Cellular Components and Gene Regulatory Basis Underlying Pod Length Variations in Cowpea (*Vigna unguiculata* L. Walp). Plant Biotechnology Journal 15:547–557. doi:10.1111/Pbi.12639.

CHAPTER 7

DISCUSSIONS, CONCLUSIONS, AND RECOMMENDATIONS.

7.1: Farmers' Production Constraints, Perceptions, and Preferences of Cowpeas in Buhera District, Zimbabwe

A survey approach on farmers' production constraints, perceptions, and preferences was carried out (i) to determine farmers' perceptions on major constraints limiting cowpea production and (ii) to identify their preferences regarding cultivars and traits. The hypothesis tested was that farmers are very much aware of production constraints and that they prefer certain traits and cultivars in cowpeas. The formal survey involved individual interviews with 100 farmers from the district and from various wards. This enabled individual farmers to express their own views freely and in a relaxed setting. At the ward level, farmer selection for the survey was random but based on their knowledge and experience of growing cowpeas. A semi-structured questionnaire was used in the survey. The questionnaire had four components: demographic information, cowpea farming systems (farm size, land allocated to cowpea and other main crops, and varieties grown), cowpea production constraints, and trait preferences of cowpea varieties.

The results revealed that 52% of the surveyed population were women, while men occupied 48%. This confirms that cowpea production is mainly performed by women in Buhera District. Cowpea yield varied considerably among the farmers, with 48% harvesting 200kg of cowpeas per season; other crops grown were sorghum, millet, maize, finger millet, and sunflower. Most farmers interviewed highlighted the unavailability and cost of fertiliser, lack of quality seeds, and labour as the major constraints in cowpea production. Farmers identified aphids as major pests, as well as thrips, legume pod borers and stem and stalk borers. The farmers interviewed ranked heat, drought, and soil fertility as the most important abiotic factors. Even though farmers ranked CBC1, IT 18 and *Chibundi Chitsvuku* respectively as their favourite cowpea accessions, in the screen houses these did not perform well and the closest cowpea accession was *Chibundi Mavara*. This explains why there should be new cowpea varieties to suit the rapidly changing climatic conditions in the area. Most farmers grew cowpeas without the use of inorganic fertilisers; they are perceived as expensive and unaffordable in this drought-prone region. Finally, most farmers agreed that they needed high-yield and drought-tolerant cowpea

cultivars, which should be introduced in a timely fashion, as most farmers solely rely on rain-fed agriculture.

7.2: Genetic Diversity Analysis of Cowpea [*Vigna unguiculata* (L.) Walp.] Accessions Using Diversity Arrays Technology Sequencing (DArTSeq) Technique

The objective of this study was to assess genetic diversity and population structure of 85 cowpea genotypes collected from different geographic origins using DArTSeq genotype by sequencing technique. A total of 85 cowpea accession (45 from IITA, 25 from South Africa, and 15 from Zimbabwe) were investigated in this study using 18,284 single nucleotide polymorphisms (SNPs).

Twenty-six percent of the SNPs had genetic diversity values of greater than 0.40, suggesting that the genotypes were highly genetically diversified. In this study, a high gene flow (N_m) of 4.89 was observed between Zimbabwean and South African accessions, indicating a high germplasm exchange among these neighbouring countries. The analysis of molecular variance revealed highly significant variation among individual accessions and low variation within individuals. The accession showed significant ($p < 0.001$) but low levels of differentiation among geographic regions. The level of polymorphism detected in this study may be attributed to the self-pollinated reproduction mechanism of cowpea and a bottleneck restriction induced by a single domestication event in cowpea. Cluster analysis of the 85 accessions generated by the unweighted pair group method with arithmetic mean (UPGMA) procedure clustered the accessions into three distinct groups. The clustering patterns showed that accessions collected from the same geographic regions tended to cluster in the same group. In this study, SNP data analysis indicated the existence of high levels of genetic diversity among cowpea accessions collected from southern Africa and Nigeria.

7.3: Phenotyping Cowpea Accessions at the Seedling Stage for Drought Tolerance Using the Pot Method in a Controlled Environment

The objective of this study was to phenotype 60 cowpea genotypes for seedling drought tolerance in screen houses.

The highest and lowest day temperatures recorded in the greenhouse were 35.75 °C and 27.67 °C, respectively, with a mean day temperature of 32.24 °C. The highest and lowest night temperatures recorded in the greenhouse were 26.87 °C and 19.99 °C, respectively, with a mean night temperature of 23.98 °C. The highest and lowest day temperatures recorded in the glasshouse were 36.4 °C and 19 °C, respectively, with a mean day temperature of 26.06 °C. The highest and lowest night temperatures recorded in the glasshouse were 23.64 °C and 18.5 °C, respectively, with a mean night temperature of 21.42 °C. The PC plot and biplot showed that the number of pods (NP), seeds per pod (SP), survival count (SC), pod weight (PWT), and stem wilting in week one after drought imposition (WWK1) had the most significant contributions to genetic variability in the drought tolerance of cowpea accessions as well as yield. Based on the PC, biplot, and scatter plot analyses, the accessions IT 07-292-10, RV 343, and IT 95K-2017-15 had the maximum variabilities in NP, SP, SC, PWT, and WWK1.

There were significant differences among most drought related traits at the seedling stage, with the exception of environment × genotype on days to emergence (DTE) and stem greenness at week 1 after the imposition of water stress (SGWK1). Thirty-seven cowpea accessions from both screen houses were tolerant to drought, while 23 were susceptible.

7.4: Association Mapping for Drought Tolerance and Yield-Related Traits in Cowpea Accessions

The objective of this study was to conduct association mapping for drought tolerance at seedling stages and yield-related traits in cowpeas. Sixty cowpea accessions were used in the study.

The populations of the cowpea accessions were analysed using STRUCTURE 2.3.4. The peak of delta K in the greenhouse showed seven sub populations, whereas the peak of delta K in the glasshouse indicated the presence of six sub populations. Single-nucleotide polymorphisms (SNPs) discovered through genotyping by sequencing (GBS) were used for genotyping. Association mapping was conducted using single-marker regression (SMR) in Q Gene, and general linear model (GLM) and mixed linear model (MLM) built in TASSEL. The population of the cowpea accessions were analysed using STRUCTURE 2.3.4 and the peak of delta K in the greenhouse showed seven population types, whereas the peak of delta K in the glasshouse indicated the presence of six population types. One SNP marker, 14083649|F|0-9 was associated with NP with a p value <0.001. Fifty SNP markers were associated with PWT at

$p < 0.001$. Four SNP markers, 14074781|F|0-16, 100047392|F|0-36, 14083801|F|0-28 and 100051488|F|0-49 were associated with AVSPD at $p < 0.001$. SNP markers, 14074781|F|0-16, 14083801|F|0-28 and 100051488|F|0-49 were associated with PL at $p < 0.001$. Five SNP markers, 100047392|F|0-36, 14083801|F|0-28, 100072738|F|0-34, 14076881|F|0-49 and 14076881|F|0-49 were associated with PWDTH at $p < 0.001$. The major difference that was observed in association mapping in the two environments was due to temperature variations. The 65 SNP markers identified can be used in cowpea molecular breeding to select for AVSPD, NP, PL, PWDTH, PWT, and RR through marker assisted selection (MAS).

7.5: Conclusions

Based on the findings of the study, the following actions are recommended:

- There is an inherent need to provide uninterrupted extension services in the smallholder farming sector, especially with the negative effects of climate change on cropping, food security, and choices of crops to grow. Farmers also need knowledge on how best to grow drought-tolerant crops such as cowpea. Efforts must also be made to provide quality seeds in a timely fashion, so that farmers can fully utilise their rain-fed agriculture systems.
- It is imperative to widen the genetic base of cowpea accessions available by importing from other regions, such as West Africa. This may be achieved through the use of improved cowpea accessions either from IITA or closely related wild relatives, which would enhance the genetic base of the crop. These could be used in the identification of new genes that are associated with desirable agronomic attributes, such as high tolerance to biotic and abiotic stress factors.
- Screening and determining drought tolerant at the seedling stage is very important. The stability of accessions, with no or minimal variation can serve as a genetic pool for breeding drought-tolerant cowpea cultivars.
- Traits such as AVSPD, NP, PL, PWDTH, PWT, and RR are ideal for population–association analyses at seedling stage for drought tolerance in cowpeas. The use of these

traits can be useful and effective in improving locally well-adapted germplasm by the marker-assisted introgression of desirable alleles.

7.6: Recommendations

- In any breeding programme, farmer preferences have to be taken into perspective for ease of adoption of the cultivars.
- Multi-environment testing of cultivars is important for yield and stability in cowpeas.
- The marker-assisted selection of important SNPs in cowpea breeding should be an ongoing process so as to release cultivars faster, especially with the threat of droughts. Marker assisted selection at seedling stage can greatly quicken the breeding of cowpea genotypes and eliminate undesirable genotypes as well.
- In this study, genetic diversity studies were done on 85 cowpea accessions while association mapping was done on 60 cowpea accessions. It is important to increase the cowpea accessions to around 300 for both drought tolerance phenotyping and genotyping through DNA sequencing to have SNPs. The SNPs can be used to conduct genome wide association study in order to identify genes for drought tolerance and use them in cowpea breeding.
- Future studies must assess these cowpea accessions under field conditions.

APPENDIX

Cowpeas Production Constraints and Variety Preference Manicaland Province, Zimbabwe

A. **General information** Date _____

District _____

Municipality _____

Village/sublocation _____

Name of respondent _____

M

F

Age (range) _____

Number of Household _____

Education level: Tick the highest level: None _____ Primary _____ Secondary

_____ College _____ University _____

B. Cowpea farming systems

1. What is the approximate size of your farm?
2. On how many hectares do you plant cowpeas?
3. How long have you been growing cowpeas?
4. Have you been trained in cowpea production?

i. Yes

ii. No

5. Do you plant any improved cowpea varieties

i. Yes

ii. No

6. Do you have cowpeas for canning in Zimbabwe?

i. Yes

ii. No

7. If available would you like to grow it and why?

i. Yes

ii. No

8. If yes, what improved varieties do you plant- Names?

9. Why do you prefer the improved varieties?

10. What other local cowpea cultivars do you plant- give names?

11. Why do you prefer the local varieties?

12. Do you intercrop cowpeas with other crops?

Yes

No

13. What is your average cowpea yield approximately (bag= 50 kg)?

14. What are the main uses of the cowpeas you grow?

- i. Home consumption
- ii. Animal feed
- iii. Green manure
- iv. Other _____

15. How many bags do you require for the family consumption every year?

- i. Less than 5 bags
- ii. 6 to 10 bags
- iii. More than 10 bags

16. What type of food preparation you make from cowpeas?

17. Do you sell your cowpeas?

Yes

No

18. Which part? _____

C. Constraints to cowpeas production

19. What are the cowpea production constraints that you face in order of importance?(the constraints may be ranked in order to see their importance)

Cowpea production constraints				
1	High cost of inputs	Fertilizers	Tick appropriately	Rank
		Seed		
		Labor		
2	Storage pests	Weevils		
		Larger grain borer		
		Beetles		
3	Field pests	Aphids		
		Thrips		
		Legume pod borer		
		Stem and stalk borers		
		Sucking bugs		
4	Diseases	Downy mildew		
		Anthracnose		
		Phoma Leaf Spot		
		Zonate Leaf Spot		
		Bacterial leaf spot		
		Bacterial streak		
		Northern Leaf Blight		
		Rust		
		Leaf smut		
		Blotches		
		Stem rots		
		Fusarium wilt		
		Blight		
		Storage rots		
	Parasitic weeds	Striga		
	Other	Birds		
5	Abiotic	Drought		
		Soil fertility and acid soil		

		Heat		
6	Policies	Low market prices		

D. Farmers' variety preference

20. Which cowpea varieties do you grow in your area? List them

21. List factors you consider when selecting cowpea varieties and rank them

Factor	Reason	Rank
High Yield		
Resistance to disease/pest		
Tolerance to drought/heat		
Resistance to storage pests		
Resistance to birds		
Maturity period		
Grain colour		
Grain size		
Head size		
Head shape		
Taste		
Plant height		
Biomass		
Other		

Taste: 1= Sweet, 2= Non-sweet, 3= Bitter Colour: 1 White, 2= Tan, 3= Brown, 4= Red

Head shape: 1= Compact, 2= Semi-compact, 3= Loose

22. How often do you eat cowpeas in a week?

23. What other crops do you grow in your area? Rank them in order of importance

Crops	Rank

24. In your opinion, what should be the focus of the current cowpea research efforts in order of priority?

25. In your opinion, what would be an ideal cowpea variety?

26. Do you wish to continue growing cowpeas in the foreseeable future?

Yes

No