Breeding cowpea (*Vigna unguiculata* [L.] Walp) for improved yield and related traits using gamma irradiation

By

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Thesis summary

Cowpea is an important grain legume widely grown in sub-Saharan Africa for food and feed. In Namibia cowpea productivity is considerably low due to a wide array of abiotic and biotic stresses and socio-economic constrains. The overall objective of this study was to develop farmers' preferred cowpea varieties with enhanced grain yield and agronomic traits through mutation breeding. The specific objectives of the study were to: (1) assess farmers'- perceived production constraints, preferred traits and the farming system of cowpea, and its implication for breeding in northern Namibia, (2) determine an ideal dose of gamma radiation to induce genetic variation in selected cowpea genotypes, (3) identify desirable cowpea genotypes after gamma irradiation of three IITA acquired cowpea varieties widely grown in Namibia including Nakare (IT81D-985), Shindimba (IT89KD-245-1) and Bira (IT87D-453-2) through continuous selections from M_2 through M_6 generations, (4) determine G x E interaction and yield stability of elite mutant cowpea selections and to identify promising genotypes and representative test and production environments, and (5) select elite cowpea varieties that meet farmers' needs and preferences through farmers' participation and indigenous knowledge.

Participatory rural appraisal (PRA) study was conducted across four selected regions of northern Namibia including Kavango East, Kavango West, Oshikoto and Omusati where cowpea is predominantly cultivated involving 171 households. The majority of respondent farmers (70.2%) grow local unimproved cowpea varieties. About 62.6% of interviewed farmers reported low yields of cowpea varying from 100-599 kg/ha, while 6% of respondents achieved good grain harvests of 1500-1999 kg/ha. Farmers who grow local unimproved avarieties also indicated that the local varieties were not readly available and most have lost them to prolonged droughts and poor rainfall. Most farmers (59.1%) produced cowpea for home consumption, while 23.4% indicated its food and market value. Field pests such as aphids (reported by 77.8% respondents), leaf beetles (53.2%) and pod borers (60%) and bruchids (100%) were the major constraints. *Striga gesnerioides* and *Alectra Vogelii* (Benth) were the principal parasitic weeds reported by 79.5% respondent farmers. Soil fertility levels were reported to be very low across regions and all farmers did not apply any fertilizers on cowpea. Farmers-preferred traits of cowpea included a straight pod shape (reported by 61.4% respondents), a long pod size bearing at least 10 seeds (68.4%), white grain colour (22.2%) and high above ground biomass (42.1%). Inter-cropping of cowpea with sorghum or pearl millet was the dominant cowpea farming system in northern Namibia. About 68.4% of farmers used a relatively smaller proportion of their land (<1 ha) for cowpea production, while only 9.9% allocated more than 5 ha^{-1}.

Before a large scale mutagenesis an appropriate dose of radiation should be established on target genotypes. Therefore, seeds of the following three cowpea genotypes widely grown in Namibia: Nakare (IT81D-985), Shindimba (IT89KD-245-1) and Bira (IT87D-453) were gamma irradiated using seven doses (0, 100, 200, 300, 400, 500 and 600 Gy) at the International Atomic Energy Agency, Austria. The optimum doses at LD_{50} for the genotypes Nakare and Shindimba were 150 and 200 Gy, respectively while genotype Bira tolerated high dose of 600 Gy. Using linear regression model, the LD_{50} for genotypes Nakare, Shindimba and Bira were established to be 165.24, 198.69 and 689 Gy, respectively.

Large scale mutagenesis were undertaken through gamma irradiation using seeds of the three varieties (Nakare, Shindimba and Bira). Field experiments were conducted in order to identify agronomically desirable cowpea genotypes. Substantial genetic variability was detected among cowpea genotypes after mutagenesis across generations including flowering ability, maturity, flower and seed colours and grain yields. Overall 34 elite cowpea mutants were selected from 37 genotypes including 3 parental lines showing phenotypic and agronomic stability. The selected 34 promising mutant lines along with the 3 parents were recommended for adaptability and stability tests across representative agro-ecologies for large-scale production or breeding in Namibia. The lines were subjected to G x E study conducted at three selected sites (Bagani, Mannheim and Omahenene) and two cropping seasons (2014/2015 and 2015/2016) providing six environments. The following four promising mutant genotypes: G9 (ShL3P74), G10 (ShR3P4), G12 (ShR9P5) and G4 (ShL2P4) were identified with better grain yields of 2.83, 2.06, 1.99 and 1.95, $t.ha^{-1,}$ in that order. The parental lines designated as G14 (Shindimba), G26 (Nakare) and G37 (Bira) provided mean grain yields of 1.87, 1.48 and 1.30 $t.ha^{-1}$, respectively. The best environments in discriminating the test genotypes were Bagani during 2014/15 and Omahenene during 2014/15.

Participatory cowpea varietal selection was undertaken in the northern Namibia using a set of newly developed 34 elite cowpea varieties. Genotypes were evaluated along with the three parents. Field evaluations were conducted across three selected villages in Omusati Region of northern Namibia where the crop is predominantly cultivated. Test varieties were independently assessed and scored using nine agronomic traits involving 114 participating farmers. Overall, the following 10 farmers-preferred cowpea varieties were selected: R9P5 (Sh200), R3P4 (Sh100), R4P1 (Sh100), L3P74 (Sh100), R1P12 (Nk100), R8P9 (Nk150), R5P1 (Nk150), R2P9 (Nk150), R10P5 (Nk150) and R11P2 (Bi600) for their larger seed size, white grain colour, high pod setting ability, insect pest tolerance, early maturity, longer pod size, drought tolerance, high biomass and pod yields.

Generally, the study identified valuable cowpea mutants derived from three local varieties Shindimba, Bira and Nakare using gamma irradiation. The identified genotypes are phenotypically and agronomically stable and recommended to distinct, uniformity and stability (DUS) trials for varietal registration and release in northern Namibia.

Declaration

I, Lydia Ndinelao Horn, hereby declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research
2. This thesis has not been submitted for any degree or examination at any other University
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from those persons
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Lydia Ndinelao Horn (Candidate)

As the candidate's supervisor, I agree to the submission of this thesis:

Signed: …… Date: ……

Prof. Hussein Shimelis (Supervisor)

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Dedication

This thesis is dedicated to my beloved children; Given and Nadia Horn, Panduleni Nghishikungu and my loving mother Epifania Johanna - Nakuti Nghipangwa for the love and support during my studies. Special dedication goes to the future plant Breeders of Namibia in their efforts to develop successful seed industry in the country.

Publications pertaining to this thesis

Chapter 2

Horn, L., H. Shimelis and M. Laing. 2015. Participatory appraisal of production constraints, preferred traits and farming system of cowpea in the northern Namibia: implications for breeding. Legume Research 38: 691-700.

Chapter 3

Horn L., Shimelis H (2013). Radio-sensitivity of selected cowpea (*Vigna unguiculata*) genotypes to varying gamma irradiation doses. Scientific Research and Essays 8: 1991-1997.

Chapter 4

Horn, L., H.M. Ghebrehiwot and H. Shimelis. 2016. Selection of novel cowpea genotypes derived through gamma irradiation. Frontiers in Plant Science 7:262.

Chapter 6

Horn, L., H.M. Ghebrehiwot., Fatma Sarsu and H. Shimelis. 2017. Participatory varietal selection among elite cowpea genotypes in northern Namibia. Legume Research.

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Thesis Introduction

Background

Cowpea (*Vigna unguiculata* (L.) Walp.; 2n =2x = 22) is an important legume crop widely grown under low input production systems in arid and semi-arid agro-ecologies of the world. Cowpea grain composed of high levels of protein (17 to 25 %) which is rich in two essential amino acids, lysine and tryptophan (Ibro *et al.,* 2014). China, Turkey, India, Brazil and USA are the leading producers of cowpea in the world (Pasquet, 2000; Ba *et al.,* 2004). West Africa is the major cowpea producing region in sub-Saharan Africa (SSA), where Nigeria and Niger stand first and second respectively covering 80% of the total regional production during the past 14 years (Aboki and Yuguda, 2013). It is one of the most preferred crops and a valuable component in the farming systems of the majority of resource poor rural households in SSA for its various attributes (Gnanamurthy *et al.,* 2012).

In Namibia cowpea is the third most important staple crop after pearl millet (*Pennisetum glaucum* (L.) R. Br.) and sorghum (*Sorghum bicolor* [L.] Moench) (McDonagh and Hillyer, 2003). It is grown by 95% of small-scale farmers in the north and central regions of the country including Oshikoto, Oshana, Ohangwena and Omusati (Fleissner and Bagnall-Oakeley, 2001). In the country cowpea is prepared in various food forms such as boiled grains, or peeled grains pounded into a mash or soup (Fleissner and Bagnall-Oakeley, 2001). The yields of cowpea have been low varying from 100-599 kg/ha (Horn *et al.,*2015) compared to potentially attainable yields of 1500 to 3000 kg/ha reported elsewhere (Gbaye and Holloway, 2011).

Constraints to cowpea production in Namibia

In northern Namibia, about 70% of smallholder farmers still grow local unimproved cowpea varieties (Horn *et al.,* 2015). Only 62.6% of interviewed farmers reported low yields of cowpea varying from 100-599 kg/ha, while 6% achieved good grain harvests of 1500-1999 kg/ha. Farmers who grow local unimproved avarieties also indicated that the local varieties were not readly available and most have lost them to prolonged droughts and poor rainfall. Only three introduced cowpea varieties are officially available in the country namely Nakare [IT81D-985], Shindimba [IT89KD-245-1] and Bira [IT87D-453-2]. Farmers however, reported poor yield response of the introduced varieties due to their susceptibility to drought and heat stresses. Other major production constraints reported affecting cowpea production in Namibia were field and storage pests (aphids, leaf beetles, pod borers and bruchids) and low soil fertility.

Furthermore, parasitic weeds such as *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* (Benth.) cause major yield losses of cowpea in Namibia (Horn *et al.,* 2015). Various national research programs and the International Institute of Tropical Agriculture (IITA) are actively involved in developing improved cowpea varieties globally. Consequently, nematode resistant (Oliveira *et al.,* 2012) and *Striga* and *Alectra* tolerant varieties were developed and released through conventional breeding techniques. To enhance crop production and productivity, the Government of Namibia in collaboration with the International Atomic Energy Agency (IAEA) initiated a mutation breeding project during 2007. This project is being coordinated by the Ministry of Agriculture, Water and Forestry (MWAF) /Namibia. Through this initiative seeds of the above three traditional cowpea varieties were gamma irradiated with varied doses for breeding. As part of this initiative, the present study was undertaken to develop improved and farmers-preferred and locally adapted cowpea varieties using gamma irradiation for sustainable production and productivity of the crop.

Rationale for breeding cowpea using gamma irradiation

Cowpea is the most important staple food crop in Namibia. According to Fleissner and Bagnall-Oakeley (2001), at least 95% of farmers in northern Namibia grow cowpea, pearl millet and sorghum. Cowpea is mostly intercropped with pearl millet, sorghum or maize. Through a collaborative research with the International Institute of Tropical Agriculture, three varieties were introduced and released in Namibia during 1997 (Fleissner and Bagnall-Oakeley, 2001). These varieties are poor yielders with grain yields of 250 to 350 kg/ha and are susceptible to pest, and changing climatic conditions such as drought stress. Therefore, there is a need to cowpea germplasm development and genetic enhancement towards high yield, insect and pest resistance, and drought tolerance in the country. Despite the rich germplasm collections available by various national breeding programs and the IITA, the genetic base for most self-pollinating crops including cowpea is narrow for economic traits such as grain yield, yield components, drought and insect pest tolerance (Tshilenge-Lukanda *et al*., 2012)

Genetic variation is the basis for plant breeding programs. Mutation breeding is helpful in pre-breeding or genetic enhancement aimed to develop suitable germplasm with farmers preferred attributes. Artificial mutagenesis may bring about fast and direct results to select useful mutants. Conventional breeding methods take longer cycle of selections after extensive crosses and genetic advancement (Novak and Brunner, 1992). Gamma irradiation has been routinely used by the IAEA and national breeding programs to induce genetic variation and to develop mutant cultivars (Mba *et al.,* 2010; Tshilenge-Lukanda *et al.,* 2012). Optimizing the

right dose of radiation treatment for each crop genotype is an important pre-requisite prior to a large scale mutagenesis through gamma irradiation. This has to be followed up with continuous selfing and selection of desired mutants from the M_2 through advanced generations. Following rigorous selection of promising genotypes, it is necessary to test their adaptability and stability across representative agro-ecologies for large-scale production or targeted breeding. Participatory varietal selection (PVS) is advocated for identification of farmers-preferred genotypes for large-scale production or ultimate adoption.

Overall research objectives

The overall goal of the study was to contribute for food security strategy of Namibia through improving yield and productivity of cowpea. To achieve this, a mutation breeding project was conducted aiming to develop farmers-preferred, locally adapted and high yielding cowpea varieties with wide adaptation and better performance.

Specific objectives

The specific objectives of the study were:

1. To assess farmers'-perceived production constraints, preferred traits, the farming system of cowpea, and their combined implications for breeding cowpea for northern Namibia.
2. To determine the ideal dose of gamma radiation to induce genetic variation in selected cowpea (*V. unguiculata*) genotypes.
3. To identify desirable cowpea genotypes after gamma irradiation of three imported cowpea varieties officially released in Namibia from IITA, Nakare (IT81D-985), Shindimba (IT89KD-245-1) and Bira (IT87D-453-2) through continuous selections from M_2 through M_6 generations.
4. To determine G x E interaction and yield stability of elite mutant cowpea selections and to identify promising genotypes and representative test and production environments.
5. To select elite cowpea varieties that meet farmers' needs and preferences through farmers' participation and indigenous knowledge.

Research hypotheses

The current study was based on the following hypotheses:

1. Participatory rural appraisal will facilitate identification of farmers'-perceived production constraints, preferred traits, and farming systems of cowpea in northern Namibia to establish long-term breeding goals.
2. Ideal dose of gamma irradiation will be established before large-scale mutagenesis is undertaken in the selected cowpea genotypes.
3. Mutation breeding technique using gamma radiation allows selection of desirable cowpea genotypes with farmers' preferred and economic traits.
4. Selected elite mutants are subject to G x E interaction and desirable genotypes could be identified with high yield, yield stability and desirable farmer-preferred agronomic traits.
5. Farmer-centred participatory varietal selection enables identification of elite cowpea varieties that meet farmers' needs and preferences.

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Chapter 1 A Review of the literature

Abstract

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important grain legume which is widely grown in sub-Saharan Africa (SSA) for food and feed. Its grain composed of high levels of protein, energy and micro- and macro-nutrients which are essential for human nutrition. Young and succulent leaves of cowpea are consumed as cooked vegetables in some parts of SSA. In SSA including Namibia cowpea productivity is considerably low due to a wide array of abiotic and biotic stresses and socio-economic constrains. Therefore, breeding improved varieties incorporating farmers-preferred traits remains an overriding consideration to boost the productivity of cowpea in the region. This review summarizes challenges and constraints to cowpea production, breeding methods and progress, genetic variation and analysis of cowpea. Furthermore, information on participatory varietal selection (PVS) is presented to highlight farmers' desire and preference in the selection of cowpea varieties for large-scale production and ultimate adoption. The literature presented herein may serve as baseline information for cowpea breeders, agronomists or producers in Namibia or similar agro-ecologies in SSA.

Keywords: breeding, genetic variation, cowpea, mutation breeding, Namibia, participatory variety selection

1.1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.; 2n =2x = 22) is an important legume crop widely grown under low input production systems and in arid and semi-arid agro-ecologies of the world. Cowpea grain composed of high proportion of protein (17 to 25 %) which is rich in two essential amino acids, lysine and tryptophan (Ibro *et al.* 2014). Cowpea is also known as southern pea, black eye pea, crowder pea, lubia, niebe, coupe or frijole. Reports (Padulosi and Ng, 1997; Agbogidi, 2010) account that cowpea belongs to the family Fabaceae and sub-family Faboideae. It is predominantly a self-fertilizing crop. China, Turkey, India, Brazil and USA are the leading producers of cowpea in the world (Pasquet, 2000; Ba *et al.,* 2004). West Africa is the major cowpea producing region in SSA, where Nigeria and Niger stand first and second respectively covering 80% of the total regional production during the past 14 years (Aboki and Yuguda, 2013).

Cowpea is one of the most preferred crops and a valuable component in the farming systems of the majority of resource poor rural households in SSA for its various attributes (Gnanamurthy *et al.,* 2012; Molosiwa *et al.,* 2016). The crop has the ability to grow under harsh environmental conditions where other major crops fail to grow. Its foliage is regarded as an important source of high-quality livestock feed. Cowpea has the ability to restore soil fertility through nitrogen fixation useful in crop rotation with the major cereal crops (Dugje *et al.,* 2009; Gnanamurthy *et al.,* 2012). In Namibia, cowpea is the third important staple crop after pearl millet and sorghum. In the country cowpea is prepared in various food forms such as boiled grains, or peeled grains pounded into a mash or soup (Fleissner and Bagnall-Oakeley, 2001).

There was no systematic cowpea research and development program over the past years in Namibia. Therefore, in the country cowpea yields have been low varying from 100-599 kg/ha (Horn *et al.,* 2015) compared to potential yields of 1500 to 3000 kg/ha reported elsewhere (Gbaye and Holloway, 2011). Using a participatory rural appraisal (PRA) study conducted across four cowpea growing regions, it was found that 70.2% farmers in the northern Namibia still grew local unimproved cowpea varieties, while only 29.8% used improved varieties either singly or in combination (Horn *et al.,* 2015). In the country only the following three improved varieties previously obtained from the IITA: Nakare [IT81D-985], Shindimba [IT89KD-245-1] and Bira [IT87D-453-2] were commercialised but not readly accessible to farmers. During the same study farmers reported poor yields of the local varieties due to their susceptibility to drought and heat stresses. In the study areas, farmers indicated other constrains affecting cowpea production such as field and storage pests (aphids, leaf beetles, pod borers and bruchids) and low soil fertility. Furthermore, 79.5% of the farmers indicated that parasitic

weeds such as *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* (Benth.) affected cowpea production in Namibia (Horn *et al.,* 2015). Various national research programs and the International Institute of Tropical Agriculture (IITA) are actively involved in developing improved cowpea varieties globally. Consequently, nematode resistant (e.g. varieties CE-31, Frade Preto, CE-28, CE-01, CE-315and CE-237) *et al.,* 2012) or *Striga* and *Alectra* tolerant varieties were developed and released through conventional breeding techniques (Timko *et al.,* 2007; Kabambe *et al.,* 2013). Furthermore, early maturing, high yielding and pest resistant cultivars have been developed by the IITA and the Agricultural Research Institute of Senegal (ISRA) (Dugje *et al.,* 2009) which are widely grown in Nigeria, Niger and Senegal . In Namibia a well-established cowpea improvement program is required to develop farmers-preferred and locally adapted varieties for sustainable production and productivity.

1.2 Production constraints to cowpea

1.2.1 Biotic constraints

1.2.1.1 Fungal diseases

The most destructive fungal disease of cowpea includes leaf smut (false smut or black spot), caused by *Protomycopsis phaseoli* (Bailey *et al.,* 1990; Singh, 2005). Fungal diseases cause leave smut, stem rot as well as root rot (Bailey *et al.,* 1990). Yield losses varying from 20 to 100% have been reported due to fungal diseases (Mbeyagala *et al.,* 2014). Sources of resistance to fungal pathogens have been identified, and screening techniques are well developed (Adejumo *et al.,* 2001; Gbaguidi *et al.,* 2013; Pujari *et al.,* 2015). Yield losses due to fungal diseases have been reported in several African countries. However, serious epidemics were reported in Nigeria, the Sudan savanna and Sahel (Adejumo *et al.,* 2001; Singh, 2005). So far there is no study that reported fungal diseases of cowpea in Namibia.

1.2.1.2 Viral diseases

Thottappilly and Rossel (1992); Adejumo *et al.,* (2001) reported eight virus strains affecting cowpea production and productivity in Africa. Cowpea viruses are transmitted by aphids, beetles and other parasitic pests that live and feed on the crop. The common cowpea viruses include yellow mosaic comovirus, mottle virus, and southern bean mosaic sobemovirus, which are beetle-transmitted. Aphid-borne viruses of cowpea include mosaic potyvirus and cucumber mosaic cucumovirus. Some cowpea viruses are transmitted by whitefly such as cowpea golden mosaic virus and cowpea mild mottle carlavirus. The red mosaic virus have negative effect on rhizobium bacterial growth and development that led to a reduction of 20 to

45% root nodulation (Taiwo *et al.,* 2014). Mbeyagala et al. (2014) suggested that introducing new cowpea genotypes into a new growing environment may bring viral epidemics. A number of landrace cowpea varieties such as WC32, WC18, NE43, NE15, and WC35B were reported to be resistant to virus strains (Taiwo *et al.,* 2014). It is not known whether viral diseases are causing economic yield losses of cowpea production in Namibia. In the country no study has been conducted on parasitic plants or pathogens limiting cowpea production.

1.2.1.3 Bacterial diseases

The common and serious bacterial diseases of cowpea reported in the literature are bacterial blight caused by *Xanthomonas campestris* pv. *vignicola* and bacterial pustule caused by *Xanthomonas campestris* pv. *vignaeuguiculatae* (Viswanatha *et al.,*2011). The two pathogens were reported to cause yield reductions reaching up to 71% in pod, 68% in seed and 53% in fodder in susceptible varieties in India (Viswanatha *et al.,* 2011). The bacteria cause yellowing of the leaves progressively showing irregular to round spots during moderate infection. This will lead to senescence and dropping of leaves. Some bio-control agents have been reported being effective in controlling bacterial blight disease of cowpea (Reddy *et al.,* 2013). There is no information available on bacterial diseases of cowpea in Namibia.

1.2.1.4 Root-knot nematodes

Root-knot nematodes cause major loss in cowpea production hindering nutrient and water absorption from the soil (Haegeman *et al.,* 2012). Gheysen and Mitchum (2011) reported the negative effect of nematodes in cowpea growth and development including interfering and limiting auxin transport and plant cell differentiation pathways. The root-knot nematode species, *Meloidogyne incognita* and *M. javanica,* are frequently prevalent in cowpea fields (Oliveira *et al.,* 2012). Some transgenic cowpea cultivars such as CE-31, Frade Preto, CE-28, CE-01, CE-315and CE-237 were reported possessing considerable resistance to nematodes (Oliveira *et al.,* 2012). Nematode infestation in cowpea production can also be prevented through cultural practices such as cleaning of field from infected crop residues after harvest and crop rotation practices (Gheysen and Mitchum, 2011).

1.2.1.5 Parasitic weeds

Striga gesnerioides (Willd.) Vatke and *Alectra vogelii* (Benth) are the two major parasitic weeds affecting cowpea production in SSA. The weeds grow and attach themselves on the root surfaces of the host where they absorb nutrients (Figure 1.1). *Alectra* causes serious yield losses in cowpea production in Namibia (Horn *et al.,* 2015). Various authors

(Noubissietchiagam *et al.,* 2010) documented the negative effects of *Striga* on cowpea production. Seeds of the parasitic weed are able to remain dormant in the soil for over 20 years making it difficult to control using traditional methods (Kabambe *et al.,* 2002; Kabambe *et al.,* 2013). One of the possible ways in controlling *Striga* and *Alectra* is by reducing its seed bank in the soil. This can be achieved by removing the parasitic weeds after germination and before flowering and seed set. Timko *et al.* (2007); Kabambe *et al.* (2013) reported some of the progress made in breeding cowpea for resistance to *Striga* and *Alectra* using conventional breeding methods.

Figure 1.1 Cowpea field infested by *Alectra vogelii* (Benth) (left) and an uprooted Alectra plant attached to cowpea roots as parasitic weed (right). Photos taken at Bagani research station in Namibia during 2014/15 cropping season.

1.2.1.6 Insect pests

Insect pests attack cowpea both in the field and in-stores. Several studies (Ngakou *et al.,* 2008; Boukar and Fatokun, 2009; Dugje *et al.,* 2009) reported the major field pests of cowpea including *Aphis craccivora* (Koch), bruchids (*Callosobruchus maculatus* (Fabricius), beetles *(Ootheca mutabilis*), maruca, leafhoppers and foliage beetles. The pests occur throughout the vegetative growing stages of the plant, feeding on the leaves and also act as virus vectors. In Namibia, farmers described the predominant field pests including aphids causing yield losses of 77.8%, leaf beetles (53.2%) and pod borers (60%) and bruchids (100%) (Horn *et al.,* 2015). In SSA bruchids are the leading pests of cowpea affecting stored grains (Figure 1.2). Bruchids damage cowpea grains which may lead losses reaching up to 100% (Stejskal *et al.,* 2006; Gbaguidi *et al.,* 2013; Horn *et al.,* 2015). Swella *et al.,* (2007); Ilesanmi and Gungula, (2011) reported of some effective control methods such as treating seeds with Actellic dust and black pepper powder to have significantly lowered percentages of seeds damages in cowpea. Black pepper powder and coconut oil were also recommended for their potential in protecting

cowpea against bruchid damage. Studies to determine the effects of neem (*Azadirachta indica* (A. Juss)) and moringa (*Moringa oleifera)* seed oils revealed that treated seeds with various concentrations were not infested by bruchids (Ilesanmi and Gungula, 2011).

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Figure 1.2 Cowpea seeds infested by bruchids (*Callosobruchus maculatus*) at Omahenene Research Station of Namibia during 2013/2014 season.

1.2.2 Abiotic constraints

Drought and heat stresses and poor soil fertility are the major abiotic factors affecting cowpea production and productivity. The negative effects of heat and drought stress in sub-Saharan Africa including Namibia have been reported since 1968. Abiotic stresses led to the loss of many landraces varieties of crops including pearl millet, sorghum and legumes (Hall, 2004). Cowpeas are sensitive to severe droughts especially during pod setting and grain filling stages (Hall, 2004). Heat stress above a threshold temperature of 16°C caused 4 to 14% loss in pod set and grain yield. Poor soil fertility is another major constraint limiting cowpea production. In Namibia, cowpea production is carried out in soils with poor fertility levels and most farmers did not apply fertilizers (Horn *et al.,* 2015).

1.2.3 Socio-economic constraints

Sabo et al. (2014); Horn et al. (2015) outlined a number of socio-economic constraints adversely affecting cowpea production in sub-Sahara Africa. These includes non-availability

of market preferred varieties, low yield potential, high cost of farmland preparation, lack of improved production and harvesting tools, high cost and absence of labour, high cost and adulteration of pesticides, poor harvest prices, and underdeveloped marketing channels.

Other major constrains to cowpea production in many SSA countries is lack of defined value chain and poor development of cowpea as a commodity crop. There is no efficient transport systems and cowpea trading is not organized due to limited value addition and lack of cowpea enterprises (Fakayode *et al.,* 2014). In Nigeria and other west African countries farmers solely survive on cowpea farming which is the major economic mainstay and business (Aboki and Yuguda, 2013). It is also reported in west Africa cowpea trades enables farmers to buy other cereal grains and farm inputs such as fertilizers making it easy to have acces to agricultural inputs (Fakayode *et al.,* 2014). In Namibia farmers earn cash incomes from sales of cowpea grains (Horn *et al.,* 2015) though the monetary values of cowpea products are low. The full economic potential of cowpea will only be realized if other value added products especially those targeted at the ever growing urban population, are introduced. Waddington et al. (2010) suggested that converting cowpea into baby food might bring about a rise in the price of the commodity which will also bring higher returns to the producer. Cowpea is an important weaning food in many communities in Africa and Asia. In SSA its demand is particularly high (Ibro *et al.,* 2014). Raising the average yield per hectare of the crop will therefore increase the annual global production and hence the revenue.

Various reports indicated that that the potential yields of cowpea can reach up to 3,000 kg/ha if most of the constraints are alleviated (Aboki and Yuguda, 2013). Therefore, targeted cowpea breeding is needed to improve production and productivity of the crops incorporating farmers' and consumers and preferences. Introduction of new value added cowpea products into the market would significantly raise revenues from cowpea production.

1.3 Genetic diversity and analysis in cowpea

Genetic diversity is fundamental in plant breeding programs. The genetic diversity of cowpea has declined due to various biotic and abiotic factors (Fang *et al.,* 2007). Farmers in Namibia reported loss of their local varieties overtime due to damage by insect pests both in the field and in storage and due to frequent droughts (Stejskal *et al.,* 2006; Horn *et al.,* 2015). Loss of genetic diversity may also arise due to artificial selection of better performing varieties, while discarding poor performing types from a narrow genetic base. Genetic variation may be restricted within specific breeding programs in the absence of a complementary pre-breeding programs (Gbaguidi *et al.,* 2013). Studies conducted using germplasms collections from the

continents of north America, Asia and Africa revealed a narrow genetic base of cowpea (Fang *et al.,* 2007). The same study further indicated a strong genetic relatedness among germplasm collections of US and Asia with that of African cowpea collections. The authors indicated that most cowpea genotypes in the world are originated from Africa. Genetic variation arises at a slow pace under natural evaluation especially in cowpeas where the predominant mode of reproduction is through self-fertilisation. Gbaguidi et al. (2013), reported loss of genetic diversity of cowpea in Africa at a rate of 28 to 60% in some agro-ecologies.

A well-characterized germplasm is useful to incorporate economic traits through designed crosses. Genetic diversity analysis can be carried out using DNA markers such as amplified fragment length polypomrshism (AFLP), simple sequence repeat (SSR), randomly amplified polymorphic DNA (RAPD) and single nucleotide polymorphism (SNPs). DNA based molecular markers are more reliable and robust methods for the characterization of genetic diversity. These genetic markers are successfully applied in genetic diversity analysis of many crop plants including cowpea (Ogunkanmi *et al.,* 2008; Tantasawat *et al.,* 2010; Adetiloye *et al.,* 2013).

Genetic diversity is routinely assessed using agro-morphological or phenotypic markers. In cowpea breeding both quantitative and qualitative phenotypic characters are extensively used in germplasm characterization, classification and selection. Quantitative traits include: number of branches per plant, days to 50% flowering, days to 50 maturity, number of pods per plant, pod length, pod width, seed weight, number of seeds per pod, seed yield (Molosiwa *et al.,* 2016). Uses of phenotypic characteristics is a common approach because they form the most direct measure of the phenotype, readily available and relatively cheaper requiring simple equipment. However, phenotypic markers are subject to environmental influences in the field that may mask the concrete genetic variation among genotypes. The combined use of phenotypic and molecular markers may allow estimation of genetic diversity more reliably and efficiently. Effective field-based high-throughput phenotyping platforms (HTPPs) are recently advocated which may improve the efficiency of selection in plant breeding programs (Araus and Cairns, 2014) .

1.4 Breeding cowpea

Various national and international research programs notably the IITA are actively developing improved cowpea cultivars with high yields, early maturity, and pest and disease resistance (Dugje *et al.,* 2009). Most of these breeding programs use conventional and molecular breeding tools to harness cowpea genetic variation for breeding. Furthermore, the

international atomic energy agency (IAEA) has been supporting member states in genetic improvement of various crops including cowpea through the use of artificial mutagenesis such as gamma rays, x-rays, and Ethyl methanesulphonate (EMS) (Mba *et al.,*2010). This has led to development and release of improved cowpea cultivars in Africa, Asia, and Latin America (Viswanatha *et al.,* 2011; Reddy *et al.,* 2013). Further, most cowpea breeding initiatives lead in broadening the genetic bases of the crop to adapt various cropping systems and agro-ecologies, and in the development of consumer-preferred varieties with enhanced nutritional quality (Singh *et al.,* 2003; Lima *et al.,* 2011). The following breeding methods have been widely used in cowpea improvement programs:

1.4.1 Pure-line selection

The concept of this selection method was proposed by the Danish botanist Johanssen in 1903 on the basis of his studies on Princess beans (*Phaseolus vulgaris*). This method is suitable for highly self-fertilizing crop species such as wheat, barley, sorghum, peas, cowpea etc. Pure-line selection involves selection of promising individuals from a large number of segregating populations after systematic crosses or induced mutagenesis. Selected individuals are harvested individually and continuously selfed and selected to develop and release pure-line cultivars.

1.4.2 Pedigree breeding

Unlike pure-line breeding, pedigree breeding maintains detailed record of the relationship between the selected plants and their progenies. In this method each progeny in every generation can be traced back to the F2 plant from which it was selected from. It is commonly applied in selection of desirable plants from the segregating populations of self- pollinated crops. Pedigree method is useful especially when improving some specific traits lacking in an already established variety. It is widely used in the selection of new and superior recombinant individuals. It is a useful procedure in transgressive breeding scheme to select individuals with unique attributes such as disease resistance, plant height or maturity.

1.4.3 Backcross breeding

Backcross breeding was proposed by Harian and Pope in 1922. It is mainly used to transfer few genes into an established cultivar of self- or cross-fertilising crop. Backcrossing, leads to increased homozygosity allowing selection of desirable genotype in homozygous and desirable genetic backgrounds.

1.4.4 Single seed descent selection method

This selection procedure was first suggested by Goulden in 1941 and subsequently modified by in Brim 960. In this method, only a single seed collected from each of the F2 plants is kept and bulked to grow the F3 generation. This process continues up to the F5 and F6 generations, whereby a desired level of homozygosity is achieved. In the F6, large number of single plants are selected and their progeny grown separately. In the F7 and F8, selection of best performing lines are selected for preliminary and national yield traits.

1.4.5 Bulk population breeding

Bulk population method is also known as mass selection or population breeding. It was first used by Nilsson Ehle in 1908. It refers to a population grown in bulk plot from F1 to F5 with or without selection. A portion of the bulk seed is used to grow the next generation and individual plant selection is often started in the F6 or later generation. Bulk selection method is useful to increase the frequency of desirable types through positive mass selection. It is suitable for studies on the survival of genes and genotypes in populations and it offers greater chances of isolation of transgressive segregants than pedigree method.

1.4.6 Mutation breeding

Mutations are the ultimate source of genetic variation, a raw material for plant breeding programs (van Harten, 1998). Induced mutation derived through the use of gamma rays, x-rays, or EMS is a powerful tool for crop genetic enhancement and breeding. Appropriate dose of radiation should be established on target genotypes before large scale mutagenesis is undertaken (Tshilenge-Lukanda *et al.,* 2012). Optimizing the dose of radiation is the first step in induced mutation breeding. This is important because its predictable value guide the researcher in the choice of the ideal dose depending on the plant materials and desired outcome (Horn and Shimelis, 2013). Induced mutations provides considerable genetic variation within a reasonably short period of time when natural genetic variation of the crop is limiting for breeding. Mutagens bring about desirable changes including plant height, growth types, genetical, biochemical, physiological or morpho-genetical changes (Girija and Dhanavel, 2009). Parry *et. al.,* (2009) reported that the mutation breeding process is fast forward in developing diverse germplasm and it may take only up to 6 generations (M_6). This can be followed by further generations by single seed descent to generate near-homozygous material as opposed to the conventional breeding techniques. It is however recommended to have a very large populations of induced mutations in order to ensure that gene of interest carries sufficient significant mutations. The size required is dependent on the dosage of

mutagen and the level of gene duplication created by recent or ancient polyploidization events. This can be labour intensive and requires a large labor force to detect mutation evenst during selection. According to Mba *et. al.,* (2010), the Joint FAO/IAEA Division of the Nuclear Techniques in Agriculture in Vienna offer irradiation services to member countries at no cost.

Various improved cultivars of major crops such as wheat, rice, barley, cotton, peanuts, beans have been developed through induced mutation platforms of the Joint FAO/IAEA (Food and Agriculture Organisation of the United Nations) and the International Atomic Energy Agency division of the Nuclear Techniques in Agriculture in the 1950s (Ahloowalia and Maluszynski, 2001; Slabbert *et al.,* 2004). Maluszynski (2001) outlined some of the major success of induced mutation breeding and varieties released globally. The Netherlands, USA and Japan are classified as top countries in releasing cultivars derived through mutation breeding techniques. About 1142 mutant cultivars were released in Asia, the highest number in the world, while only 48 mutant varieties were released in Africa (Maluszynski, 2001). The Mutant Varieties Database (MVD) of FAO and the IAEA) maintained a list of 2,252 crop cultivars developed through artificial mutations (Nielen, 2004). These cultivars were released across 59 countries worldwide, mainly in the continental Asia (1142 cultivars), Europe (847) and North America (160) (Maluszynski, 2001; Maluszynski *et al.,* 2009). Studies indicate that induced mutagenesis has successfully modified several plant traits such as plant height, maturity, seed shattering resistance, disease resistance, oil quality and quantity, malting quality, size and quality of starch granules of cowpea (Goyal and Khan, 2010; Singh *et al.,* 2013).

Despite its importance and significant contribution to plant breeding and genetics, there is ilimited information that induced mutation could have negative impact on the environment or on organisms. Furthermore, it was found that most research papers only discussed the importance without reporting the possible negative impact (Mba *et al.,* 2010; Tulmann Neto *et al.,* 2011). Chopra (2005) and Slabbert *et al.,* (2004) gave details on varieties and the techniques to induce mutation from different countries including USA, China and India. In generalinduced mutation technique has been in use for over 100 years (Shu, 2008). This give a clear indication that the method have been used and accepted for over 100 years without harmfull effects resulting from its use or application. Suprasanna (2015) reported that the mutant varieties developed and released in major crops have been cultivated by farmers in large areas and have resulted in increased food production, thus contributing to food security.

1.5 Genotype by environment interaction

Genotype by environment interaction (G × E) is a differential response of genotypes when grown across environments (Yan and Hunt, 1998; Annicchiarico, 2002). Multi-environmental trials (METs) are required to quantify the magnitude of genotype × environment interaction and to recommend varieties with narrow or broader adaption (Ramburan *et al.,* 2012). G. x e trials are valuable for cultivar recommendation or for the final stages of selection of elite breeding material (Annicchiarico, 2002). Data generated through G × E interaction studies may assist crop ecologists, agronomists and plant breeders to define ecological regions, mega-environments and ecotypes (Annicchiarico *et al.,* 2011). Two types of genotype × environment interaction (GEI) are distinguishable: cross-over or qualitative and non-cross-over or quantitative (Annicchiarico and Iannucci, 2008). Cross-over or qualitative interaction is observed when there is change in ranking of cultivars when grown in different environments, while non-cross-over interaction is the interaction that is observed when genotypes show changes in magnitude of performance but the rank order of genotypes across environments remains unchanged (Jalata, 2011). For cultivar development, the cross-over type of interaction is not desirable as non-cross-over type. This is because the cross-over interaction complicates the selection of high yielding genotypes due to inconsistent performance of test genotypes across locations (Annicchiarico *et al.,* 2010; Jalata, 2011).

Genotype × environment interaction has an advantage to crop improvement that targets broad adaptation, but it can also represent opportunities to genetic improvement for specific sites (Annicchiarico *et al.,* 2010). G × E interactions may present a barrier to crop improvement because it can contribute to temporal and spatial instability of crop yields (Annicchiarico, 2002). The advantage of G × E interactions is that it can offer opportunities for selection and adoption of genotypes showing positive or negative interaction with the location and its environmental conditions allowing the exploitation of specific or broad adaptation and yield stability (Gurmu *et al.,* 2009; Mohammed *et al.,* 2016).

Several methods have been proposed to analyse and interpret the genotype × environment interaction. These include: contrasts (Yan and Hunt, 1998), linear regression (Finlay and Wilkinson, 1963), additive main effect and multiplicative interaction (AMMI) (Fleischmann *et al.,* 2016) and multivariate analysis such as principal component analysis. Also, the genotype plus the genotype by environment interaction (GGE) biplot has been reported as a method of choice in analysing g x e data (Aruna *et al.,* 2011; Adinurani *et al.,* 2015). The GGE biplot has been used in mega-environment analysis (Yan and Rajcan, 2002; Casanoves *et al.,* 2005), genotype and test environment evaluation (Yan and Rajcan, 2002; Blanche *et al.,* 2009), trait

association (Yan and Rajcan, 2002) and heterotic pattern analysis (Blanche *et al.*,, 2007). The GGE biplot is constructed by plotting the two principal components (PC1 and PC2) derived from the singular value decomposition (SVD) of environmental centred data (GGE matrix) such that three component matrices are generated; the singular value matrix (array), the genotype eigenvector matrix, and the environment eigenvector matrix. The GGE biplot is powerful than other tools and has the merit of showing graphically the which-won-where pattern of data (Yan and Wu, 2008; Adinurani *et al.,* 2015) compared to other methods of analysing genotype by environment interaction and stability. In this situation, both genotype and genotype × environment interaction can be effectively exploited by selecting superior genotypes for each mega-environment (Yan and Rajcan, 2002). Two concepts of stability have been reported, the static or biological and the dynamic or agronomic stability (Kang, 1998). Under the static concept, a genotype is indicated to be stable when its performance does not change with change in environmental conditions while under the dynamic concept a genotype is considered to be stable when it yields well relative to the productive potential of test environments.

1.6 Participatory rural appraisal (PRA) and participatory variety selection (PVS)

Participatory research techniques have been successfully used to identify farmers' perceived production constraints, preferred crop varieties and traits for deployment of production packages and suitable crop varieties (Alam and Ihsan, 2012). Depending on the breeding goal and the environment, farmers could contribute significantly at different stages of crop cultivar design, development, release and adoption (Nkongolo *et al.,* 2008). Participatory variety selection is an approach to provide choices of varieties to the farmers for increasing production in their diversity of socioeconomic and agro-ecological condition (Belay *et al.,* 2006). PVS is a more rapid and cost effective way of identifying farmer preferred cultivars if a suitable choice of cultivars exists. Various researchers including (Hoffmann *et al.,* 2007; Rusinamhodzi and Delve, 2011; vom Brocke *et al.,* 2010) have reported the importance of PVS. Understanding farmers' requirements and trait preferences, as well as their farming systems, is essential for wide adoption of newly developed crop varieties and production technologies (Rusinamhodzi and Delve, 2011; vom Brocke *et al.,* 2010). The major objectives for PVS are to promote the adoption and dissemination of new varieties and site-specific resource conservation technologies; to obtain farmers' assessments of new improved lines/varieties and specific traits; to understand farmers' criteria in evaluating improved germplasm; to obtain feedback from farmers for breeding purposes and finally to demonstrate the value of combining

improved varieties with resource conservation techniques (Hoffmann *et al.,* 2007). In PVS, the participants are selected based on their indigenous knowledge and selection is done based on farmers' selection criteria such as diseases, pest and drought tolerance, yield, grain characteristics etc. (vom Brocke *et al.,* 2010). According to Nkongolo *et al*. (2008), field extension workers and the village chiefs are more familiar with farmers in the study sites and are often helpful during PVS. It is therefore recommended for current and future breeding programme to be conducted towards meeting the specific farmers' needs and preferences. Moreover, breeding aiming at specific agricultural practices and production constraints for specific region and develop cultivar with wide adaptation is encouraged.

1.7 Conclusions

Cowpea is the major food crop and a source of cheap protein for most of resource poor households in SSA including Namibia. This literature showed the gap in global research efforts directed at improving cowpea, one of the orphan crops globally. Concerted research and development efforts is required to develop improved cultivars of cowpea for sustainable and enhanced production. The need of multi-disciplinary collaborations between breeders, farmers, processors, consumers, traders and gene banks should not be overlooked to boost cowpea production and beneficiation along the value chains. In the past various international organizations such as the IAEA and IITA and national breeding programs contributed significantly in developing improved cowpea germplasm and generation of scientific knowledge. These programs developed and released useful cowpea varieties. Evaluation of developed genetic resources is essential under the target environments prior to recommendation for large scale production. In Namibia cowpea research and development is in its infancy. Only three improved cultivars are available and widely grown in the country over the years. The country requires a cowpea breeding program focusing on developing varieties with short maturity, drought, and pest and disease tolerance. In the country farmers face yield losses due to parasitic weeds (*Striga* and *Alectra)* and insect pests. Farmers reported to have lost their cowpea germplasm overtime. This requires creation of genetic pool of the crop for cultivar development incorporating farmers-preferred traits. Mutation breeding is an important tool for genetic enhancement and breeding improved crop varieties for specific environments. Mutation breeding can be regarded as an efficient breeding tool and procedure for cowpea breeding which is the main focus of this study.

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Chapter 2 Participatory appraisal of production constraints, preferred traits and farming system of cowpea in the northern Namibia: implications for breeding

Abstract

Cowpea [*Vigna unguiculata* (L.) Walp.] productivity is low in the subsistence farming systems due to biotic, abiotic and socio-economic constraints. The objective of this study was to assess farmers' perceived production constraints, preferred traits and the farming system of cowpea, and its implication for breeding in northern Namibia. A participatory rural appraisal study was conducted across four selected regions of northern Namibia including Kavango East, Kavango West, Oshikoto and Omusati where the crop is predominantly cultivated. Data was collected using structured interviews involving 171 households. Results showed that 70.2% farmers grow local unimproved cowpea varieties and 29.8% used improved varieties either singly or in combination of two or three. About 62.6% of interviewed farmers reported low yields of cowpea varying from 100-599 kg/ha, while 6% achieved good grain harvests of 1500-1999 kg/ha. Most farmers (59.1%) produced cowpea for home consumption, while 23.4% indicated its food and market value. Field pests such as aphids (77.8%), leaf beetles (53.2%) and pod borers (60%) and bruchids (100%) were the major constraints. *Striga gesnerioides* and *Alectra Vogelii* (Benth) were the principal parasitic weeds reported by 79.5% cowpea farmers. Soil fertility levels were reported to be very low across regions and all farmers did not apply any fertilizers on cowpea. Farmers-preferred traits of cowpea included a straight pod shape (61.4%), a long pod size, bearing at least 10 seeds (68.4%), white grain colour (22.2%) and above ground biomass (42.1%). Inter-cropping of cowpea with sorghum or pearl millet was the dominant cowpea farming system in northern Namibia. Of all the farmers, 68.4% used a relatively smaller proportion of their land (<1 ha) for cowpea production while only 9.9% allocate more than 5 ha. Breeding for high grain yield and farmers-preferred traits and availability of seed and production input are the most important strategies to increase cowpea production and productivity by subsistence farmers in the northern regions of Namibia.

Key words: agro-ecology, cowpea, farming system, Namibia, aarticipatory rural appraisal

2.1 Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the widely cultivated and consumed grain legumes globally, especially in the arid and semi-arid tropics (Noubissietchiagam *et al.,* 2010). It is able to grow in harsh environments under dry-land condition, making it one of the most widely grown legume crops in sub-Saharan Africa (Baidoo and Mochiah, 2014). World production of cowpea was estimated at 5,249,571 tonnes in 2007, of which over 64% were produced in Africa (Gbaguidi *et al.,* 2013). The leading producers of cowpea include: Nigeria with 5 million ha, 2.1 million tonnes, Niger with 3 million ha, 0.6 million tonnes and Brazil with 1.9 million ha, 0.7 million tonnes (Singh *et al.,* 2003; Awurum, 2013).

In Namibia cowpea is the third most important staple crop after pearl millet [(Pennisetum glaucum (L.) R. Br.)] And sorghum (*Sorghum bicolor* [L.] Moench) (McDonagh and Hillyer, 2003). It is grown by 95% of small-scale farmers in the north and central regions including Oshikoto, Oshana, Ohangwena and Omusati (Fleissner and Bagnall-Oakeley, 2001). On the other hand, cowpea productivity and market supply in Namibia has declined in recent years due to several challenges such as low yields, unavailability of improved seeds, drought stress and damages by field pests including *Aphis craccivora* (Koch), storage pests including *Callosobruchus maculatus*, and parasitic weeds such as *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* (Benth) (Matanyaire, 1996). Fleissner and Bagnall-Oakeley (2001) reported that only 5,000 tonnes of cowpea were produced annually by the Namibian communal farming households. Furthermore, the reported cowpea yields vary from 250 to 350 kg/ha-1 per household, which is relatively low compared to the yield potential of 1500 to 3000 kg/ha-1 (Stejskal *et al.,* 2006). In the country only three cowpea varieties were made available to growers during 1997 (Fleissner and Bagnall-Oakeley, 2001). The three varieties: Nakare [IT81D-985], Shindimba [IT89KD-245-1] and Bira [IT87D-453-2] are relatively low yielding selections made from an introduced pool of genetic resources acquired from the International Institute of Tropical Agriculture (IITA).

Understanding farmers' requirements and trait preferences, as well as their farming systems, is essential for wide adoption of newly developed crop varieties and production technologies (Hoffmann *et al.,* 2007). This can be effectively studied through the PRA approach where farmers are fully involved in the various stages of development of the technologies. This approach considers the value of stakeholders' knowledge, their preferences, abilities and innovation (Chandra and Sharma, 2010). In Namibia, there is no recent participatory research documentation on the production status, farmers' production constraints or varietal preferences among the major cowpea growing aglo-ecologies (Matanyaire, 1996; Fleissner

and Bagnall-Oakeley, 2001; Stejskal *et al.,* 2006). Therefore, the objective of this study was to assess farmers'-perceived production constraints, preferred traits, the farming system of cowpea, and their combined implications for breeding cowpea for northern Namibia.

2.2 Materials and methods

This study was conducted across four selected northern regions of Namibia: Kavango East, Kavango West, Oshikoto and Omusati. The regions are known for their production of various crops including cowpea (Matanyaire, 1996). Kavango East and Kavango West are located in the north east while Oshikoto and Omusati are situated in the north central areas of Namibia. The annual rainfall in Namibia increases from 300 mm in the North West to 700 mm in the north east (McDonagh and Hillyer, 2003). The maximum temperatures of the regions vary from 25-36°C. A systematic sampling procedure was followed to identify cowpea farmers in the cowpea growing constituencies of four regions in northern Namibia. In Kavango East region, two constituencies (Mukwe and Kapako), and in Kavango West, two constituencies (Kahenge and Ndiyona), were sampled. In the Oshikoto region, four constituencies (Omuthiya, Onayena, Omuntele and Olukonda) were sampled while in Omusati four constituencies (Outapi, Okalongo, Ruacana, Otamanzi) were represented. In each constituency 15 cowpea growing farmers (households) were sampled, making a total of 180 households. However, only 171 households were interviewed because some farmers were not available during interviews. Data were collected through interviews using a structured questionnaire. 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 farmers' trait preferences of cowpea varieties. The staff of the Agricultural Extension and Engineering Service Department of the Ministry of Agriculture and Forestry assisted in locating the constituencies where cowpea growing households were selected. Interviews were conducted using the local languages (Rukavango and Oshiwambo) with the help of enumerators selected from these areas. The collected information was then translated to English at the same time. Data were subjected to analyses using cross-tabulation procedure and contingency chi-square values calculated for significant tests using SPSS (Release 16.0) computer package (SPSS Inc., 2007).

2.3 Results and discussion

The study determined farmers' perceived production constraints, preferred traits in the farming systems of cowpea and its implication for breeding in four regions of Northern Namibia. The results of this study are presented in Table 2.1 to Table 2.11. Age categories of respondents ranged between 20 to 95 years (Table 2.1). About 59.1% of farmers produced cowpea for home consumption, while 23.4% indicated its food and market value (Table 2.2. Cowpea fresh biomass is regarded as a very important trait by many farmers (Table 2.3). Farmers indicated that cowpea production is hindered by various field pests and parasitic weeds (Table 2.3), which usually lead to severe crop damages. Cultural practices were among the methods used to combat parasitic weeds and pests (Table 2.5).

Table 2.1. Age categories of farmers across four selected regions of northern Namibia who participated in the study.

		Age category (years)											**Number of**
Regions	**Class**	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**70-79**	**80-89**	**90-95**	**df**	X^2	**P-value**	**valid cases**
Kavango East	Count	0.0	3.0	9.0	7.0	5.0	0.0	1.0	0.0	21	64.241	0.00	25
	Expected	0.4	1.6	4.5	6.1	6.3	4.8	0.9	0.3				
Kavango West	Count	3.0	4.0	11	10	2.0	0.0	0.0	0.0				30
	Expected	0.5	1.9	5.4	7.4	7.5	5.8	1.1	0.4				
Omusati	Count	0.0	2.0	9.0	13	19	16	1.0	1.0				61
	Expected	1.1	3.9	11.1	15.0	15.3	11.8	2.1	0.7				
Oshikoto	Count	0.0	2.0	2.0	12	17	17	4.0	1.0				55
	Expected	1.0	3.5	10.0	13.5	13.8	10.6	1.9	0.6				
	Total%	1.8	6.4	18.1	24.6	25.1	19.3	3.5	1.2				

df = degrees of freedom and X^2 = Chi-Square.

A smaller portion of land is usually allocated for cowpea production (Table 2.6). Results showed that 70.2% farmers grow local unimproved cowpea varieties and 29.8 % used improved varieties (Table 2.7). Eventhough 70.2% indicated that they grow unimproved cowpea varieties, it was reported that they have lost most of these materials due to mostly drought. The use of improved or unimproved varieties or their combinations showed highly significant differences among the regions (P<0.00). Farmers reported that Nakare, Shindimba and Bira cowpea varieties are performing poorly with the yield between 100-500 kg/ha (Table 2.8). Nearly 68.4% of farmers indicated that the local cowpea varieties produce pods containing less than ten seeds per pod (Table 2.9). Farmers are interested in growing straight

pod cowpea with white grain colour; however they would also grow other shapes (Table 2.10 and Table 2.11).

Table 2.2 various uses of cowpea in northern Namibia.

		Uses								Number of valid cases
Regions	**Class**	**Food**	**Market**	**Food and Feed**	**Food and market**	**Food, feed and market**	**df**	**X^2**	**P-value**	
Kavango East	Count	18.0	1.0	0.0	6.0	0.0	15.0	15.191	0.438	
	Expected	14.8	0.1	3.4	5.8	0.9				25
Kavango West	Count	20.0	0.0	1.0	9.0	0.0				
	Expected	17.7	0.2	4.0	7.0	1.1				30
Omusati	Count	19.0	0.0	21.0	16.0	5.0				
	Expected	36.0	0.4	8.2	14.3	2.1				61
Oshikoto	Count	44.0	0.0	1.0	9.0	1.0				
	Expected	32.5	0.3	7.4	12.9	1.9				55
	Total%	59.1	0.6	13.5	23.4	3.5				

df = degrees of freedom and X^2 = Chi-Square.

There was a highly significance difference between the age groups of cowpea growing farmers across the four selected regions (P<0.00) (Table 2.1). The majority of farmers were 50-59 and 60-69 years old at 24.6% and 25.1%, respectively. Farmers of 70-79 years old constituted 19.3% followed by 40-49 years at 18.1%. It was observed that the youth (20-29 years) are not actively involved in crop farming, making up only 1.8% of the sample. As expected, the older groups, 80-89 and 90-95 years, were not involved in cowpea farming activities and represented only 3.5 and 1.2% of the sample, respectively. It is suggested that a negative attitude of rural youths in agricultural activities as among the major reason for their movement to the urban area. However, Blackie (2010) indicated that youth movement to the cities could be resolved if they become practically involved in agriculture to produce and sell to earn income.

About 59% farmers indicated that they grew cowpea for home consumption or food while 23.4% grew for home consumption and sale, some 13.5% use the crop foliage for animal feed during excess production (Table 2.2). The results are in line with Maredia et al. (2000) findings who reported that farmers used cowpea leaves and young pods as fresh vegetable. (Kapewangolo *et al.,* 2007) indicated the use of aboveground biomass of cowpea as animal fodder. Previous report indicated that the dried cowpea grains composed of protein varying from 23-25%, carbohydrate (50-67%) and fat (1.9%) and are used as supplementary diet for growing children in Namibia (McDonagh and Hillyer, 2003).

A highly significant difference between regions was found on importance of the fresh biomass of cowpea (P<0.00). Nearly 42.1% of farmers regarded cowpea fresh biomass as a very important trait while 41.5% of farmers reported it as not-important (Table 2.3). Legume pod borer (*Maruca virtrata* Fab.) was one of the major field pests identified by farmers as causing serious yield losses across the four regions. The responses of farmers varied significantly (P<0.00) across regions reporting damages caused by the pest from non- important (22%) to very important (60%) (Table 2.3). Baidoo and Mochiah (2014) indicated Maruca as one of the field pest that contributed significantly to low yield in the local variety of cowpea.

Field and storage pests were reported by most farmers causing yield losses every year (Table 2.3 and Table 2.5). Pod borers and cowpea leaf beetles (*Ootheca mutabilis*) feed on immature pods and grains making it unsuitable for human consumption. Matanyaire (1996), reported that most farmers lost their varieties because of several constrains including pest infestation. *Aphis craccivora* (Koch) aphids were reported as another major field pest with 77.8% of farmers indicated its importance (Table 2.5). Baidoo and Mochiah (2014) also reported aphids as one of the major cowpea pest leading to stunted growth and plant death.

In addition, field pest causes damage to cowpea as reported by Moussa et al. (2011) and Gbaguidi et al. (2013) that the damages could sometimes reach up to 100% yield loss in cowpea. *Callosobruchus maculatus* (Fabricius) bruchids was the only storage pest identified as being economically important by all (100%) interviewed farmers across the four regions. Other authors reported losses of 70% quality after *Callosobruchus* attack (Murdock *et al.,* 2003; Stejskal *et al.,* 2006). Studies on indigenous knowledge practice on pests of stored crops in the Northern Namibia established that farmers mix cowpea seeds with ash and store grains in sealed clay pots, calabash or any possible container to prevent insects from hatching (Stejskal *et al.,* 2006). Many farmers in the study area confirmed the effectiveness of this method. The use of ash as a protecting agent against storage pests was also reported by (Murdock *et al.,* 2003) among West African farmers. Similarly, Moussa et al. (2011) and Murdock *et al.,* (2003) documented various methods to minimize losses of storage pests including storing seeds in airtight containers using of ash and solar heater.

Table 2.3 The relative degree of importance of cowpea fresh biomass, pod borers, sting beetles and parasitic weeds across four Regions in northern Namibia.

		Regions				
Variable	**Class**	**Kavango**	**Kavango**	**Omusati**	**Oshikoto**	**Total%**
Importance of cowpea fresh biomass	Very Important					
	Count	19.0	14.0	10.0	29.0	42.1
	Expected	10.5	12.6	25.7	23.2	
	Less important					
	Count	2.0	3.0	11.0	12.0	16.4
	Expected	4.1	4.9	10.0	9.0	
	Not Important					
	Count	4.0	13.0	40.0	14.0	41.5
	Expected	10.4	12.5	25.3	22.8	
	Df					6.0
	P-Value					36.798
	X^2					0.000
	Number of Valid	25	30	61	55	
Importance of legume pod borer (*Maruca virtrata*)	Very Important					
	Count	24.0	26.0	29.0	23.0	59.6
	Expected	14.9	17.9	36.4	32.8	
	Less important					
	Count	1.0	4.0	20.0	7.0	18.7
	Expected	4.7	5.6	11.4	10.3	
	Not Important					
	Count	0.0	0.0	12.0	25.0	21.6
	Expected	5.4	6.5	13.2	11.9	
	Df					6.0
	P-Value					50.934
	X^2					0.000
	Number of Valid	25	30	61	55	
Importance of sting beetle	Very Important					
	Count	21	30.0	13.0	27.0	59.6
	Expected	13.3	16.0	32.5	29.3	
	Less important					
	Count	3.0	0.0	15.0	17.0	18.7
	Expected	5.1	6.1	12.5	11.3	
	Not Important					
	Count	1.0	0.0	33.0	11.0	
	Expected	6.6	7.9	16.1	14.5	
	Df					6.0
	P-Value					70.438
	X^2					0.000
	Number of Valid	25	30	61	55	
Importance of parasitic weeds (*Striga gesnerioides* (Wild.) and and (*Alectra Vogelii* (Benth)	Very Important					
	Count	24.0	30.0	36.0	46.0	59.6
	Expected	19.9	23.9	48.5	43.7	
	Less important					
	Count	1.0	0.0	13.0	3.0	18.7
	Expected	2.5	3.0	6.1	5.5	
	Not Important					
	Count	0.0	0.0	12.0	6.0	21.6
	Expected	2.6	3.2	6.4	5.8	
	Df					6.0
	P-Value					29.338
	X^2					0.000
	Number of Valid	25	30	61	55	

In the present study farmers also stressed increased occurrence of the parasitic weeds *Alectra vogelii* (Benth) and *Striga gesnerioides* (Wild.) (Table 2.3). Alectra has been documented by Kabambe et al. (2013).

Table 2.4 Effects of aphids on cowpea production across four Regions in northern Namibia.

		Importance						
Regions	**Class**	**Very important**	**Less important**	**Not important**	**df**	***X²***	**P-value**	**Number of valid cases**
Kavango East	Count	23.0	2.0	0.0	6.0	13.667	0.034	25
	Expected	19.4	3.9	1.6				
Kavango West	Count	28.0	2.0	0.0				30
	Expected	23.3	4.7	1.9				
Omusati	Count	41.0	15.0	41.0				61
	Expected	47.4	9.6	47.4				
Oshikoto	Count	41.0	8.0	41.0				55
	Expected	42.8	8.7	42.8				
	Total%	77.8	15.8	6.4				

df = degrees of freedom and X^2 = Chi-Square.

Various authors including Noubissietchiagam et al. (2010) discussed the establishment and effects of Striga on cowpea production. Some authors suggested growing of resistant varieties or crop rotation practices as important strategies to reduce Striga seed bank in the soil (Kabambe *et al.,* 2002). These strategies could be explored to determine their efficiency in combating damages caused by Alectra. Serious economic loss in crop production due to the above parasitic weeds have been reported in Malawi and other Southern Africa countries (Kabambe *et al.,* 2002). Farmers indicated that employing various cultural practices such as crop rotation, regular weeding, and early planting could minimize weed infestations. However, most of these practices were less effective except for hoeing and plucking pests with 43.9% (Table 2.5).

Often farmers' allocate cropping land according to crops of choice or importance. The most valued crops receive larger areas of cultivation. Typically cowpea was intercropped with millet or sorghum in all the selected regions of this study. Intercropping cowpea with pearl millet and sorghum in Northern Namibia was also reported by (McDonagh and Hillyer, 2003). Soil fertility levels were reported to be very low across regions and all farmers did not apply any fertilizers on cowpea believing that it does not require fertilizer. There was a highly significant difference among respondents in allocation of farm lands to cowpea production across regions ($P<0.00$).

Table 2.5 Cultural practices used by farmers to control field pests and weeds in four Regions in northern Namibia.

		Cultural practice								
Regions	**Class**	**Crop rotation**	**Early planting**	**Timely weeding**	**Use of resistant varieties**	**Hoeing and plucking pests**	**df**	***X²***	**P-value**	**Number of valid cases**
Kavango East	Count	1.0	0.0	3.0	1.0	20.0	12.0	1.726	0.000	25
	Expected	4.5	3.4	3.7	2.5	11.0				
Kavango West	Count	0.0	0.0	0.0	0.0	30.0				30
	Expected	5.4	4.0	4.4	3.0	13.2				
Omusati	Count	25.0	2.0	0.0	16.0	18.0				61
	Expected	11.1	8.2	8.9	6.1	26.8				
Oshikoto	Count	5.0	21.0	22.0	0.0	7.0				55
	Expected	10.0	7.4	8.0	5.5	24.1				
	Total%	18.1	13.5	14.6	9.9	43.9				

df = degrees of freedom and X^2 = Chi-Square.

About 68.4% of farmers used a smaller proportion of their land (<1 ha) for cowpea production. Only 9.9% of farmers grew cowpeas on larger farm areas of >5 ha (Table 2.6).

Table 2.6 Land allocations for cowpea production across four selected regions of northern Namibia.

		Area allocated to cowpea production (ha)							
Regions	**Class**	**0-1**	**2-3**	**4-5**	**>5**	**df**	***X²***	**P-value**	**Number of valid cases**
Kavango East	Count	16.0	6.0	0.0	3.0	9.0	32.645	0.000	25
	Expected	17.1	5.3	0.1	2.5				
Kavango West	Count	17.0	13.0	0.0	0.0				30
	Expected	20.5	6.3	0.2	3.0				
Omusati	Count	35.0	12.0	1.0	13.0				61
	Expected	41.7	12.8	0.4	6.1				
Oshikoto	Count	49.0	5.0	0.0	1.0				55
	Expected	37.6	11.6	0.3	5.5				
	Total%	68.4	21.1	0.6	9.9				

df = degrees of freedom and X^2 = Chi-Square.

Results showed that 70.2% farmers grow local unimproved cowpea varieties and 29.8% used improved varieties either singly or in combination of two or three (Table 2.7), which are low yielders and prone to drought and pest. However the study identified low productivity of the existing cowpea varieties as another production constraint in northern Namibia. Farmers reported that Nakare, Shindimba and Bira cowpea varieties were poor performers with the yield response varied from 100-500 kg/ha (Table 2.8). This yield level is significantly low when compared to achievable yields of 1500 to 3000 kg/ha reported in Egypt and Malawi (Nabirye *et al.,* 2003). Namibia together with Botswana, Zambia, Zimbabwe, Mozambique and the

Republic of South Africa are considered to be the centre of diversity of cowpea due to the presence of most primitive and wild botanical varieties including rhomboidea, prottracta, tennis and stenophylla (Ng and Marachel, 1985). However, farmers described that they have lost most of known cowpea landraces due to environmental calamities notably of recurrent droughts in the region. Loss of cowpea varieties and genetic variability is also reported in West Africa, (Gbaguidi *et al.,* 2013). Consequently, farmers used poor genetic materials which are prone to many biotic and abiotic factors (Gbaguidi *et al.,* 2013).

A long pod is an important trait for farmers when selecting cowpea variety. Farmers indicated that longer pods often set several seeds, an important determinant of grain yield. Also longer pods were preferred by farmers for their potentially tender cowpea pods when cooked and consumed as a fresh vegetable. Cowpea growers estimated the number of seeds they counted per pod in their preferred varieties grown across regions (Table 2.9). There was a highly significance difference ($P<0.00$) between farmers' who counted less or more than 10 seeds per pod when using local unimproved cowpea varieties across the four regions. 68.4% of farmers indicated that the local cowpea varieties produce pods containing less than ten seeds per pod while 31.6% indicated counting >10 seeds.

Farmers-preferred traits of cowpea in the study areas were a straight pod shape, a long pod size, white seed colour, and a high above ground biomass. There was a highly significance difference in pod shape preference by farmers across Regions ($P<0.00$). Accordingly, 61.4% of farmers preferred a straight shape cowpea pods, 33% indicated the insignificance of pod-shape as their selection criterion while 5.3% expressed that they can grow both straight and coiled shaped cowpea pods (Table 2.10).

Furthermore, cowpea displays a mosaic of seed colour however there was a highly significant difference ($P<0.000$) observed between farmers across regions for their grain colour requirements. The most preferred seed colour of cowpea was white at 22.2% followed by brown and chocolate each with 0.6%. The remaining percentage was allocated to combination of various colours (Table 2.11). It suggested that a white coloured grain was most preferred due to its popularity in use as relish and also for its rough coat which is less preferred by storage pests. Bruchids prefer smoothed coat grains for oviposition (Baidoo and Mochiah, 2014).

Table 2.7 The relative proportion of farmers who grow local unimproved landraces and improved cowpea varieties such as Nakare, Shindimba or Bira or their combinations across four regions of northern Namibia.

		Varieties grown											
Regions	**Class**	**Landraces**	**Nakare**	**Shindimba**	**Bira**	**Nakare + Shindimba**	**Nakare + Bira**	**Shindimba + Bira**	**Nakare+Bira +Shindimba**	df	X^2	P-value	Number of valid cases
Kavango East	Count	14.0	1.0	4.0	1.0	1.0	0.0	1.0	3.0	21.0	62.691	0.00	25
	Expected	17.5	0.3	2.9	0.7	0.3	0.9	0.6	1.8				
Kavango West	Count	17.0	0.0	10.0	0.0	0.0	0.0	3.0	0.0				30
	Expected	21.1	0.4	3.5	0.9	0.4	1.1	0.7	2.1				
Omusati	Count	41.0	1.0	1.0	4.0	0.0	6.0	0.0	8.0				61
	Expected	42.8	0.7	7.1	1.8	0.7	2.1	1.4	4.3				
Oshikoto	Count	48.0	0.0	5.0	0.0	1.0	0.0	0.0	1.0				55
	Expected	38.6	0.6	6.4	1.6	0.6	1.9	1.3	3.9				
	Total%	70.2	1.2	11.7	2.9	1.2	3.5	2.3	7.0				

df = degrees of freedom and X^2 = Chi-Square.

Table 2.8 Average grain yield (kg/ha) of local cowpea reported by households during the main growing season across four regions of northern Namibia.

Regions	Class	Grain yield (Kg/ha) ≤100	100-599	600-1499	1500-1999	2000-2999	3000-4000	df	X^2	P-value	Number of valid cases
Kavango East	Count	7.0	11.0	7.0	0.0	0.0	0.0				25
	Expected	5.8	15.6	3.1	0.1	0.1	0.1				
Kavango West	Count	7.0	21.0	2.0	0.0	0.0	0.0				30
	Expected	7.0	18.8	3.7	0.2	0.2	0.2				
Omusati	Count	11.0	41.0	7.0	0.0	1.0	1.0	15.0	15.191	0.438	61
	Expected	14.3	38.2	7.5	0.4	0.4	0.4				
Oshikoto	Count	15.0	34.0	5.0	1.0	0.0	0.0				55
	Expected	12.9	34.4	6.8	0.3	0.3	0.3				
	Total%	23.4	62.6	12.3	06	0.6	0.6				

df = degrees of freedom and X^2 = Chi-Square.

Table 2.9 Farmers' estimation of the number of seeds per pod when growing local unimproved cowpea varieties across four regions of northern Namibia.

		Number of seeds per pod					
Regions	**Class**	**≤ 10**	**>10**	**df**	***X²***	**P-value**	**Number of valid cases**
Kavango East	Count	12.0	13.0				25
	Expected	7.9	17.1				
Kavango West	Count	22.0	8.0				30
	Expected	9.5	20.5				
Omusati	Count	11.0	50.0	3.0	38.400	0.00	61
	Expected	19.3	41.7				
Oshikoto	Count	9.0	46.0				55
	Expected	17.4	37.6				
	Total%	31.6	68.4				

df = degrees of freedom and X^2 = Chi-Square.

Table 2.10 Preference of pod shape by farmers across the four regions used in the study.

		Pod shape preference						
Regions	**Class**	**No preference**	**Straight**	**Straight and coiled**	**df**	***X²***	**P-value**	**Number of valid cases**
Kavango East	Count	2.0	19.0	4.0				25
	Expected	8.3	15.4	1.3				
Kavango West	Count	1.0	28.0	1.0				30
	Expected	10.0	18.4	1.6				
Omusati	Count	17.0	41.0	3.0	6.0	53.921	0.000	61
	Expected	20.3	37.5	3.2				
Oshikoto	Count	37.0	17.0	1.0				55
	Expected	18.3	33.8	2.9				
	Total%	33.3	61.4	5.3				

df = degrees of freedom and X^2 = Chi-Square.

Table 2.11 Farmers' preferences of grain colours in cowpea across four regions in northern Namibia.

		Grain colour(s)													
Regions	**Class**	**White**	**Brown**	**Chocolate**	**Any**	**White+ Brown**	**White+ Red**	**White+ Brown+ Red+**	**White+ Brown+ Red+ Chocolate**	**White+ Brown+ Red+ Chocolate +Speckled**	**White+ Brown+ Chocolate**	**White+ Red +Chocolate**	**df**	***X²***	**P-value**	**Number of valid cases**
Kavango East	Count	13.0	1.0	0.0	0.0	1.0	2.0	0.0	0.0	1.0	3.0	4.0				25
	Expected	5.6	0.1	0.1	2.3	1.3	2.9	1.8	1.2	2.8	2.8	3.9				
Kavango West	Count	14.0	0.0	0.0	0.0	0.0	6.0	0.0	0.0	3.0	3.0	4.0				30
	Expected	6.7	0.2	0.2	2.8	1.6	3.5	2.1	1.4	3.3	3.3	4.7				
Omusati	Count	3.0	0.0	0.0	10.0	1.0	9.0	9.0	5.0	10.0	5.0	9.0	33.0	77.548	0.000	61
	Expected	13.6	0.4	0.4	5.7	3.2	7.1	4.3	2.9	6.8	6.8	9.6				
Oshikoto	Count	8.0	0.0	1.0	6.0	7.0	3.0	3.0	3.0	5.0	8.0	10.0				55
	Expected	12.2	0.3	0.3	5.1	2.9	6.4	3.9	2.6	6.1	6.1	8.7				
	Total%	22.2	0.6	0.6	9.4	5.3	11.7	7.0	4.7	11.1	11.1	15.8				

df = degrees of freedom and X^2 = Chi-Square.

2.4 Conclusions

It is concluded that the present study provided insights on production constraints, preferred traits and farming systems of cowpea in the Northern communal areas of Namibia. Breeding for high grain yields, resistance to field and storage pests and farmers-preferred cowpea varieties are the most important strategies to increase cowpea production and productivity by subsistence farmers in the northern regions of Namibia. Furthermore availability of improved seeds and production input are overriding considerations to boost cowpea productivity in the country.

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Chapter 3 Radio-sensitivity of selected cowpea (*Vigna unguiculata*) genotypes to varying gamma irradiation doses

Abstract

An appropriate dose of radiation should be established on target genotypes before large scale mutagenesis undertaken. The objective of this study was to determine an ideal dose of gamma radiation to induce genetic variation in selected cowpea (*Vigna unguiculata*) genotypes. Seeds of three Namibian released cowpea genotypes were gamma irradiated using seven dozes at the International Atomic Energy Agency, Austria. Experiments were laid out in the completely randomised design with three replications and important data collected. Data were subjected to analysis to identify optimal lethal dose aiming LD_{50}. Results revealed that genotype Nakare tolerated the radiation dose of 200 Gy providing germination of 43.33%. Seeds of genotypes Nakare and Shindimba failed to germinate above 400 Gy. However, genotype Bira showed germination of 46.67% at 600 Gy, the highest dose used in the study. The optimum doses at LD50 for genotypes Nakare and Shindimba are at 150 and 200 Gy, respectively while genotype Bira tolerated increased dose of 600 Gy. Using linear regression model, the LD_{50} for genotypes Nakare, Shindimba and Bira calculated at 165.24, 198.69 and 689 Gy, respectively. The findings may assist as reference doses for large-scale gamma irradiation of cowpea genotypes to induce genetic variation.

Key words: cowpea, gamma radiation, LD_{50}, radio sensitivity, *Vigna unguiculata*

3.1 Introduction

Cowpea (*Vigna unguiculata* L. Walp., 2n=2x=22) is one of the important food legumes and a useful component of the traditional cropping systems in the semiarid tropics (Ayisi *et al.,* 2000; Singh *et al.,* 2002). Cowpea adapts to harsh environments including extreme temperatures, drought and poor soil fertility. In poor environments it yields comparatively better than other food legumes (Shimelis and Shringani, 2010). The crop originated and domesticated in Southern Africa, which was later spread to east and West Africa and Asia (International Institute for Tropical Agriculture [IITA], 2004). Southern Africa including Namibia, Botswana, Zambia, Zimbabwe, Mozambique and the Republic of South Africa is reportedly considered the center of diversity of *V. unguiculata* where the primitive and wild relatives are found (Ng and Marachel, 1985).

It is reported that at least 95% of farmers in northern Namibia grow cowpea, pearl millet and sorghum. In the country, cowpea ranks second after pearl millet making a crop of importance in the agricultural system. However, cowpea productivity is generally low (250 to 350 kg/ha) since farmers grow unimproved landraces as a result of unavailability of improved and locally adapted cultivars. Further, poor cultural practices, insect pest infestation and photoperiod sensitivity contribute to low productivity. The crop is susceptible to a number of fungal, bacterial, and viral diseases and such stress factors are considered to be the major production constraints of cowpea in Namibia (Thottappilly and Rossel, 1992).

In Namibia, since the early nineties, several research activities have been conducted involving cowpea adaptation trials by the Ministry of Agriculture, Water and Forestry in collaboration with the IITA. Consequently, three introduced varieties were released during 1993. However these varieties are less-preferred by farmers due to their proneness to damages caused by inspect pests such as aphids, thrips and storage pests particularly weevils. The yield level in the country is below the achievable yield of 1500-3000 kg/ha such as reported in Egypt and Malawi (Adeola *et al.,* 2011). Therefore, there is utmost need of cowpea germplasm development and enhancement towards high yield, insect and pest resistance, and drought tolerance in the country.

Despite the rich germplasm collections available by various national breeding programs and the IITA, the genetic base for most self-pollinating crops including cowpea is narrow for economic traits such as grain yield, yield components, drought and insect pest tolerance (Mudibu *et al.,* 2012). Mutation breeding is helpful in pre-breeding or genetic enhancement aimed to develop suitable germplasm. Artificial mutagenesis may bring about fast and direct

results to select useful traits unlike the conventional methods in which up to ten years of selections after extensive crosses are required in genetic advancement (Novak and Brunner, 1992). Mutations are the ultimate source of genetic variation, and provide unique germplasm, the raw material for plant breeders (van Harten, 1998). Mutation breeding has been used for generating genetic variation and breeding new varieties during the past decades (van Harten, 1998; Ahloowalia *et al.,* 2004; Tambe and Apparao, 2009). Recently the technique is being applied to generate mutants with altered agronomic traits for genetic studies and to predict the gene function through identification of an allelic series by Targeting Induced Local Lesions IN Genomes (TILLING) (Till et al., 2003; Xin *et al.,* 2008).

Physical mutagenic agents such as radiation and chemical mutagens e.g. Ethyl methane sulfonate (EMS) can be used to induce mutations at a higher frequency to generate genetic variation from which desired mutants may be selected. Similar work was conducted on neglected but important crop species such as in Amaranth, Bambara groundnut and sorghum (Girija and Dhanavel, 2009).

Gamma irradiation is one of the main physical mutagens used to induce genetic variation. An appropriate dose of radiation should be established on target genotypes before large scale mutagenesis undertaken (Tshilenge-Lukanda *et al.,* 2012). Radio sensitivity or determination of the optimum dose of radiation is a term describing a relative measure of the quantity of recognizable effects of a radiation exposure on the irradiated material (Owoseni *et al.,* 2007). Optimizing the dose of radiation is the first step in induced mutation breeding where it's predictable value guide the researcher in the choice of the ideal dose depending on the plant materials and desired outcome. According to Mba *et al*. (2010) the dose of mutagen that is regarded as the optimal is one that achieves the optimum mutation frequency with least possible unintended damage. Tshilenge-Lukanda *et al.* (2012) described that the optimum mutation doses can be determined by recording the percentage seed germination, epicotyl and hypocotyl lengths, among others. In seed propagated crops such as cowpea, Mba et al. (2010) suggested preliminary ranges of gamma irradiation doses of 0 to 600 Gy that should be tested to determine the optimal treatment condition on test genotypes. However these studies did not report an optimal dose of recommendation for cowpea due to differences in genotypic response to the treatment. In groundnut, Tshilenge-Lukanda et al. (2012) tested varied radiation doses of 0, 100, 200, 400 and 600 Gy to determine the optimum dose for mutagenesis.

Research and development collaboration was initiated on mutation breeding in 2009 between the Namibian government and the International Atomic Energy Agency (IAEA). This created a platform to further develop pre-breeding and breeding of high yielding and drought resistant genotypes of cowpea and cereals such as pearl millet [*Pennisetum glaucum* (L.) R. Br.; 2n=2x=14] and sorghum (*Sorghum bicolor* L. Moench; 2n=2x=20). The project focused on improving selected crops using induced mutation breeding techniques especially gamma irradiation. Gamma irradiation was recommended by the Namibian Radiation Regulatory Authority as a better option without any impact on the environment. Once the seed is irradiated under a controlled environment mutants can be assayed without radiation contamination. Therefore, the objective of this study was to determine the ideal dose of gamma radiation to induce genetic variation in selected cowpea (*V. unguiculata*) genotypes.

3.2 Materials and methods

3.2.1 Plant material and study site

The study used seeds of three Namibian released cowpea genotypes obtained from a selection originated from the IITA, Nakare (IT81D-985), Shindimba (IT89KD-245-1) and Bira (IT87D-453-2). The genotypes were different in seed colour as well as in hilum pattern (Fig 3.1). Dry, healthy and quiescent seeds were prepared for irradiation. Preliminary germination and viability tests were conducted and provided 100% germination. The study was conducted at the International Atomic Energy Agency (IAEA), Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria, through a dedicated fellowship grant to the first author under the Technical Cooperation Project (TCP) NAM5009/10 between the IAEA and the Namibian Government.

Figure 3.1 Unique seed shape and colour of three Namibian released cowpea genotypes which were irradiated. From left Bira, Shindimba and Nakare.

3.2.2 Gamma irradiation

Thirty seeds per genotype were gamma irradiated in three replications using the gamma irradiation facility at the IAEA. The study used seven irradiation doses (0, 100, 200, 300, 400, 500 and 600 Gy) making seven different envelopes per genotype replicated three times. The 0 Gy dose served as a comparative control. The seeds were packed in separate seed envelopes and placed in desiccators for three days to attain the desired moisture level of 8%. Irradiation was applied using a CO_{60} source Gammacell Model No. 220. The various doses were used to establish the optimum irradiation level that can achieve optimum mutation frequency with least possible and unintended damage (Mba et al. 2010).

3.2.3 Growing plants, experimental design, data collection and analysis

The radio sensitivity (the biological effects of the mutagen treatments on plants) was studied following the methods described by Mba et al. (2010) and Tshilenge-Lukanda et al. (2012). Irradiated seeds were planted in seedling trays with a medium that consisted peat, sand and vermiculate at a ratio of 2:1:1, respectively. Trials were established under environmentally controlled greenhouse with temperatures of 22-35 C° and light regime kept at 12 hours photoperiod. The experiment was set up in the completely randomised design with three replications. Seedlings were watered twice per week to ensure adequate soil moisture. Seven days after planting germination was recorded and expressed in percentage. Lengths of epicotyl and hypocotyl were measured 14 days after planting. These variables are regarded as suitable indicators in estimating the damage caused by mutagenic treatments. The epicotyl height was measured above the soil surface to the tip of the primary leaf using a ruler and expressed in cm. Data were subjected to the standard analysis of variance procedure using Genstat version 11 (Payne *et al.,* 2008) statistical package to compare genotypes and identify the optimal lethal dose aiming LD_{50}. The LD_{50} for each genotype was estimated through the simple linear regression model by fitting the straight line equation $y= mx+c$; where y is the response variable (percent germination), x is the independent variable (irradiation dose), while m and c represent the slope and constant, respectively.

3.3 Results

Table 3.1 summarizes the analysis of variance on percent germination, epicotyl and hypocotyl lengths between cowpea genotypes, radiation dose and their interaction. A significant (P<0.01) interaction occurred between genotypes and irradiation doses suggesting differential responses of cowpea varieties for the tested irradiation doses. The mean and standard deviation of percent germination, epicotyl and hypocotyl lengths are presented in Table 3.2. Germination persentage decreased drastically in all the three varieties with increased Gy doses (Figure 3.2). Germination was not observed for both Nakare and Shindimba above 300 Gy and 400 Gy, respectively. Genotype Bira could withstand the radiation doses of up to 600 Gy and displayed 47% germination at this dose (Figure 3.2).

Table 3.1 Analysis of variance on percent germination, epicotyl and hypocotyl lengths among three cowpea genotypes tested using seven irradiation doses in three replications.

Source of variation	DF	Germination %		Epicotyl length		Hypocotyl length	
		Mean Square	F value	Mean Square	F value	Mean Square	F value
Replication	2	350.00	3.47 ns	0.18	0.64 ns	1.69	1.27ns
Genotype	2	14551.03	144.31**	8.63	30.86**	21.15	15.78**
Dose	6	6746.39	66.91**	6.76	24.17**	40.47	30.177**
Genotype*Dose	12	1038.95	10.30**	1.29	4.62**	3.77	2.81**
Error	40	100.83		0.28		1.34	
Total	62						

df = degrees of freedom; ** denote significant differences at 1% probability level ns=not significant.

The germination response of Nakare, Shindimba and Bira against irradiation dosses are given by the linear equations: y=-0.17x + 78.09, y=-0.16x + 81.79 and y=0.08x + 105.12, respectively (Figure 3.2). Aiming germination response, y, at 50 the LD_{50} values of genotypes Nakare, Shindimba and Bira were calculated at 165.24, 198.69 and 689 Gy, respectively (Figure 3.2).

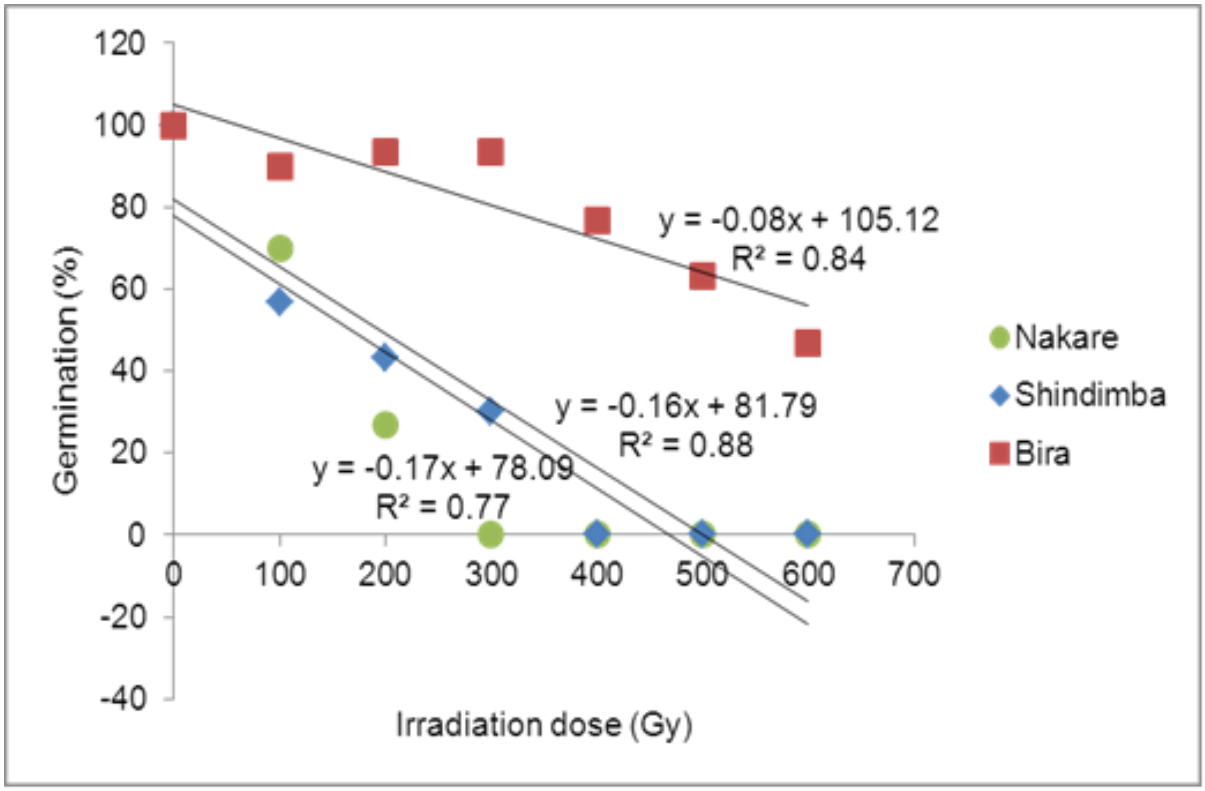

Figure 3.2 Germination % and fitted straight lines to estimate the LD50 in three cowpea genotypes when subjected to seven gamma radiation doses.

The higher the LD_{50} value, the stronger is the resistance shown by the test variety to irradiation and therefore relatively high dose is needed to induce mutagenesis and isolate mutants from the 50% surviving plants. The overall mean summarised in Table 3.1 suggests that radiation dose of > 300 Gy rendered relatively low germination. The variation in germination was explained by 94% (R^2 = 93.90) due to genotypic differences and radiation doses (Table 3.1). The coefficient of variation on percent germination was estimated at 21.2% which is relatively low compared to CVs of lengths of epicotyl and hypocotyl. In genotypes Nakare and Shindimba both epicotyl and hypocotyl lengths significantly reduced when applying gamma radiation above 200 Gy when compared to the control (Figure 3.2 and Figure 3.4). At the 0 Gy level Nakare showed the highest epicotyl and hypocotyl lengths at 3.11 and 6.71 cm, receptively. Data shown that the lengths of epicotyl and hypocotyl were significantly short in Shindimba and Bira when compared to Nakare at 0 Gy. It appears that in Bira the radiation dose of 100 Gy rendered relatively increased epicotyl length at 2.94 cm and hypocotyl length of 6.19 cm in comparison with the control which recorded 2.86 and 5.69 cm, respectively.

Table 3.2 Mean and standard deviation on percent germination, epicotyl and hypocoyl lengths among three cowpea genotypes tested using seven irradiation doses.

Genotype	Irradiation Dose (Gy)	Parameters		
		Germination (%)	Epicotyl length (cm)	Hypocotyl Length (cm)
Nakare	0	100.00±0.00	3.11±0.76	6.71±2.50
	100	70.00±20.00	3.06±0.51	6.37±2.09
	200	26.67±23.09	1.35±1.31	1.90±1.77
	300	0.00±0.00	0.00±0.00	0.00±0.00
	400	0.00±0.00	0.00±0.00	0.00±0.00
	500	0.00±0.00	0.00±0.00	0.00±0.00
	600	0.00±0.00	0.00±0.00	0.00±0.00
Shindimba	0	100.00±0.00	2.79±0.22	5.19±0.62
	100	56.67±15.26	2.30±0.16	4.66±0.80
	200	43.33±15.26	2.21±0.47	3.96±0.88
	300	30.00±0.00	1.26±1.01	0.79±1.06
	400	0.00±0.00	0.00±0.00	0.00±0.00
	500	0.00±0.00	0.00±0.00	0.00±0.00
	600	0.00±0.00	0.00±0.00	0.00±0.00
Bira	0	100.00±0.00	2.86±0.06	5.69±1.46
	100	90.00±10.00	2.94±0.49	6.19±1.76
	200	93.33±11.56	2.75±0.28	4.98±0.37
	300	93.33±11.56	2.75±0.28	3.54±0.28
	400	76.67±15.28	2.22±0.12	3.28±1.69
	500	63.33±15.28	1.88±0.87	3.36±1.86
	600	46.67±11.55	1.82±0.59	0.67±0.29
Mean	0	100.00±0.00	2.46±0.63	5.86±0.62
	100	72.22±19.86	2.92±0.52	5.74±1.64
	200	54.44±33.58	2.77±0.51	3.62±1.69
	300	41.11±41.67	2.10±0.94	1.44±1.7
	400	25.56±39.09	1.33±1.30	1.09±1.84
	500	21.11±32.58	0.74±1.11	1.12±1.92
	600	15.56±24.04	0.63±1.04	0.22±0.36
Grand mean		47.14	1.58	2.73
R^2 (%)		93.90	83.6	81.2
CV (%)		21.30	33.49	42.4

The straight line equations showing the trends of the epicotyl and hypocotyl lengths against the seven gamma irradiation doses of the three genotypes are shown in Figure 3.3 and Figure 3.4. As expected, the epicotyl and hypocotyl lengths showed decreasing trend as the gamma irradiation doses increased. The coefficient of determination (R^2) estimated in the straight lines were considerably high ranging from 76 to 93% suggesting notable association between the reduction of epicotyl and hypocotyl lengths due to increased radiation doses (Figure 3.3 and Figure 3.4). The overall mean summarised in Table 3.1 suggests that radiation dose of $\geq$ 300 Gy rendered relatively low germination. The variation in germination was explained by 94% (R^2=93.90) due to genotypic differences and radiation doses (Table 3.2). The coefficient of

variation on percent germination was estimated at 21.2% which is relatively low compared to CVs of lengths of epicotyl and hypocotyl. In genotypes Nakare and Shindimba both epicotyl and hypocotyl lengths significantly reduced when applying gamma radiation above 200 Gy when compared to the control (Figure 3.3 and Figure 3.4). At the 0 Gy level Nakare showed the highest epicotyl and hypocotyl lengths at 3.11 and 6.71 cm, receptively.

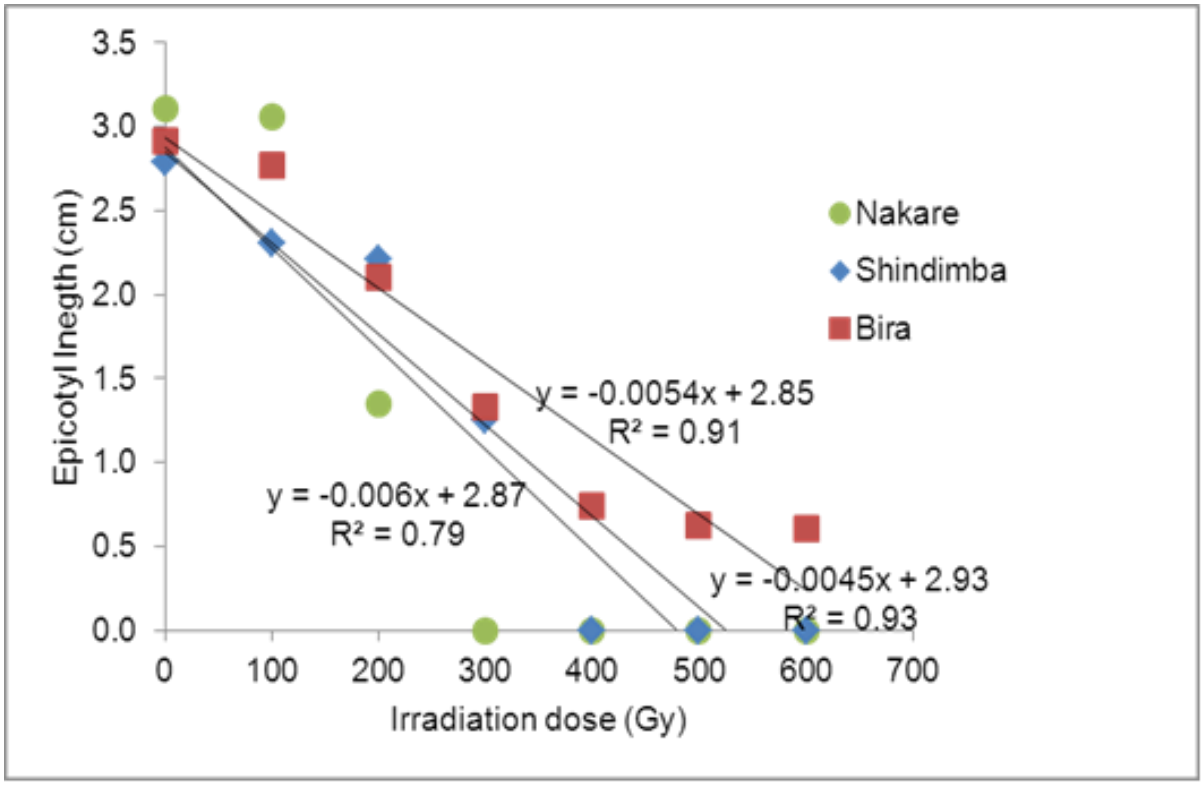

Figure 3.3 Epicotyl length (cm) and fitted straight lines of three cowpea genotypes when tested by seven gamma radiation doses.

Data shown that the lengths of epicotyl and hypocotyl were significantly short in Shindimba and Bira when compared to Nakare at 0 Gy. It appears that in Bira the radiation dose of 100 Gy rendered relatively increased epicotyl length at 2.94 cm and hypocotyl length of 6.19 cm in comparison with the control which recorded 2.86 and 5.69 cm, respectively. The straight line equations showing the trends of the epicotyl and hypocotyl lengths against the seven gamma irradiation doses of the three genotypes are shown in Figure 3.3 and Figure 3.4. As expected, the epicotyl and hypocotyl lengths showed decreasing trend as the gamma irradiation doses increased. The coefficient of determination (R^2) estimated in the straight lines were considerably high ranging from 76 to 93% suggesting notable association between the

reduction of epicotyl and hypocotyl lengths due to increased radiation doses (Figure 3.3 and Figure 3.4).

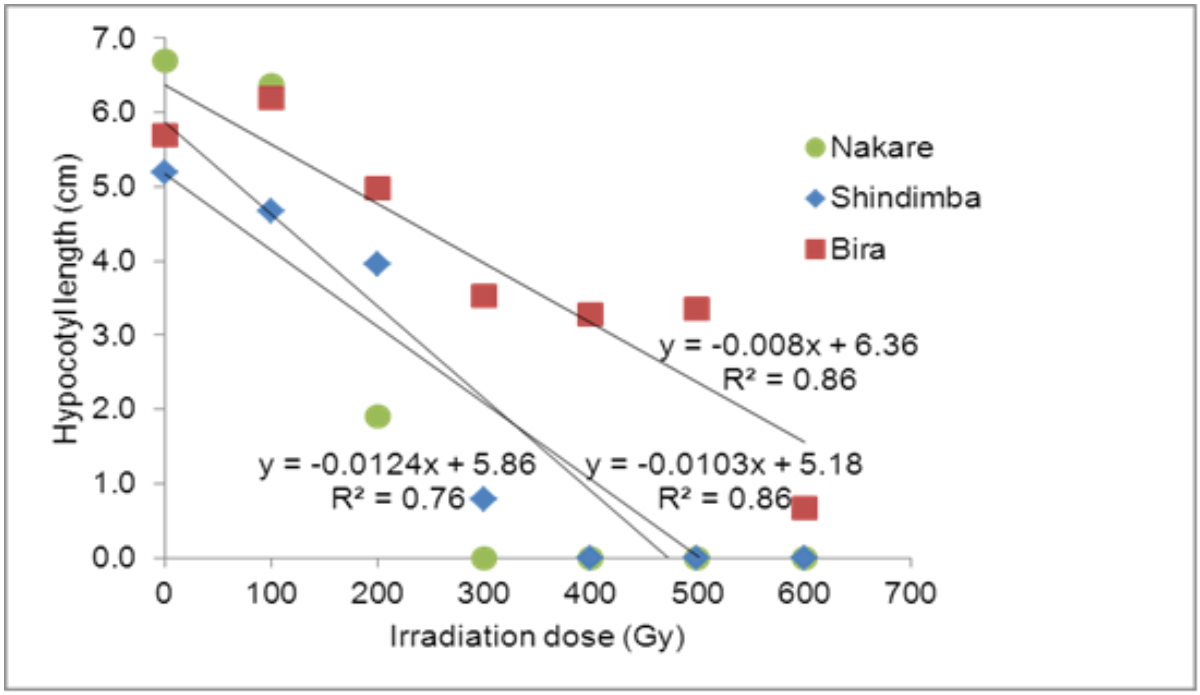

Figure 3.4 Hypocotyl length (cm) and fitted straight lines of three cowpea genotypes when tested by seven gamma radiation doses.

3.4 Discussion

The present study compared the responses of three Namibian grown cowpea genotypes using seven gamma radiation doses to establish the LD_{50} and to determine associated effects on early growth characters. Results revealed that seed germination, epicotyl and hypocotyl lengths decreased substantially with increased gamma radiation dose. The germination percent dropped from 100% (at 0 Gy, control treatment) to 0% when applying 300 and 400 Gy to genotypes Nakare and Shindimba, respectively (Fig 3.1). The decrease was proportional to the increased dose on the two genotypes. Bira tolerated the doses up to 600 Gy providing germination of 47%. This genotype was more resistant to doses of 200-300 Gy in comparison with Nakare and Shindimba. Mudibu et al. (2012) described that heavy doses of the radiation treatment is associated with toxicity and leads to undesirable changes including chromosomal aberrations, lethality, injury, and sterility. These anomalies are measured as the reduction in germination, survival, plant growth and fertility as well as increase in frequency of chromosomal aberrations and chlorophyll deficient chimeras.

This study found that the LD_{50} for genotypes Nakare, Shindimba and Bira were achieved at 168.54, 194.89 and 671.38 Gy, respectively. Nakare required low gamma irradiation dose to achieve the expected LD_{50} compared to Shindimba and Bira. Conversely, genotype Bira was the most tolerant to heavier dose of radiation and only reached to the desired LD_{50} at 671 Gy. Mba et al. (2010) and Owoseni et al. (2007) described that the irradiation level for generating mutants in crop improvement programmes should be carried out within a range of ± 5 units of the experimentally determined optimal dose. Further, the present findings showed that there has been a progressive reduction in the mean height of epicotyl and hypocotyl in both genotypes as the radiation dose increased. Manju and Gopimony (2009) pointed out that the reduction in the survival of plants is an index of post germination mortality resulting from cytological and physiological disturbances due to the effect of irradiation. Decreased plant height and growth was also observed in a similar experiment on rice varieties in Sierra Leon (Harding *et al.,* 2012). The authors indicated that the percentage survival of germinated seedlings decreased significantly within 8 to 14 days with increase in radiation doses up to 600 Gy in a laboratory condition. According to Sparrow and Evans (1961) the reduction in lengths of the epicotyl and hypocotyl could be attributed to the destruction of the plant growth hormone, auxin, and possibly influenced by the ionizing radiation causing genetic loss due to chromosomal aberration. Summarily, the current study confirmed that varied doses of gamma radiation applied on three different cowpea genotypes differentially affected germination, and

early growth and development significantly. Experimentally selected dose of the gamma radiation may help as a generic treatment dose to induce large scale mutagenesis in cowpeas.

3.5 Conclusions

Based on the differences between the irradiated and non-irradiated plant materials, different germination, epicotyls and hypocotyls length were observed. Through this study the doses leading to an average of 50% damage (LD_{50}) to seed germination in genotypes Nakare, Shindimba and Bira varieties were determined. These baseline doses are important for large scale mutagenesis and to increase genetic variation among crop varieties such as in cowpea. The result demonstrated that cowpea genotypes required specific irradiation dose to undertake large-scale mutagenesis. It should, however, be taken into consideration that induced mutations are random events and as such published irradiation conditions might not result the same mutation events for different genotypes.

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Chapter 4 Selection of novel cowpea genotypes derived through gamma irradiation

Abstract

Cowpea (*Vigna unguiculata* [L.] Walp.) yields are considerably low in Namibia due to lack of improved varieties and biotic and abiotic stresses, notably, recurrent drought. Thus, genetic improvement in cowpea aims to develop cultivars with improved grain yield and tolerance to abiotic and biotic stress factors. The objective of this study was to identify agronomically desirable cowpea genotypes after mutagenesis using gamma irradiation. Seeds of three cowpea varieties originally from the IITA and officially released in Namibia including Nakare (IT81D-985), Shindimba (IT89KD-245-1), and Bira (IT87D-453-2) were gamma irradiated with varied doses and desirable mutants were selected from M_2 through M_6 generations. The three were selected for this study because their agronomic traits were known and for the popularity when they were firstly introduced. Substantial genetic variability was detected among cowpea genotypes after mutagenesis across generations including in flowering ability, maturity, flower and seed colours and grain yields. Ten phenotypically and agronomically stable novel mutants were isolated at the M_6 each from the genetic background of the above three varieties. The selected promising mutants' lines are recommended for adaptability and stability tests across representative agro-ecologies for large-scale production or breeding in Namibia or similar environments. The novel cowpea genotypes selected through the study are valuable genetic resources for genetic enhancement and breeding.

Keywords: cowpea, gamma radiation, mutation breeding, mutants, legume improvement

4.1 Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is a leguminous species used as food, forage, and vegetable crop mainly in the tropics (Steele, 1972). The grains are an excellent source of food and feed; a vital nutrient for healthy growth both for humans and livestock. The leaves, green pods, and grains are consumed as a dietary source of protein (Ghaly and Alkoaik, 2010). The cowpea grain contains 23% protein and 57% carbohydrate, and the leaves contain 27–34% of proteins. The crop originated and domesticated in Southern Africa, which was later spread to east and West Africa and Asia (International Institute for Tropical Agriculture [IITA], 2004). In semi-arid West and Central Africa, it is consumed as a pulse where it supplements the daily diet (Bressani, 1985). Thus, cowpea production remains the most prominent food legume cultivated by farmers majorly in most sub-Saharan African countries. The main reasons being the natural ability of the crop to withstand moderate episodes of drought and its adaptation to grow in nutrient limited soils. Cowpea is also able to fix atmospheric nitrogen in marginal soils where farmers are unable to adequately fertilize their crops due to unaffordability or inaccessibility (Steele, 1972).

Accounts indicate that greater than 16,000 genotypes of cowpea are registered in trust for the World Bank by the International Institute of Tropical Agriculture, (IITA) Ibadan, Nigeria. Such a huge genotype bank is believed to provide a wide range of information on the agronomy and potential benefits of the crop. The southern African region is reportedly considered the centre of diversity of *V. unguiculata* which includes Namibia, Botswana, Zambia, Zimbabwe, Mozambique, and the Republic of South Africa (Ng and Marachel, 1985). In Namibia, cowpea is the third most important crop next to pearl millet. Nearly, 95% of the smallholder farmers in the northern part of the country grow cowpea for food security and/or livelihoods. However, cowpea yields of the available cultivars are considerably low (250–350 kg/ha) predominantly due to lack of improved varieties and biotic and abiotic stresses notably recurrent severe drought. Previously introduced varieties lost their popularity due to poor performance over the years. Farmers complained that the official released varieties were no longer performing to their expectation as they were prone to insect pest, drought and heat. Hence, genetic improvement in cowpea requires systematic breeding and development of genotypes associated with higher yielding capacity and drought resilience. Genetic variation is the basis for plant breeding programs.

Most conventional crop improvement programs rely on natural genetic variation present among germplasm pools (Ceccarelli and Grando, 2007). Mutations can be induced in various

ways, such as exposure of plant propagules, including seeds, tissues, and organs, to physical and chemical mutagens (Mba *et al.,* 2010). Induced mutagenesis has the potential to create genetic variation for genetic enhancement and breeding in a relatively shorter time unlike natural mutation or controlled crosses of especially unrelated parents (Singh *et al.,* 2006; Wani, 2006; Tulmann Neto *et al.,* 2011). Gnanamurthy *et al*. (2012) reported that induced mutations have been successfully used in breeding of seed propagated crops since 1940s. The Mutant Varieties Database (MVD) of FAO (Food and Agriculture Organisation of the United Nations) and the International Atomic Energy Agency (IAEA) maintained a list of 2,252 crop cultivars developed through artificial mutations (Nielen, 2004). These cultivars were released across 59 countries worldwide, mainly in the continental Asia (1,142 cultivars), Europe (847), and North America (160) (Maluszynski, 2001; Maluszynski *et al.,* 2009). Studies indicate that induced mutagenesis has successfully modified several plant traits such as plant height, maturity, seed shattering resistance, disease resistance, oil quality and quantity, malting quality, size and quality of starch granules of cowpea (Goyal and Khan, 2010; Singh *et al.,* 2013).

In South Africa, cowpea mutants were developed through selections from the M_2 to M_4 generations. These included the drought tolerant mutants such as 447, 217, and 346, and mutants such as 447, MA2, and 217 isolated for their high yielding ability under well-watered conditions (De Ronde and Spreeth, 2007). Furthermore, early maturing cowpea mutants with leaflets containing tendrils, broad leaves, and light green pods were developed through gamma irradiation in Nigeria (Adekola and Oluleye, 2007). The use of gamma irradiation at different doses has been reported to change the proximate and anti-nutritive compositions in pulses (Udensi *et al.,* 2012). Some varieties of groundnut were developed in Congo through gamma irradiation (Tshilenge-Lukanda *et al.,* 2012). Wani (2006) reported a significant increase in the mean values of the fertile branches per plant, pods per plant and seed yield per plant (SYP) in mutant varieties of mungbean (*Vigna radiata* [L.] Wilczek) derived through gamma irradiation. In light of this, a collaborative research was developed in 2009 between the Namibian Government and the IAEA under Technical Cooperation project on induced mutation breeding using Gamma irradiation. This created a platform for pre-breeding and breeding of high yielding, drought tolerant and insect pest resistant genotypes of cowpea. Gamma irradiation was recommended by the Namibian Radiation Regulatory Authority as an alternative option to create new crop genotypes in a short period of time without any negative impact to the environment. Therefore, the objective of this study was to identify desirable cowpea genotypes after gamma irradiation of three traditional cowpea varieties widely grown

in Namibia including Nakare (IT81D-985), Shindimba (IT89KD-245-1), and Bira (IT87D-453-2) through continuous selections from M_2 through M_6 generations.

4.2 Materials and methods

4.2.1 Plant material and gamma irradiation

Three cowpea genotypes originally from the IITA grown and officially released in Namibia, namely, Nakare (IT81D-985), Shindimba (IT89KD-245-1) and Bira (IT87D-453-2) (Chapter 3, Figure 3.1) were obtained from Likorerere Farmers Co-operatives at Kavango Region, Namibia. The seeds (M_0) were irradiated at the International Atomic Energy Agency (IAEA), Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria using a CO_{60} source Gammacell Model No. 220 to obtain the M_1. Various doses were used to establish the optimum irradiation level that can achieve optimum mutation frequency with least possible and unintended damage. The three varieties were gamma irradiated as follows: Bira [0, 75, 150, 300, 450, and 600 Gy], Nakare [0,100, 150, 200, 250, and 300 Gy] and Shindimba [0, 100, 150, 200, 300, and 400 Gy]. Preliminary tests showed that the three varieties differed in their optimal requirement of irradiation doses and was used as the bases for using different doses for each genotype (Horn and Shimelis, 2013). The 0 Gy dose served as a comparative control.

4.2.2 Study sites, experimental design, and field establishment

A series of selection experiments were conducted at three different sites; namely Mannheim 19º10'10.05 S, 17º45'52.45E, Bagani 18º05'44.89 S, 21º33'43.28 E and Omahenene 17º26'40.53 S, 14º47'21.37 E. Mannheim Research Station is located in Oshikoto region along the north central of Namibia and it is situated at an altitude of 1234 m above sea level (masl). Bagani Research Station is located at (1007 masl) north east in the Kavango East region, whereas Omahenene research station is situated in the Omusati Region in North-Western Namibia at altitude of 1109 masl. In general, climatic, biological conditions of the selection sites vary considerably. Physicochemical properties of the sites are provided in Table 4.1. The M_1 and M_2 generations were evaluated at Mannheim Research Station during the 2009/2010 and 2010/2011 seasons, respectively. The M_3 generations were established at Bagani research station during the 2011/2012 season. The M_4 and M_5 were established at Omahenene Research Station in 2012/2013 and 2013/2014 season, respectively. Plots were arranged in a randomized complete block design using two replications. Plants were established using intra-row spacing of 20 cm and inter-row spacing of 75 cm. Seedlings were thinned to one plant per hill after 2 weeks from planting. Weeds were controlled manually.

Planting of the M_1 seeds was done under normal growing conditions with supplemental irrigation during dry spell. Each row of the M_1 generation contained 26 individuals, making a total of 104 plants per irradiation dose. At harvest the M_2 seeds were bulked in separate bags according to irradiation doses (Figure 4.1). During the M_2 to M_5 generations' variable number of individual plants ranging from 50 to 100 per irradiation dose were assayed for qualitative and quantitative observations.

Table 4.1 Physicochemical properties of soils Mannheim, Bagani and Omahenene research sites.

Sample/parameter	**Research station (study site)**		
	Mannheim	**Bagani**	**Omahenene**
Soil pH	7.87	7.5	8.2
Total Nitrogen%	0.06	0.06	0.05
Organic carbon%	0.38	0.48	0.60
Phosphorus (ppm)	18	58.2	14
Potassiumme%	0.17	0.9	0.99
Calcium me %	1.6	1.3	1.38
Magnesium me%	4.74	1.7	4.80
Manganese me%	0.05	0.18	0.17
Copper (ppm)	0.6	0.6	0.5
Iron (ppm)	0.5	0.7	0.5
Zinc (ppm)	0.6	0.5	0.4
Sodium %	0.10	0.09	0.07
EC mS/cm	0.29	0.18	0.36

ppm= part per million, me = milliequivalent, EC=Electrical conductivity.

4.2.3 Selection procedure

The selection procedure was undertaken based on methods adapted from Maluszynski et al. (2009). The selection procedure used in the study is illustrated in Figure 4.1**.** The irradiated seeds (M_1) were planted in the field at Mannheim research station under standard cultural practices. All the pods, from the M_1 plants that survived were harvested and bulked according to their respective radiation doses and genotypes. Consequently, the harvested M_2 seeds were planted in the field at Mannheim as M_2 population during 2010/2011 season in the form of progeny rows for individual plant selection and to develop the M_3 seeds. The M_3 seed from selected M_2 plants were planted at Omahenene and Bagani Research Station during 2011/2012 for evaluation. The M_3 plants at both sites were evaluated in the field using morphological and agronomical attributes. Pods from selected M_3 plants were harvested. During 2012/2013, the M_4 seeds obtained from the selected M_3 population were planted at Omahenene Research Station as single-plant progenies and segregants were selected with desired traits. During 2013/2014 the M_5 seeds obtained from the selected M_4 population were planted at Omahenene Research Station as single-plant progenies and selection were made toward desired trait on single plant basis. Uniform, non-segregating mutant progenies, were

bulked at this stage to hasten the breeding cycle. During 2014/2015 the M_6 generation was evaluated at Omahenene, Bagani, and Mannheim using suitable lines selected for seed yield and related traits.

4.2.4 Data collection and analysis

Both quantitative and qualitative data were collected during evaluations from the M_2 to M_5 generations. The data collected included: days to 50% germination (DG), percent seed emergence (ES%), number of abnormal individuals or visual phenotype mutants (ABN), total number of surviving plants per plot (TNP), number of main branches (NMB) averaged over 10 randomly selected and tagged plants, days to 50% flowering (DTF), days to 50% pod setting (DPS), days to 50% maturity(DMT), number of pods per plant (NPP) averaged over five pods per selected plant, pod length (PL) expressed in cm and averaged over five pods per plant, pod weight per plant (PW) in gram, number of seeds per pod (NSP) averaged over five pods per plant, 100 seed weight (HSW) in gram and SYP in gram. The qualitative data collected included variation in flower color (FC) and seed color (SC) during the M_1 and M_2 generations. Additional qualitative data such as, pod shape (PS), pod color (PC), seed coat texture (SCT), and growth habit (GH) were collected from M_2 to M_5 generations. Data were analysed and descriptive statistics summarized using the SAS statistical program (SAS, 2002).

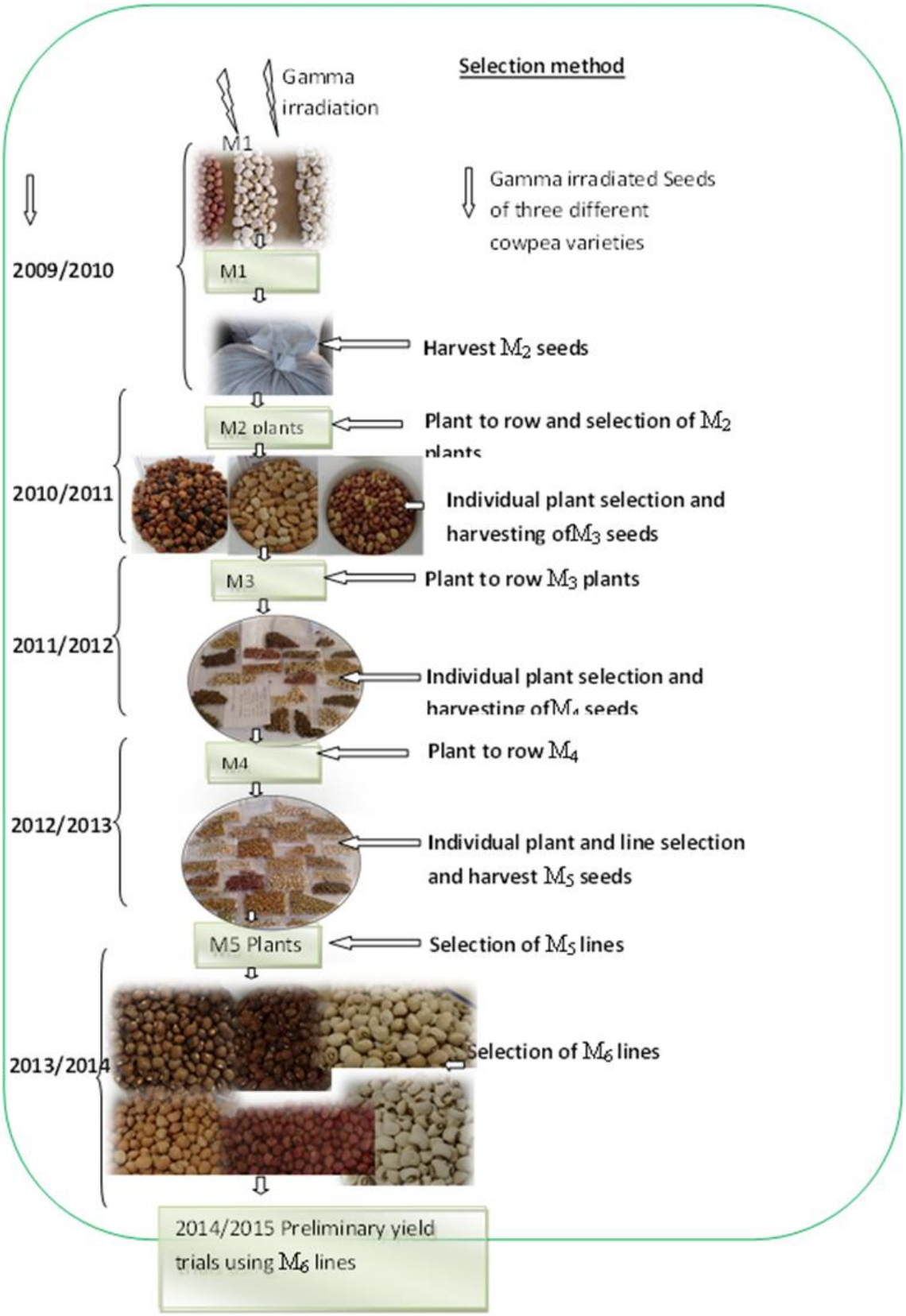

Figure 4.1 Illustration of the selection methods during 2009 to 2014.

4.3 Results

4.3.1 Phenotypic characterization of mutants qualitative and quantitative traits at M_1 and M_2

During the M_1 and M_2 generations the percentage field establishment (ES) ranged between 79 to 89%, respectively (Table 4.2). Nakare and Shindimba mutants had ES of 0% at irradiation does of 250, 300, and 400 Gy. Phenotypic abnormalities such as albinism, leaf deformity, single stem, seedless pods or short pod sizes were invariably observed at the following doses and genotypes: 450 and 600 Gy (Bira); 150 and 200 Gy (Nakare); and 100, 150, and 200 Gy (Shindimba) (Figure 4.2). Segregation of flower colour (white and purple) were observed at the M_2 with the following doses and genotypes: 300, 450, and 600 Gy (Bira), 100 and 200 Gy (Nakare), and 100, 150, and 200 Gy (Shindimba) (Figure 4.3). SC variations were observed during the M_2 Figure 4.4). White, brown, red, and cream seed colour were common in Bira mutants across all irradiation doses.

Table 4.2 Phenotypic characteristics of mutants observed during the first two season 2009/2010 and 2010/2011at Mannheim Research station.

Variety		M_1 (2009/2010)					M_2 (210/2011)				
	Dose (Gy)	ES%	ABN	FC	SC	SYP	ES%	ABN	FC	SC	SYP
Bira	0	89	0	2	3	2.9	99	0	2	3	98
	75	80	0	2	3	2.9	88	0	2	1,2,3,4	150
	150	87	0	2	3	3.1	89	0	2	1,2,3,4	162
	300	82	1	2	3	2.0	90	1,2,3	1,2	1,2,3,4	160
	450	81	1,2	2	3	1.6	93	1,2,3	1,2	1,2,3,4	158
	600	79	1,2,3,4,5	2	3	1.1	97	1,2,3,5	1,2	1,2,3,4	200
Nakare	0	86	0	1	1	1.6	89	0	1	1	90
	100	49	0	1	1	1.3	88	0	1,2	1,2,4,5,6	75
	150	46	1,2,3,4	1	1	0.3	86	1,2,3	1,2	1,2,4,5,6	81
	200	8	1,2,3,4	1	1	0.5	80	1,2,3,4,5	1,2	1,2,4,5,6	71
	250	0	N/A	N/A	N/A	0.0	N/A	N/A	N/A	N/A	N/A
	300	0	N/A	N/A	N/A	0.0	N/A	N/A	N/A	N/A	N/A
Shindimba	0	88	0	1	1	1.9	95	0	1	1	70
	100	35	1,2,3,4	1	1	1.4	86	1,2,3	1,2	1,2,4,5,6	66
	150	37	1,2,3,4	1	1	0.8	93	1,2,3	1,2	1,2,4,5,6	65
	200	18	1,2,3,4	1	1	0.1	90	1,2,3,4,5	1,2	1,2,4,5,6	60
	300	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	400	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

ES% = percent seed emergence, ABN= Abnormalities observed, where 0= normal, 1= Albino, 2= leafy type, 3 = upright single stem, 4=seedless pods and 5= short dwarf pods, FC= Flower colour, where 1= white and 2 purple. SC= Seed colour, where 1= White, 2= Brown, 3= Red, 4= Cream, 5= speckled, 6=chocolate, 7=light brown, 8= Black, 9= Mixed, 10 = dark brown. SYP= seed yield per plot,

In addition to these Nakare and Shindimba had speckled, chocolate, light brown, black, mixed and dark brown SC when subjected to irradiation doses of 100, 150, and 200 Gy (Figure 4.4 and Table 4.3). Bira mutants displayed relatively high seed yields varying from 98 to 200 g/plant at 0 and 600 Gy, respectively (Table 4.2).

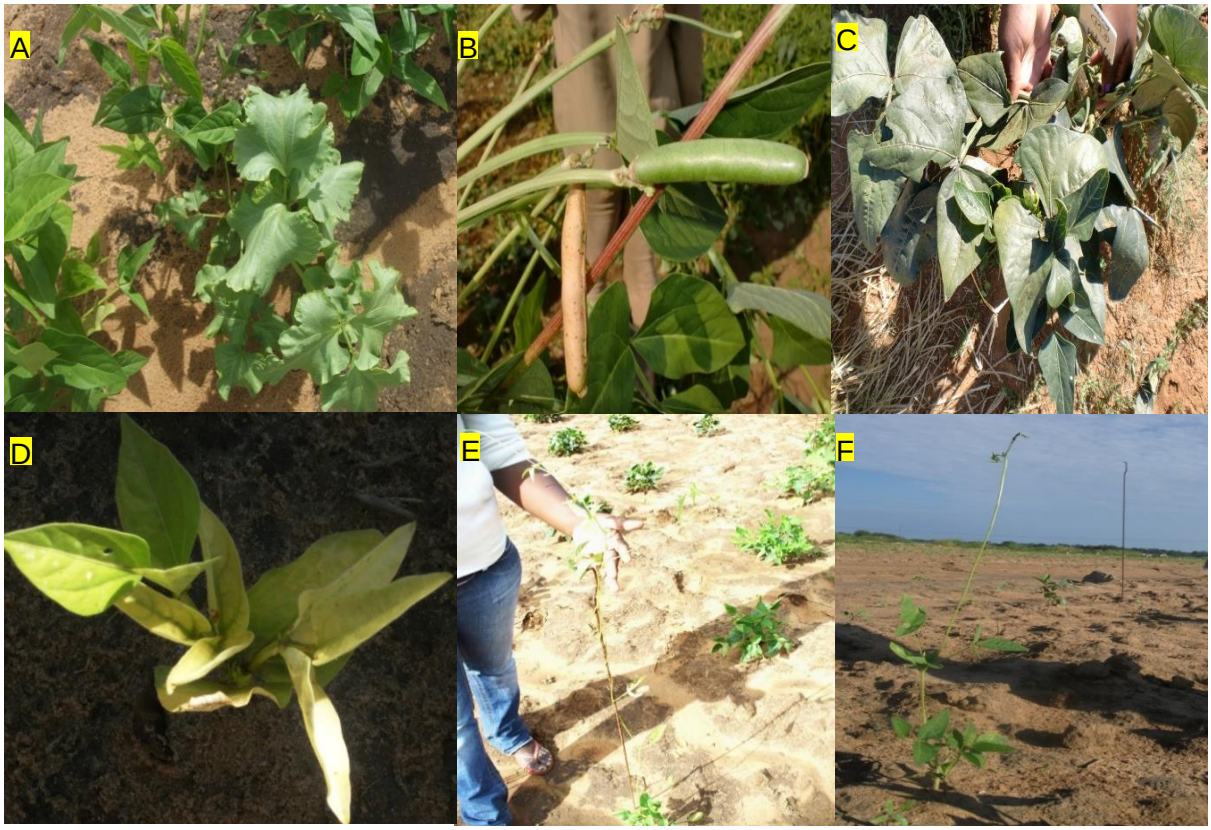

Figure 4.2 Some of the common abnormalities at M_3 observed at Bagani Research Station: A- spinach like leaves, B-short pods, C- broad dark leaves while D- chlorophyll mutant, -single stem E and F observed at Omahenene research Station.

Figure 4.3 Variation in flower colour A-white flower colour, B-purple flower and field plant stands of M_5 Nakare mutants observed at Omahenene Research Station.

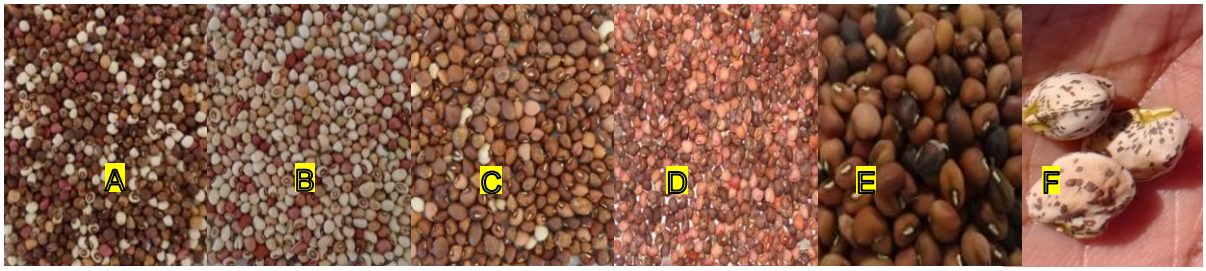

Figure 4.4 Different M_3 seed colors (A–F) observed among all mutants at all locations.

Figure 4.5 Variation among Shindimba mutant lines over the generation (A-coiled pods, B-Semi-coiled pods observed at Mannheim during the M_2 generation, C- white flower with semi-coiled pods and D- Purple flowers observed at Omahenene during the M_5 generation).

4.3.2 Qualitative traits evaluated during the M_3 to the M_5

Variable number of individual plants was available for selection during M_3 to M_5 generations, because of the strength of irradiation treatment and segregation. The following doses allowed successful selections of mutants during the M_3 to M_5: 300, 450, and 600 Gy (Bira), 100 and 150 Gy (Nakare), and 100 and 200 Gy (Shindimba). Surviving and phenotypically stable individuals were advanced at each selection generation at Omahenene and Bagani Research Stations. Qualitative traits had limited variation during M_3 to M_5 (Table 4.3). Bira mutants displayed purple FC irrespective of doses and test generations, while Nakare and Shindimba segregated for white and purple FC (Figure 4.3). Both Bira and Nakare mutants had straight PS similar to the controls. However, Shindimba segregants had straight and coiled pod types (Figure 4.5). Variable SCs including white, brown, red, cream, speckled, chocolate, light and dark brown, black and mixed were observed during the M_3 to M_5. Bira mutants had smooth

SCT, while Nakare and Shindimba had mainly rough and smooth seed texture. Bushy, erect and spreading GHs were detected during the M_3 to M_5 (Table 4.3).

Table 4.3 Qualitative traits observed among the mutant lines at the M_3, M_4, and M_5 at Omahenene and Bagani Research Stations.

Genotype	Dose (Gy)	FC	PS	PC	SC	SCT	GH	PI
Bira	0	2	1	1	3	1	3	1
	300	2	1	1	1,2,3,4	1	1,2,3	1
	450	2	1	1	1,2,3,4	1	1,2,3	1
	600	2	1	1	1,2,3,4,5,6,7,8	1	1,2,3,	1
Nakare	0	1	1	1	1	2	2,	1
	100	1,2	1	1	1,2,3,6,7,9,10	1,2	1,2,	1
	150	1,2	1	1	1,2,3,6,7,9,10	1,2	1,2,	1
Shindimba	0	1	2	1	1	1,2	2	1
	100	1,2	1,2	1	1,2,3,7,9,10	1,2	1,2,	1
	200	1,2	1,2	1	1,2,3, 7,9,10	1,2	1,2,	1

Flower colour (FC), where 1= white and 2 purple; Pod shape (PS), where 1= Straight and 2=coiled or curved; Pod colour (PC), where 1= Cream; Seed colour (SC), where 1= White, 2= Brown, 3= Red, 4= Cream, 5= speckled, 6=chocolate, 7=light brown, 8= black, 9= mixed, 10 = dark brown; Seed coat texture (SCT), where 1= smooth and 2=rough; Growing habit (GH), where 1=bushy, 2=Erect and 3= crawling; pest infestation (PI)where 0=none, 1= mild and 2=sever.

4.3.3 Quantitative traits observed from M_3 to M_5

Quantitative traits of agronomic importance were measured during the M_3 to M_5 (Table 4.4). The percent seed emergence (ES%) reduced significantly with increased irradiation dose. Maximum seed germination was achieved 3 days after planting irrespective of irradiation doses (Table 4.4 –Table 4.6). Shindimba mutants relatively flowered early (40 days) at the M_3 (Table 4.4). At the M_4 a relatively shorter days to flowering (44 days) was recorded at 300 Gy (Table 4.5). Contrastingly, the number of days to flowering was 37 days at the M_5 at using 600 Gy (Table 4.6). Nakare derived mutants flowered relatively earlier (10 days) at 100 Gy at the M_3 (Table 4.4). At the M_5 Nakare mutants recorded a minimum of 61 days to flowering at 0 and 150 Gy (Table 4.5). At the M_3, Shindimba mutants displayed a minimum of 15 and a maximum of 84 days to flowering at 200 and 100 Gy, respectively (Table 4.4). Nakare mutants recorded the lower days (25) for pod setting (DPS) at the M_5 when using 100 Gy. Comparatively, the higher number of DPS (98 days) was measured in Shindimba at 200 Gy. At the M_4 a minimum DPS of 48 days was recorded for Bira derivatives at 300 Gy. A maximum DPS of 86 days was recorded for Bira mutants at 400 Gy, Nakare at 100 and 150 Gy and Shindimba at 100 and 200 Gy (Table 4.5). At the M_5, Nakare mutants recorded the lower DPS (41 days) at 100 Gy, while Bira genotypes had the higher DPS of 88 days at 300 Gy, (Table 4.6). During the M_3, Nakare mutants matured 32 days after planting at 100 Gy. At the same

dose rate Shindimba displayed late maturity (98 days) at the M_3 (Table 4.4). During the M_4 Bira mutants matured earlier (54 days) at 450 Gy. Delayed maturity (115 days) were recorded for Nakare at 150 Gy and Shindimba at 100 and 200 Gy (Table 4.5).

Table 4.4 Quantitative characteristics of M_4 cowpea mutant lines irradiated at different gamma radiation doses (Gy) in relation to their parental.

Variety	Gy	TNP	ES%		DG	DTF	DPS	DMT	NPP	PL	PW	NSP	HSW	SYP
				Min	3	47	51	57	3	13	7	7.6	13	3
	0	**330**	**100**	**Max**	3	58	63	74	57	20.6	136	18	18	94
				Mean	3	50	55	64	28.6	15.1	64.9	14.5	15.1	40.9
	300			**Min**	3	40	49	52	4	10.4	3	9	9	1
Bira		**330**	**90.6**	**Max**	3	80	86	92	5439	20.0	4003	19	25	3500
				Mean	3	52	58	63	322.6	16.9	211.9	10.8	14.4	136.6
	450			**Min**	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		330	**60.6**	**Max**	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
				Mean	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
				Min	3	46	50	57	1	5	1	1.8	4	1
	600	**330**	**93**	**Max**	3	59	63	74	120	21.3	218	18.6	21	142
				Mean	3	50	54	63	16.4	14.9	36.6	12.2	14.9	22.0
				Min	3	11	25	32	11	14.2	11.5	5	16.2	1.3
	0	**330**	**45.5**	**Max**	3	35	34	43	85	19.6	220.6	12	30.5	138.8
				Mean	3	20	29	36	51.2	15.8	106.1	7.7	25.3	59.2
				Min	3	10	25	32	3	6.8	2.8	2	11.4	0.7
Nakare	**100**	**330**	**78.5**	**Max**	3	37	62	65	125	23.5	327.8	17.2	32.1	191.2
				Mean	3	19	29	37	32.3	16.7	65.2	9.0	23.5	32.9
				Min	3	43	45	46	3	5.6	3.0	1.8	6.1	N/A
	150	**330**	**60.3**	**Max**	3	78	95	96	172	22.7	360.8	18.4	109	N/A
				Mean	3	52	57	66	39	14.2	67.6	10.2	15.8	N/A
				Min	3	49	55	60	3	10	3.0	6.3	15.0	2.0
	0	**330**	**69.7**	**Max**	3	69	75	83	38	19	110.0	12.0	19.0	62.0
				Mean	3	57	62	70	23	16	44.7	9.5	17.5	22.2
				Min	3	49	54	60	3	10	3.0	5.8	14.0	1.0
Shindimba	**100**	**330**	**36**	**Max**	3	84	90	98	40	21	110.0	16.6	25.0	118.0
				Mean	3	59	63	71	21	16	35.4	9.5	17.7	17.7
				Min	3	15	47	N/A	2	1.3	1.1	3.0	8.1	N/A
	200	**330**	**69**	**Max**	3	76	98	N/A	109	21.9	220.2	15.4	48.7	N/A
				Mean	3	62	68	N/A	32	14.6	55.5	7.9	20.8	N/A

TNP = Total number of plants per plot, **ES%**= percentage establishment, DG = days to 50% germination, DTF = days to 50% flowering, DPS = Days to 50% pod setting, DMT = days to 50% maturity, NPP = number of pods per plant, PL = pod length 5 pods, PW =Pod weight, NSP = number of seeds per pod, HSW =100 seed weight, SYP = seed yield per plant and N/A=Data not available.

At the M_5 Bira measured early maturity (62 days) with the highest dose of 600 Gy. Interestingly, this genotype matured late (115 days) when subjected to irradiation dose of 300 Gy (Table

4.6). Nevertheless, Bira recorded lower NPP (1 pod/plant) at 600 Gy and higher (5 pods/plant) when irradiated at 300 Gy (Table 4.4). At the M_4, 1 pod/plant was recorded for Bira at 450 and 600 Gy and Shindimba at 200 Gy (Table 4.5). At the M_3 the longer pod size measured at 23.5 cm was recorded for Nakare at 100 Gy (Table 4.4). At the M_4, Shindimba mutants resulted from 200 Gy measured longer pod size of 31 cm (Table 4.5). Bira mutants induced with 300 Gy produced longer pod size (30 cm) (Table 4.6). Relatively heavier pod size (4003 g/plant) was recorded for Bira at 300 Gy (Table 4.4). At the M_4, Bira had pod size measured at 325 g/plant at 300 Gy. Notably this genotype had reduced pod weight (1 g/plant) at the highest irradiation dose (Table 4.5). The NSP varied significantly between irradiation doses and genotypes. At the M_3, the highest number of seeds of 18.6/pod was recorded for Bira at 600 Gy and Nakare 150 Gy (Table 4.4).

Table 4.5 Quantitative characteristics of M_4 cowpea mutant lines irradiated at different gamma radiation doses (Gy) in relation to their parental.

Variety	Gy	TNP	ES%		DG	DTF	DPS	DMT	NPP	PL	PW	NSP	HSW	SYP
				Min	3	45	49	59	8	14	13	5	10	6
	0	**330**	**69.7**	**Max**	3	48	53	72	88	20	231	20	16	187
				Mean	3	46	50	68	31	17.7	86.2	14.2	13	53.4
				Min	3	44	48	66	2	9	4.0	6	5	1
Bira	**300**	**330**	**55.0**	**Max**	3	51	55	74	97	21	325	18	79	287
				Mean	3	46	50	69	31	16.8	79.6	14.3	13	52.9
				Min	3	45	49	54	1	10	2	4	9	1
	450	**330**	**85.7**	**Max**	3	81	86	90	127	16	330	20	115	195
				Mean	3	49	54	60	26	15.8	50	17.0	15	30
				Min	3	46	50	57	1	6	1	2	4	1
	600	**330**	**85.0**	**Max**	3	59	63	74	124	22	224	19	21	160
				Mean	3	50	55	63	18.4	16.3	41.6	13	15.1	25.1
				Min	3	61	66	96	5	11	9	4	17	6
	0	**330**	**42.0**	**Max**	3	74	78	110	32	19.0	85	17	26	62
				Mean	3	70	75	104	14	15.1	29.8	9.8	22.8	22.8
				Min	3	61	66	86	2	10	4	5	5	2
Nakare	**100**	**330**	**56.0**	**Max**	3	78	86	113	70	21	227	14	59	199
				Mean	3	71	76	103	15	15.5	35.3	10.0	21.4	26.7
				Min	3	61	67	86	2	8	3	3	3	1
	150	**330**	**88.8**	**Max**	3	79	86	115	85	26	287	18	40	131
				Mean	3	71	76	103	21.9	16.5	48	11.0	17.3	33.5
				Min	3	42	68	72	7	7	7	3	12	3
	0	**330**	**93.9**	**Max**	3	75	78	85	44	29	123	13	30	91
				Mean	3	71	72	76	20.0	13	33.0	7.4	20.1	25.1
				Min	3	42	66	87	2	7	3	3	10	2
Shindimba	**100**	**330**	**82.4**	**Max**	3	80	86	115	63	23	130	19	30	91
				Mean	3	71	76	104	16.7	13.8	27.7	8.1	19.8	20.9
				Min	3	62	66	94	1	9	3	3	6	2
	200	**330**	**68.5**	**Max**	3	80	86	115	59	31	123	16	30	91
				Mean	3	72	76	104	15.5	13	26.5	8.1	18.4	19.3

TNP = Total number of plants per plot, ES%= percent seed emergence, DG = days to 50% germination, DTF = days to 50% flowering, DPS = Days to 50% pod setting, DMT = days to 50% maturity, NPP =

number of pods per plant, PL = pod length 5 pods, PW =Pod weight, NSP = number of seeds per pod, HSW =100 seed weight, SYP = seed yield per plant and N/A=Data not available.

Table 4.6 Quantitative characteristics of M_5 cowpea mutant lines irradiated at different gamma radiation dozes (Gy) in relation to their parental lines/control observed at Omahenene Research Station during 2013/2014 season.

Variety	GY	TNP	ES%		DG	DTF	DPS	DMT	NPP	PL	PW	NSP	HSW	SYP
Bira	0	330	97.0	Min	3	68	73	98	7	16	15	6	9	10
				Max	3	83	88	115	66	27	155	18	16	115
				Mean	3	74	78	102	40.7	21	88.9	14.3	12.9	61.9
	300	330	77.6	Min	3	64	69	98	3	13	6	5	4	3
				Max	3	83	88	115	150	30	325	20	29.3	213
				Mean	3	73	78	102	30.9	21	66.6	14.2	12.7	47.0
	450	330	85.8	Min	3	42	46	66	2	13	6	5	11	3
				Max	3	58	69	76	233	20	659	20	171	570.0
				Mean	3	47	51	70	31.6	17.7	81.5	14.9	16.2	60.0
	600	330	78.5	Min	3	37	42	62	1	9	1	3	4	2
				Max	3	56	61	81	78	27	276	18	19	157.0
				Mean	3	46	50.3	68.6	19.9	16.3	43.4	13.2	12.4	28.1
Nakare	0	330	42.0	Min	3	41	45	65	47	16	76	13	1	51
				Max	3	53	57	79	46	21	72	12	298	51
				Mean	3	47	50.8	69	45	16.8	70.8	12.3	62.8	50.5
	100	330	56.4	Min	3	37	41	58	1	9	1	1	1	1
				Max	3	57	60	80	144	23	375	18	81	298
				Mean	3	46	49.5	66	39	17.7	86.8	12	18.1	62.8
	150	330	59.7	Min	3	42	46	64	1	10	3	5	6	2
				Max	3	53	57	73	110	28	317	20	82	209
				Mean	3	46	50	68	29	21.0	70.6	12.5	18.4	45.6
Shindimba	0	330	93.9	Min	3	50	54	66	2	8	5	3	2	2
				Max	3	55	59	73	88	20	127	11	29	90
				Mean	3	52.3	56	69	33.8	13.6	64	8	22.0	42
	100	330	86.7	Min	3	42	46	60	1	8	1	1	2	1
				Max	3	67	70	89	122	25	392	18	29	208
				Mean	3	50.5	54.2	70	35.5	14.0	75	8	21.5	50
	200	330	83.6	Min	3	44	48	65	1	7	1	1	6	1
				Max	3	67	71	91	89	29	193	18	25	93
				Mean	3	52	56	73	20	16	39	9	16	25

TNP = Total number of plants per plot, ES%= percent seed emergence, DG = days to 50% germination, DTF = days to 50% flowering, DPS = Days to 50% pod setting, DMT = days to 50% maturity, NPP = number of pods per plant, PL = pod length 5 pods, PW =Pod weight, NSP = number of seeds per pod, HSW =100 seed weight, SYP = seed yield per plant and N/A=Data not available.

At the M_4 19 seeds/pod was achieved in the mutants of Bira at 600 Gy and Shindimba at 100 Gy. At the M_5, mutants of Bira derived from 300 and 450 Gy and Nakare 150 Gy recorded 20 seeds/pod, the highest in this trial (Table 4.6). Hundred seed weight (HSW) at M_3 was relatively heavier measured at 109 g for Nakare mutants derived from 150 Gy (Table 4.4). At the M_4 the higher HSW (115 g) was recorded for Bira at 450 Gy (Table 4.6). During the M_5 Bira displayed higher HSW of 171 g at 450 Gy (Table 6). High seed yield per plant is an economic trait for cowpea growers. At M_3, higher seed yield of 3500 g per plant was recorded for Bira mutants derived from the mutagenic treatment of 300 Gy (Table 4.4). During the M_4

generation Bira and Nakare mutants derived from 300 Gy and 100 Gy had a relatively higher seed yields of 287 and 199 g/plant, in that order (Table 4.5). At the M_5 generation Bira mutants yielded 570 g/plant, while Nakare had 298 g/plant when subjected to 450 Gy and 100 Gy, respectively (Table 4.6).

Figure 4.6 Common insect pests (A) Spiny brown bugs *Clavigralla sp.*, (B) Coreid bug *Anoplocnemis curvipes*, (C) Aphids *Aphis craccivora Koch* and Blister (D) Beetle *Mylabris phalerata* observed among the M_5 mutants at Bagani, and Omahenene Research Stations concurrently.

4.4 Discussion

The present study revealed the important roles of induced mutations in cowpea breeding. It was evident from this study that increased Gy doses above 150 Gy can be lethal for the cowpea breeding line such as Nakare, while a dose above 200 Gy is lethal for the breeding line Shindimba. Other authors have reported the negative effects of increased mutagenic doses affecting various crops' establishment and survival for breeding (Mba *et al.,* 2009). The present study showed the presence of clear phenotypic differences among the tested mutant lines when compared to their respective controls. Visual phenotypic differences including chlorophyll, leaf, and upright single stem, pod, and seed during the M_2 to M_5 generations. Chlorophyll mutants observed were plants with yellow and striped leaves, albinos or yellow to pale leaf and stem pigmentations. Virescence mutants showed broad pale green leaf breeding line such as Nakare, while a dose above 200 Gy is lethal for the breeding line Shindimba (Figure 4.2). Other authors have reported the negative effects of increased mutagenic doses affecting various crops' establishment and survival for breeding (Mba *et al.,* 2009).

According to Girija and Dhanavel (2009) and Maluszynski et al. (2009), the appearance of chlorophyll defects is a good indicator of genetic action of the mutagen. Singh et al. (2013) reported that increased Gy doses provided higher frequency of chlorophyll mutants in cowpea when compared to other mutagens such as EMS. Girija and Dhanavel (2009) outlined the effectiveness and efficiency of mutagens for selection of mutants with economic traits. The authors suggested that for effective phenotypic selection the mutation treatment should not yield unintended damages including chromosomal aberrations, physiological and toxic effects, which reduce cell survival and ultimately eliminate the mutation. Despite its negative effects on the early stages of crop growth, chlorophyll mutants are important in mutation breeding programs. Tulmann Neto et al. (2011) reported that the chlorophyll mutants were used in evaluation of the genetic effects and sensitivity of various mutagens on crops. These results are in agreement with Goyal and Khan (2010) whose studies indicated that the incidence of chlorophyll mutants were higher with increased Gy doses in earlier selection generations. In the present study, mutants at the M_2 were genetically diverse owing to phenotypic segregation.

The genetic diversity assessed in these mutants were tall/dwarf plant heights, early/late maturity, leaf shapes, branching habit, GH, PS, FC, SC and texture, seed weight and yield Table 4.4 - Table 4.6). Both the qualitative and quantitative parameters measured in the study were useful for selection of cowpea mutants. According to Maluszynski et al. (2009), induced genetic polymorphism among initial cells of the sporogenic layer influences the segregation

ratio in the M_2 generation. However, mutations of cells of somatic tissues are not transferred to the next generation.

Gnanamurthy *et al*. (2012) stipulated that easily detectable mutants' characteristics are phenotypically visible and morphologically distinct with qualitatively inherited genetic changes. These changes occur due to the effect of few major genes or oligogenes yielding macro mutations. In this study, some macro mutations observed were the changes in flower and SC. Micro mutations are the result of polygenes each with minor genetic effect showing quantitative inheritance. The effect and inheritance of minor genes is detected using quantitative genetic parameters and statistical methods (Singh *et al.,* 2006). In the current study, short plant height and one seed per pod mutants were recorded in all the breeding lines mostly at the M_3 generation. Single seeded pods were also reported by Girija and Dhanavel (2009).

In the present study, other main phenotypic changes observed were increased NMB especially in mutants with spreading GH. Mutants with bushy GH had reduced number of branches per plant. These characters are indicated to be associated with some physiological properties of the plant including leaf senescence and indeterminate GH (Hall, 2004; Martins *et al.,* 2014). It is reported that characteristics altered through mutation breeding can be combined through the conventional breeding to improve crop performance and drought adaptation (Ehlers and Hall, 1997).

The present study found that Nakare mutants had a maximum of 23 main branches per plant, while the comparative control had nine main branches (Table 4.3). According to previous studies (Singh *et al.,* 2003, 2013), the spreading and semi-spreading cowpea types yielded less grain and more fodder when planted in closer spaced rows. The present study found that mutation treatment did not significantly affect the number of days taken to germination, hence all the breeding lines germinated 3 days after planting (Table 4.3–Table 4.6). The mutation treatment had positive effect on the number of days taken to 50% flowering whereby some of the breeding lines flowered 11 days before the control. Bira mutants subjected to irradiation of 300 Gy flowered 80 days after planting (Table 4.3). Maluszynski et al. (2009) suggested that a high dose of a mutagen should yield delayed maturity. Dhanavel et al. (2008) reported that mutagenesis resulted into variation in plant development including the number of days taken to maturity. According to Singh *et al.* (2003), these variations are important to the farmers and the breeders allowing choices of planting time. The breeder will have a choice from a larger breeding stock for various breeding traits and purposes.

Significant observations made in the present study were increased PL and seed yield measured during the M_3 to M_5 in all the breeding lines. Goyal and Khan (2010) reported that mutations caused increased PL in some of the cowpea lines. Pod size may contribute to increased seed yield. The number of grains per pod increases with increased PL though this may be associated with reduced total biomass (Singh *et al.,* 2003). Other major effects of the mutation observed in the present study were the range of variations in SC. A mosaic of SCs were noted including white, brown, chocolate, red, speckled, cream, and black. Dhanavel et al. (2008) reported various SCs due to mutational events. The present findings suggested that the NMB per plant, NPP, number of grains per pod, 100-seed weight and seed yield per plant reduced significantly with increased concentration of irradiation doses. These findings are in agreement to the studies of Girija and Dhanavel (2009), who reported that mutagenesis is associated with negative and positive phenotypic effects for selection.

The present study demonstrated that most characters of cowpea which are of interest to plant breeders can be altered through mutations using the gamma irradiation technique. Furthermore, new plant attributes were created in the high yielding and well adapted local cowpea varieties. Various pests were observed on mutant cowpea during this study (Figure 4.6). Therefore, there is a need to breed for insect pest tolerance in cowpea.

Timko et al. (2007) suggested that the future of cowpea improvement programs should focus on breeding for pests and diseases resistance and other desirable traits such as early maturity, photoperiod insensitivity, suitable plant type, seed quality and yield. Overall, the present study made extensive phenotypic selections of mutants from the M_2 to M_5 generations and identified promising genotypes. The selected mutants' are recommended for adaptability and stability tests across representative agro-ecologies for large-scale production or breeding in Namibia or similar environments. The novel cowpea genotypes selected through the study are valuable genetic resources for genetic enhancement and breeding.

4.5 Conclusions

Cowpea is an important food legume and an integral part of traditional cropping systems in Namibia as well as in the semi-arid regions of the tropics. Farmers depend on its contribution to soil fertility and for its highly nutritious value. A lack of locally improved cowpea varieties is hindering production in the country. Induced mutation breeding technique are available for crop improvement and to enhance genetic diversity. It is also found that it is possible to induce new features which did not exist in the available range of variability in a high yielding and well adapted variety. This experiment has demonstrated that most of the characters which are of interest to plant breeders can be either altered or amended by mutations. The future breeding in cowpea improvement should focus on resistances to numerous pests and diseases and other desirable traits such as those governing maturity, photoperiod sensitivity, plant type, and seed quality. Based on the results of the preliminary evaluation, promising genotypes were selected for further test under multi-location trials for various agronomic traits.

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Chapter 5 Genotype-by-environment interaction of elite varieties of cowpea derived through mutagenesis

Abstract

Grain yield of cowpea (*Vigna unguiculata* L.) is considerably low in the northern communal areas of Namibia where the crop is predominantly cultivated. This is attributed to the lack of improved and well-adapted cultivars, limited water availability as well as the effects of genotype by environment (G x E) interaction. The objectives of this study were to determine G x E interaction and yield stability of elite cowpea selections derived through mutagenesis and to identify promising genotypes and representative test and production environments. The study was conducted in Namibia at three selected sites (Bagani, Mannheim and Omahenene) and two cropping seasons (2014/2015 and 2015/2016) providing six environments. The experiments were laid out using a randomised complete block design with three replications. Thirty four elite genotypes and three check lines were evaluated. Data were analysed using the Additive Main Effects and Multiplicative Interaction (AMMI) and the Genotype plus Genotype by Environment (GGE) bi-plot methods. The following four promising mutant genotypes: G9 (ShL3P74), G10 (RSh3P4), G12 (ShR9P5) and G4 (ShL2P4) were identified with better grain yields of 2.83, 2.06, 1.99 and 1.95, $t.ha^{-1,}$ in that order. The parental lines designated as G14 (Shindimba), G26 (Nakare) and G37 (Bira) provided mean grain yields of 1.87, 1.48 and 1.30 $t.ha^{-1}$, respectively. The best environments in discriminating the test genotypes were Bagani during 2014/15 and Omahenene during 2014/15. The AMMI model explained 77.49 % of the total variation in the present study. The GGE bi-plot showed that 63.57% of the total variation was explained by the first principal component (PC1), while the second principal component (PC2) explained 12% of the variation. Overall, the selected elite mutant lines with wide adpation and high grain yields are useful genetic resources for direct production or copwea breeding in Nambia. Elite mutant selections (G4, G9, G10 and G12), all derived from the parental line Shindimba were best grain yielders with straight pod shape desired by cowpea farmers in northern Namibia.

Key words: additive main effects and multiplicative interaction (AMMI), cowpea, genotype by environment (Gx E), GGE bi-plots, mutants

5.1 Introduction

Cowpea (*Vigna unguiculata* L.; 2n =2x = 22) is a highly preferred crop by most resource poor farmers especially in the sub-Saharan Africa owing to its several desirable attributes. The crop is widely grown by the majority of rural farmers in Namibia because of its ability to withstand drought stress and harsh environmental conditions (Kapewangolo *et al.,* 2007; Fleissner and Bagnall-Oakeley, 2001). Cowpea grain and succulent leaves serve for food. Furthermore, cowpea foliage is an important source of high-quality hay for livestock feed (Agbogidi, 2010). Cowpea is a valuable component of crop production because of its ability to restore soil fertility through nitrogen fixation useful in crop rotation systems.

In Namibia, the productivity of cowpea has declined over the past years. In the country farmers reported loss of useful genetic resources due to harsh climatic conditions coupled with damage by diseases, insect pests and parasitic weeds (Horn *et al.,* 2015). A project on induced mutation breeding using gamma irradiation was initiated in Namibia with the aim of developing promising genotypes with farmers' preferred traits. Consequently, suitable and promising mutants were selected through continuous selfing and selection from the M_2 to M_7 between 2009 to 2014/15 cropping seasons. These selections were done across various representative cowpea growing sites in Namibia. These led to the development of several elite mutant lines for direct production or breeding (Horn *et al.,* 2016).

Assessment of adaptability and yield stability of genotypes is an important step in cultivar selection and recommendation for production (Annicchiarico *et al.,* 2011). According to Yan and Hunt (1998), the performance of a crop cultivar is highly influenced by its adaptation to the specific environment. Thefore, candidate cultaivers should be evaluated to measure their wide or specific adaptability and yield stability. Dehghani et al. (2010) outlined the two concepts of stability (the static or biological and the dynamic or agronomic stability). Under the static concept, a genotype is indicated to be stable when its performance does not change with the change in the environmental conditions, while under the dynamic concept a genotype is considered to be stable when it yields well relative to the productive potential of test environments. Therefore multi-environmental trials (METs) are required to quantify the magnitude of genotype by environment interaction and to recommend varieties with narrow or broad adaption. Genotype by environment interaction (G × E) trials are valuable during the final stages of selection of elite breeding material (Annicchiarico, 2002). The performance of a genotype is influenced by its genetic makeup (G), the environment (E) and the interaction of genotype with the environment (G × E) (Adinurani *et al.,* 2015). Genotype by environment

interaction is a differential response of genotypes when grown across varied growing environments (Yan and Hunt, 1998; Annicchiarico, 2002). According to Fasoula and Fasoula (2003), the environmental effect often mask the genetic component which causes poor genetic gain during selection especially for quantitative traits such as grain yield and yield components. Data generated through G × E interaction studies may assist crop ecologists, agronomists and plant breeders to define ecological regions, mega-environments and ecotypes (Annicchiarico *et al.,* 2011).

Several statistical methods have been proposed and are widely adapted to analyse and interpret G × E data including the following: contrasts (Yan and Hunt, 1998), linear regression (Finlay and Wilkinson, 1963), additive main effect and multiplicative interaction (AMMI) (Fleischmann *et al.,* 2016) and multivariate analysis such as principal component analysis. Also, the genotype plus the genotype by environment interaction (GGE) bi-plot method has been reported as a method of choice in analysing G x E data (Aruna *et al.,* 2011; Adinurani *et al.,* 2015). The GGE bi-plot has been used in mega-environment analysis (Yan and Rajcan, 2002; Casanoves *et al.,* 2005), genotype and test environment evaluation (Yan and Rajcan, 2002; Blanche *et al.,* 2009), trait association (Yan and Rajcan, 2002) and heterotic pattern analysis (Blanche *et al.,* 2007). Compared to other methods of analysing genotype by environment interaction and stability, the GGE bi-plot has the merit of showing graphical presentations which are easier to visualise and interpret such as the which-won-where pattern of data (Yan and Wu, 2008; Adinurani *et al.,* 2015). Therefore, the objectives of this study were to determine G x E interaction and yield stability of elite mutant cowpea selections and to identify promising genotypes and representative test and production environments.

5.2 Material and methods

5.2.1 Description of the study sites and germplasm

The study was conducted at three selected sites (Bagani, Mannheim and Omahenene) of the Ministry of Agriculture, Water and Forestry during 2014/2015 and 2015/2016 cropping seasons under dry land conditions in Namibia (Table 5.1). This provided a total of six testing environments which are described in Table 5.1. The climatic and biological conditions of the sites varied considerably. The rainfall condition of the experimental sites is presented in (Table 5.3) while the physio-chemical properties of soils at the sites are provided in (Chapter 4, Table 4.1).

Table 5.1 List of the six environments of the study.

Environment code	Site	Year (season)	Altitude (m.a.s.l.)
E1	Bagani	2014/15	1007
E2	Bagani	2015/16	
E3	Mannheim	2014/15	1234
E4	Mannheim	2015/16	
E5	Omahenene	2014/15	1109
E6	Omahenene	2015/16	

m.a.s.l. = meters above sea level.

Thirty four elite genotypes and three check cowpea varieties were used in the study (Table 5.2). The lines were selected based on their agronomic performance mainly grain yield. The details of selection procedures that led to the development of these lines are summarised in Chapter 4 section 4.2.33. The elite lines were evaluated during 2014/2015 and 2015/2016 at the M_6 and M_7 generations, in that order.

Table 5.2 The 34 cowpea mutant genotypes and three parental lines evaluated at three sites (Bagani, Mannheim and Omahenene) during 2014/2015 and 2015/2016 cropping seasons as M_6 and M_7 generations, respectively.

Codes	ID	Genotype	Irradiation dose (Gy)	Grain colour	Cod	ID	Genotype	Irradiation dose (GY)	Grain colour
G1	1	ShL10P7	100	Cream	G20	20	NkP6R2	100	Military red
G2	2	ShL7P1	100	White	G21	21	NkR10P15	150	Military
G3	3	ShR10P10	100	White	G22	22	NkR5P1	150	White
G4	4	ShL2P4	100	White	G23	23	NkR4P5	150	Red
G5	5	ShL2P7	100	White	G24	24	NkR8P9	150	Brown
G6	6	ShL3P7-2	100	White	G25	25	NkL9P7	150	Red
G7	7	ShR4P1	100	White	G26	26	Nakare	0	White
G8	8	ShR1P4	100	White	G27	27	BrL1P12	450	Red
G9	9	ShL3P74	100	White	G28	28	BrR8P1	350	Red
G10	10	ShR3P4	100	Military	G29	29	BrR9P1	450	Red
G11	11	ShR10P12	200	Chocolate	G30	30	BrR3P1	600	Red
G12	12	ShR9P5	200	Red	G31	31	BrR5P4	300	Red
G13	13	shR2P11	200	White	G32	32	BrR1P3-2	300	Red
G14	14	Shindimba	0	White	G33	33	BrR7P12	450	Red
G15	15	NkR1P12	100	Chocolate	G34	34	BrR4P11	600	Red
G16	16	NkR10P5	150	Cream	G35	35	BrR11P11	450	Black
G17	17	NkR2P9	150	White	G36	36	BrR11P2	600	Red
G18	18	NkR9P9	100	Cream	G37	37	Bira	0	Red
G19	19	NkR1P3	150	White					

ID = Identification number; G14 = Shindimba, G27 = Nakare and G37 = Bira which were the progenitors of the 34 elite mutants.

5.2.2 **Experimental design, field management and data collection**

The experiments were laid out using a randomised complete block design with three replications. Thirty four mutant selections and three parental checks (Bira, Nakare and Shindimba) making a total of 37 genotypes were evaluated. The experimental units consisted of 8 rows of 4 m long with a spacing of 20 cm between plants and 75 cm between rows and 100 cm between plots. Fertilizers (250 kg/ha superphosphate) was broadcasted to the entire plot after ploughing, prior to planting. Weeding was done two weeks after germination and continued as necessary keeping the plots weed free. Two middle rows (net plots) were harvested per plot to estimate grain yield. Grain yield was obtained by expressing net plot grain yield on hectare basis (t $ha^{-1.}$). Details of the 37 genotypes and 6 environments are given in Table 5.1 and Table 5.2.

Table 5.3 Mean monthly and total rainfall (mm) from 2009/2010 to 2015/2016 across the three study sites.

Site	Season	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	My	Jun	Jul	Aug	Total Mm
Omahenene	2009/2010	0.00	19.00	45.50	106.40	107.00	61.90	85.80	78.00	6.80	0.00	0.00	0.00	510.40
	2010/2011	0.00	0.00	102.8	107.60	135.20	159.40	271.80	132.00	0.00	0.00	0.00	0.00	908.80
	2011/2012	0.00	0.00	84.00	136.00	104.30	87.00	115.80	0.00	0.00	0.00	0.00	0.00	527.10
	2012/2013	0.00	0.50	30.80	25.00	48.00	27.00	38.00	0.00	3.00	0.00	0.00	0.00	172.30
	2013/2014	0.00	0.50	70.00	134.00	128.00	98.50	113.00	5.00	0.00	0.00	0.00	0.00	549.00
	2014/2015	10.00	0.00	37.80	253.00	238.00	2.20	136.00	22.00	0.00	0.00	0.00	0.00	699.00
	2015/2016	0.00	0.00	5.20	84.50	38.00	208.70	160.50	0.00	0.00	0.00	0.00	0.00	496.90
Bagani	2009/2010	42.00	15.00	96.00	70.90	119.40	145.60	19.00	182.60	0.00	0.00	0.00	0.00	690.50
	2010/2011	0.00	4.00	42.60	119.10	208.00	143.20	152.00	30.50	8.00	0.00	0.00	0.00	707.40
	2011/2012	0.00	12.40	98.50	81.30	193.40	113.60	39.90	0.00	0.00	0.00	0.00	0.00	539.10
	2012/2013	0.00	44.50	50.60	120.50	150.30	27.00	0.00	3.00	0.00	0.00	0.00	0.00	395.90
	2013/2014	0.00	1.30	36.10	119.30	145.60	73.90	122.30	106.60	0.00	0.00	0.00	0.00	605.10
	2014/2015	0.00	0.00	41.50	231.60	10.80	24.50	40.00	23.00	0.00	0.00	0.00	0.00	371.40
	2015/2016	0.00	0.00	2.30	48.80	190.20	45.40	71.30	0.00	0.00	0.00	0.00	0.00	358.00
Mannheim	2009/2010	20.00	49.00	24.00	76.00	121.00	68.00	0.00	45.00	18.00	0.00	0.00	0.00	421.00
	2010/2011	0.00	0.00	156.70	107.70	260.80	208.20	74.70	65.30	0.00	0.00	0.00	0.00	873.40
	2011/2012	0.00	0.00	93.00	116.00	198.60	224.50	72.00	0.00	0.00	0.00	0.00	0.00	704.10
	2012/2013	0.00	33.00	89.00	54.50	47.80	32.30	46.00	0.00	12.00	0.00	0.00	0.00	314.60
	2013/2014	0.00	5.00	15.80	91.20	39.80	45.00	56.80	0.00	9.20	0.00	0.00	0.00	262.80
	2014/2015	0.00	2.80	5.80	65.20	83.80	22.20	39.20	57.00	0.00	0.00	0.00	0.00	276.00
	2015/2016	0.00	0.00	0.00	0.00	83.60	13.60	18.40	11.80	0.00	0.00	0.00	0.00	127.40

Data obtained from the Ministry of Agriculture, Water and Forestry (WWW.Mawf.gov.na).

5.3 Data analysis

Grain yield data was subjected to a combined analysis using the general analysis of variance (ANOVA) using GenStat Release 17 statistical software (Payne et al. 2007). The least significant difference (LSD) values were computed at P≤0.05 to separate the mean yields of genotypes. The AMMI and GGE bi-plot models based on the principal component analysis

(PCA) of environmental centred data according to Yan et al. (2000) were used to test the G × E interaction and yield stability of genotypes. Adjusted means of the genotypes were used to compute the GGE bi-plot analysis. The AMMI model is outlined as follow:

$$Y_{ge=}\mu + \alpha_g + \beta_e + \sum_{n=1}^{N} \lambda_n \Upsilon_{gn} \eta_{en} + \theta_{ge,}$$

where Y_{ge} is the yield of genotype, g, in environment, e; μ is the grand mean; α_g is the genotype mean deviation; β_e is the environment mean deviation; $\lambda_n$is the Eigen value of the principal component (PCA) axis, n; Υ_{gn} and ηen are the genotype and environmental PCA scores for the PCA axis, n; N is the number of PCA axis retained in the model; and $\theta_{ge,}$is the residual. The AMMI stability value (ASV) was used to compare stability of genotypes as described by Purchase et al (2000) as follows:

$$ASV = \sqrt{\left[\frac{IPCA1SS}{IPCA2SS}(IPCA1Score)\right]^2 + (IPCA2\ score)^2}$$

ASV= AMMI stability value; SS= sum of squares; IPCA1 and IPCA2= the first and the second interaction principal component axes, respectively. According to Das et al. (2010), genotypes with lower ASV values are considered more stable. Furthermore the combination of G x E represented by which won where pattern among test environments and genotypes were outlined using GGE bi-plots. An average environment coordinate (AEC) was drawn on the genotype bi-plot to outline their mean and stability as described by (Yan and Tinker 2006). Average environment coordinate was also used to identify the ideal environment.

5.4 Results

5.4.1 Climatic condition and physio-chemical properties of soils of the test environments

The physio-chemical properties of the soils of the testing sites varied significantly (Chapter 4 Table 4.1). The rainfall data presented in Table 5.3 covering a period of six years showed a declined trend. The total annual rainfall varied from 510.4 mm in 2009/2010 to 496.9 in 2015/2016 at the Omahenene site. The highest rainfall of 908.8 mm was received at the Omahenene site during 2010/2011 cropping season. At the Bagani site a total rainfall of 707.4 mm was recorded during 2010/2011 and lowest being 358.0 mm during 2015/2016. At the Mannheim site a total rainfall of 873.4 mm was recorded during 2010/2011 and the lowest being 127.4 mm during 2015/2016 cropping season (Table 5.3).

5.4.2 Combined analysis of variance

The combined analysis of variance of grain yield of the 37 cowpea genotypes including 3 parental lines is presented in Table 5.4. This is followed by mean grain yield for 34 cowpea mutant genotypes and their three parental lines (Table 5.5). Results of the ANOVA showed highly significant differences (P≤0.001) among genotypes, environments, and genotype by environment interaction (GEI). These results indicate the presence of genotype by environment interaction affecting the overall performance of genotypes across the test environments. Therefore, further analysis using AMMI and GGE biplot provided clear information.

Table 5.4 Combined ANOVA showing mean square and significance tests of grain yield of 34 cowpea mutant genotypes and their three parental lines tested over six environments in the northern Namibia.

Source of variation	d.f.	Mean squares
Replication	2	0.0014
Genotype (G)	36	2.4107***
Environment (E)	5	2.2986***
Genotype x Environment (G x E)	180	0.4298***
Residual	442	0.12

df = degrees of freedom, and *** = Significant at P ≤ 0.001.

Table 5.5 Mean grain yield (t.ha^{-1}) for 34 cowpea mutant genotypes and their three parental lines tested under six environments in the northern Namibia.

Genotypes	Bagani 2014/15	Bagani 2015/16	Mannheim 2014/15	Mannheim 2015/16	Omahenene 2014/15	Omahenene 2015/16	Mean (t.ha^{-1})
G1	1.41	1.06	1.32	1.37	1.30	1.07	1.25
G2	0.94	0.90	1.13	1.05	0.88	1.17	1.01
G3	1.16	1.94	1.27	1.87	1.02	1.97	1.54
G4	2.87	0.99	2.48	1.63	2.07	1.67	1.95
G5	1.56	1.27	1.64	1.55	1.32	1.61	1.49
G6	0.83	2.00	0.68	1.63	0.81	1.93	1.31
G7	1.27	1.59	1.47	1.64	1.46	1.62	1.51
G8	2.37	1.37	1.63	1.09	1.36	1.45	1.54
G9	3.62	2.07	3.62	2.13	3.26	2.30	2.83
G10	2.67	2.10	2.12	1.39	2.10	1.99	2.06
G11	2.53	1.20	1.43	1.17	1.60	1.29	1.54
G12	3.56	1.26	1.83	1.22	1.82	2.24	1.99
G13	1.45	1.39	2.40	1.39	2.95	1.53	1.85
G14	2.30	1.94	1.73	1.53	2.26	1.46	1.87
G15	1.81	1.36	1.33	1.20	1.62	1.23	1.43
G16	1.63	1.73	1.28	1.27	1.20	1.47	1.43
G17	1.11	1.61	1.50	0.91	1.57	1.00	1.28
G18	1.53	1.33	1.47	0.93	1.61	1.35	1.37
G19	1.33	1.23	0.87	1.10	2.30	1.35	1.36
G20	1.52	1.15	1.47	1.23	1.33	1.27	1.33
G21	1.30	1.13	1.17	1.09	1.23	1.32	1.21
G22	1.29	1.20	1.20	0.98	2.37	1.57	1.43
G23	1.83	1.64	1.37	1.42	1.47	1.63	1.56
G24	1.64	1.10	0.73	0.67	2.19	2.03	1.39
G25	1.33	1.13	1.10	0.94	1.73	1.43	1.28
G26	1.60	1.81	1.43	1.25	1.25	1.53	1.48
G27	1.19	1.37	1.23	1.40	1.52	1.63	1.39
G28	1.13	1.28	0.67	1.37	1.13	0.75	1.06
G29	0.84	0.78	0.77	0.59	0.73	0.71	0.74
G30	1.37	1.11	1.07	0.91	1.53	0.92	1.15
G31	1.04	1.09	1.20	1.46	0.76	1.07	1.10
G32	1.08	0.93	0.84	0.91	1.13	1.36	1.04
G33	1.43	1.44	1.43	1.44	0.80	0.89	1.24
G34	1.40	1.27	1.17	1.19	1.44	1.54	1.33
G35	0.91	0.99	1.27	1.50	1.28	1.10	1.17
G36	2.30	1.42	0.90	0.95	1.74	2.17	1.58
G37	1.76	1.42	0.74	0.95	1.80	1.13	1.30
mean	1.64	1.36	1.37	1.25	1.56	1.45	1.44
Mean	1.65	1.37	1.38	1.25	1.57	1.45	
Min	0.34	0.06	0.56	0.40	0.46	0.46	
Max	3.87	2.93	3.87	2.51	3.82	3.61	
LSD (5%)	0.53	0.66	0.51	0.50	0.45	0.68	
CV%	19.90	29.60	29.90	24.50	17.5	28.90	

See codes of genotypes in Table 5.2. Min= Minimum; Max= Maximum, CV% = Coefficient variance %.

5.4.3 AMMI analysis

The results following the AMMI analysis are presented in Table 5.6 based on grain yield of the 37 cowpea genotypes tested at three locations. A highly significant main effect (P<0.001) of genotypes, and environments, as well as their interaction was revealed by AMMI analyses (Table 5.6). The total variation contributed by the genotypes was 37.95% and the GEI

contributed to 33.83%. Only 5.05% of the variation was due to environmental effect. The AMMI model was able to discriminate and explain 77.49 % of the total variation in this experiment (Table 5.6). In addition, the interaction effect (G x E) was further partitioned into two interaction principal component axes IPCA1 and IPCA2 and the G x E residual (Table 5.6). Both IPCAs explained 44.63% and 23.41% of the total variation, respectively. The residual effect contributed to 31.96% of the total variation. The presence of significant G x E interaction indicated the inconsistency in the performance of the cowpea genotypes across environments. According to Ghaderi et al. (1980) standard analysis of variance procedure is useful for estimating the magnitude of genotype x environment interaction but fails to provide more information on the contribution of individual genotypes to genotype x environment interaction.

Table 5.6 AMMI analysis of variance for seed yield of 34 cowpea mutant genotypes and their three parental lines tested across six environments in the northern Namibia.

Source	d.f.	Mean square	Total variation Explained (%)	G x EExplained (%)
Genotypes (G)	36	2.41***	37.95	-
Environments (E)	5	2.30***	5.02	-
Block	12	0.13	0.69	-
G x E	180	0.43***	33.83	-
IPCA 1	40	0.86***	-	44.63
IPCA 2	38	0.48***	-	23.41
Genotype x Environment (G x E)	102	0.24***	-	31.96
Error	432	0.12	22.51	-

df = degrees of freedom; *** = Significant at P ≤ 0.001; IPCA = Interaction principal component axis.

The AMMI analysis was also able to identify the first four best performing cowpea genotypes at each environment (Table 5.7). Genotype G9 was ranked in the first position across all the environment making it the best candidate that can be recommended for release and wide area production (Table 5.7). All of the best genotypes identified per environment (G3, G4, G6, G9, G10, and G12) by the AMMI except G19 and G22 were derivatives of the parental line Shindimba following irradiation of seeds at 100 or 200 Gy (Table 5.2). Genotypes G19 and G22 were both developed from parental line Nakare irradiate at 150 Gy (Table 5.7). The AMMI bi-plot revealed correlation between genotypes and the environments for example genotypes G3, G6 and G7 were negatively correlated with E1, E3 and E5 , while genotypes G4, G5 and G15 showed positive correlation with the environments E1, E3 and E5 (Figure 5.1). According to Ramburan et al. (2012), the lines that connect the bi-plot origin and the markers of the environments are called environmental vectors and the angle between the vectors of the two environments relates to the correlation coefficient between them. While the environment with

the larger standard deviation (SD) and long vector are considered as most discriminatory. For example E1 showed a higher SD of 0.68, while E4 had the lowest at 0.32. Therefore E1 was the most discriminating environment (Table 5.7 and Figure 5.1). The other environments (E2, E3 and E6) displayed more or less vector lengths which is varied in its discriminatory hence they were less discriminatory test environments (Figure 5.1). Less discriminatory in this case mean that the three environments were closely related and one of them can be used to obtain similar results. When looking at the angle between the lines that connect the bi-plot origin, environment E1 and E3 as well as E2, E4 and E6 were closely related based on the smaller angle between them, while E4 and E5 were loosely correlated due to the wider angle between them (Figure 5.1). When the angle between the vectors that connect the two environment is greater than 90º then the correlation between the two points become smaller (Ramburan *et al.,* 2012). Furthermore, the AMMI Stability Value (ASV) provided more information on the variation among the 37 genotypes (Table 5.8). According to Mahmodi et al. (2011), ASV is the distance from zero in a two dimensional scatter gram of IPCA1 (Interaction Principal Component Analysis Axis 1) scores against IPCA2 scores. A stable genotype is defined as one with the ASV close to zero. Genotype G20 was the most stable with the ASV of 0.08 while G13 was the most unstable with the ASV of 0.83 (Table 5.8).

The IPCA scores of a genotype in the AMMI analysis are an indication of the stability or adaptation over environments. The greater the IPCA scores are, either negative or positive, (as it is a relative value) the more specific adapted is a genotype to certain environments. The more the IPCA scores approximate to zero, the more stable or adapted the genotype is over all the environments sampled (Crossa *et al.,* 1990).

Table 5.7 First four AMMI selections per environment.

Environment	Site	Season	Mean	Standard deviation (SD)	Score	1	2	3	4
E2	Bagani	2015/16	1.37	0.34	0.8698	G9	G3	G6	G10
E4	Mannheim	2015/16	1.25	0.32	0.8537	G9	G3	G6	G10
E6	Omahenene	2015/16	1.45	0.40	0.3623	G9	G10	G14	G13
E3	Mannheim	2014/15	1.38	0.56	-0.3685	G9	G12	G4	G10
E5	Omahenene	2014/15	1.57	0.57	-0.571	G9	G13	G22	G19
E1	Bagani	2014/15	1.65	0.68	-1.1463	G9	G12	G4	G10

G-genotype, E=environment, See codes of genotypes and environments in in Table 5.1 and Table 5.2, respecetively.

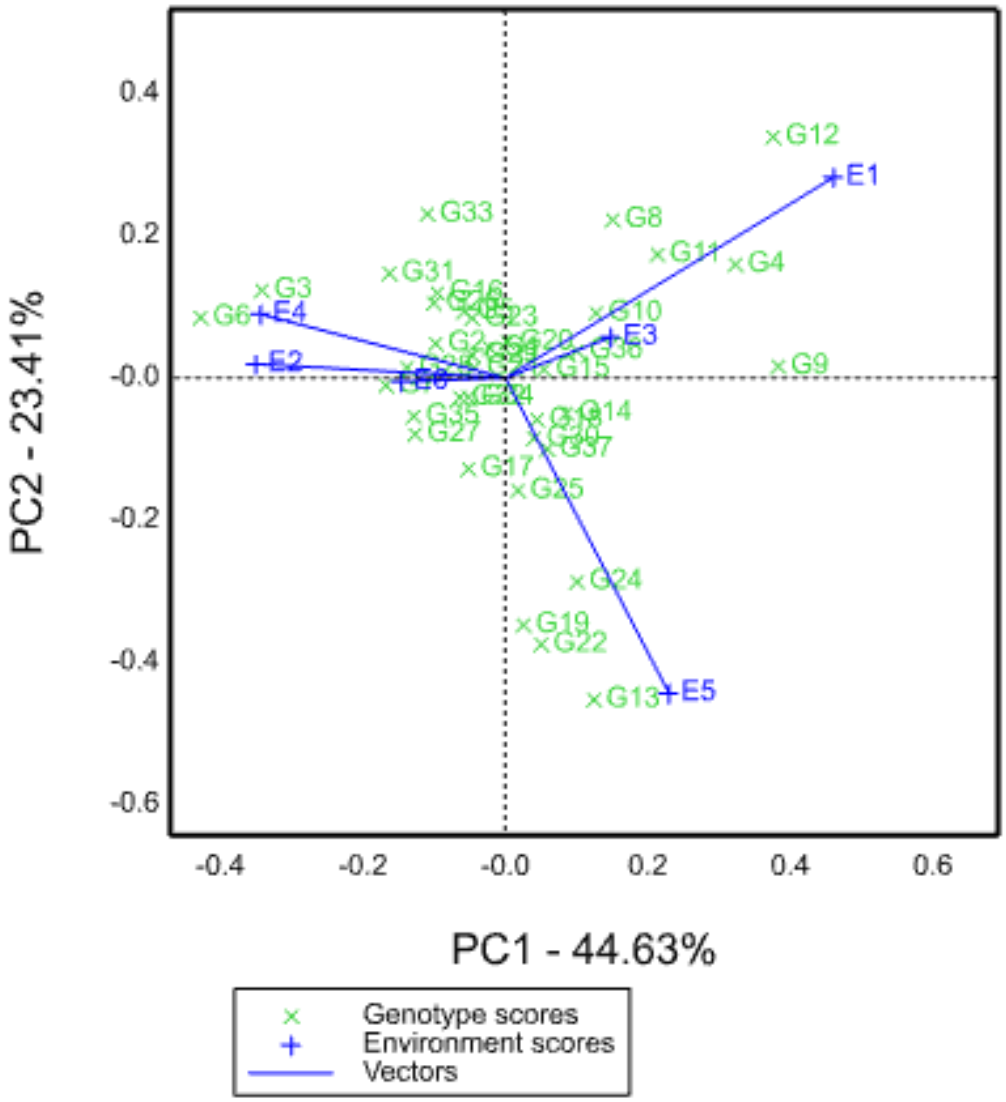

Figure 5.1 The vector view of the AMMI biplot of grain yield based on environment scaling showing the discrimination power and representativeness of the six environments against the tested 34 cowpea mutant genotypes and their three parental. See codes of genotypes and environments in Table 5.1 and Table 5.2, respecetively.

Table 5.8 AMMI adjusted combined mean grain yield (t ha-1), IPCA scores of 34 cowpea mutant genotypes and their three parental lines tested across six environments in the northern Namibia.

No.	Mean	IPCA1	IPCA2	ASV	No.	Mean	IPCA1	IPCA2	ASV
Genotypes									
G1	1.25	0.04	0.07	0.10	G20	1.33	-0.01	0.08	0.08
G2	1.01	0.18	0.08	0.35	G21	1.21	0.08	0.02	0.15
G3	1.54	0.63	0.19	1.22	G22	1.43	-0.09	-0.59	0.61
G4	1.95	-0.59	0.25	1.15	G23	1.56	0.09	0.13	0.22
G5	1.49	0.11	0.15	0.26	G24	1.39	-0.19	-0.45	0.58
G6	1.31	0.79	0.13	1.51	G25	1.28	-0.03	-0.25	0.26
G7	1.51	0.31	-0.02	0.59	G26	1.48	0.18	0.17	0.38
G8	1.54	-0.28	0.35	0.64	G27	1.39	0.23	-0.12	0.45
G9	2.83	-0.71	0.03	1.35	G28	1.06	0.25	0.02	0.48
G10	2.06	-0.23	0.14	0.46	G29	0.74	0.09	0.05	0.18
G11	1.54	-0.39	0.27	0.79	G30	1.15	-0.07	-0.13	0.19
G12	1.99	-0.69	0.53	1.42	G31	1.10	0.30	0.23	0.62
G13	1.85	-0.23	-0.71	0.83	G32	1.04	0.12	-0.04	0.23
G14	1.87	-0.16	-0.08	0.32	G33	1.24	0.20	0.36	0.52
G15	1.43	-0.10	0.02	0.19	G34	1.34	0.10	-0.04	0.19
G16	1.43	0.18	0.19	0.39	G35	1.17	0.24	-0.08	0.46
G17	1.28	0.10	-0.20	0.28	G36	1.58	-0.19	0.06	0.37
G18	1.37	-0.08	-0.09	0.18	G37	1.30	-0.11	-0.16	0.26
G19	1.36	-0.05	-0.54	0.55					
Environments									
E1	1.65	-1.15	0.82	2.34					
E2	1.37	0.87	0.06	1.66					
E3	1.38	-0.37	0.17	0.73					
E4	1.25	0.85	0.26	1.64					
E5	1.57	-0.57	-1.30	1.69					
E6	1.45	0.36	-0.02	0.69					

IPCA- interaction principal component axis; ASV= AMMI Stability Value. See codes of genotypes and environments in See codes of genotypes and environments in Table 5.1 and Table 5.2, respecetively.

5.4.4 GGE bi-plot analysis

The results of the mean grain yield of the 34 mutant cowpea genotypes and their three parental lines evaluated across three site and six environments are presented using the GGE bi-plots (Figure 5.1 - Figure 5.5) respectively. The GGE bi-plots were constructed from the mean grain yield presented in Table 5.5. Based on the bi-plots, the PC1 scores were used as the X-axis while the PC2 as the Y-axix. The GGE scatter plot (Figure 5.2) shows the polygon view of the which won where concept of multilocation mean yield data. Environmental variation of 63.57% was explained by PC1 while PC2 explained 12% of the variation. In total, 75.57% of the total variation were explained by the bi-plot. The polygon separated the biplot into 5 different sectors separated by the perpenicular lines into various directions of the polygon. By connecting the genotypes that were far from the origin with a polygon, most of the 37 genotypes were grouped within the polygon but separated from the rest of the bi-plot by two perpendicular lines from the origin that run through either side of the polygon from the origin.

among the genotypes sharing the sector with it, while G24 was the highest yielding genotype

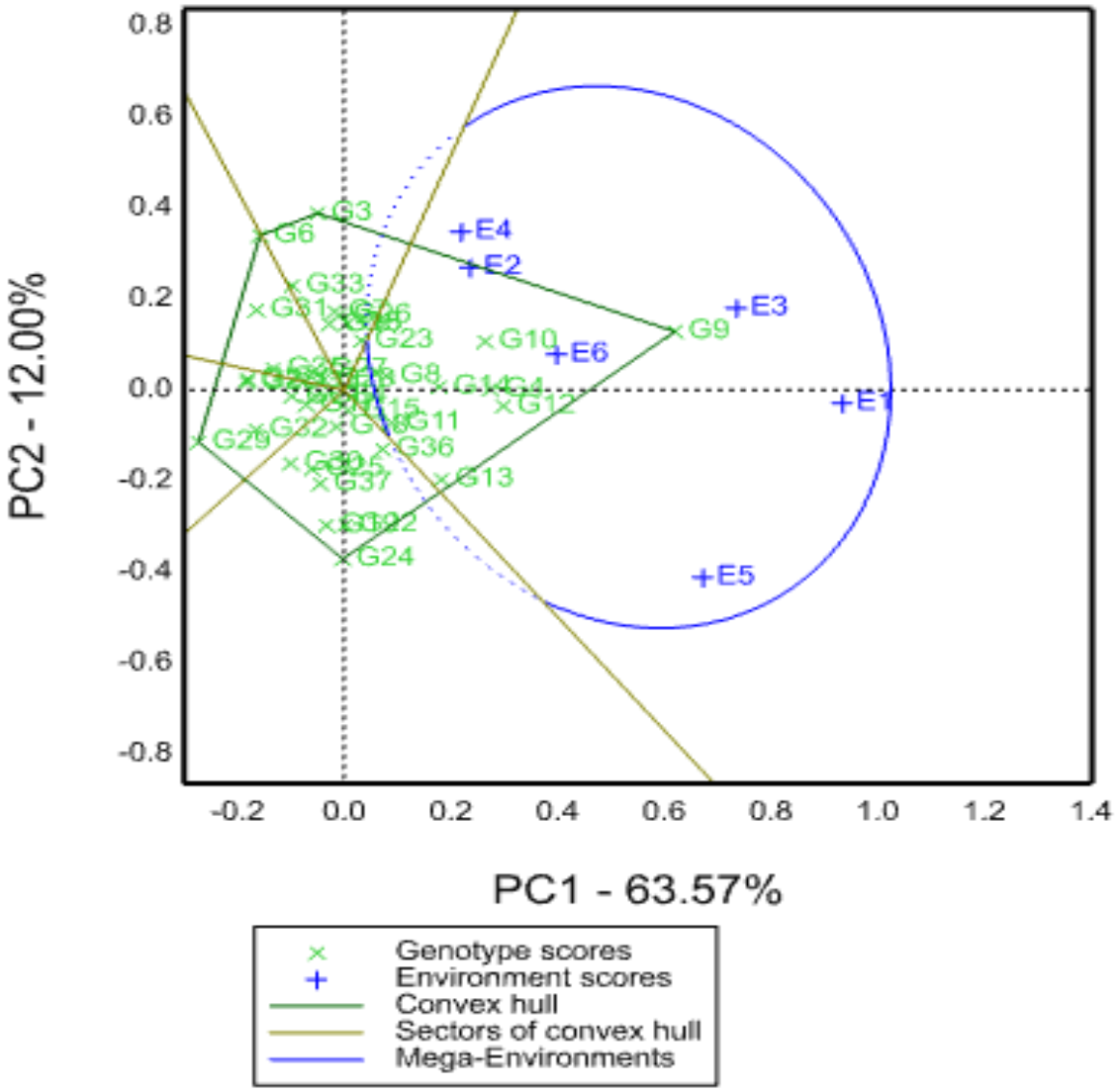

Figure 5.2 The which-won-where view of the GGE biplot to show which genotypes of the 37 performed best in which environment. See codes of genotypes and environments in See codes of genotypes and environments in Table 5.1 and Table 5.2, respecetively.

in a separate sector at Omahenene (E5) during 2014/15 (Figure 5.2). All the six test environments (E1, E2, E3, E4, E5 On the polygon, the genotypes G3,G6, G9, G24 and G29 are situated at the corners and these are the genotypes with the longest vectors and thus called vertex genotypes. In comparison to other genotypes, the vertex genotypes are among the environmentally responsive genotypes based on the vector directions to the environments. Conversly, the genotypes (G1,G2,G13,G17 and G20) located close to the origin and with similar rank were among the least responsive across the test environments (Figure 5.2). In this case G9 was the highest yielding vertex genotype in all the test environment and E6) were grouped by the bi-plot in one sector and these were separated from the rest of the bi-plot by

two perpendicular lines drawn to the respective side of the polygon (Figure 5.2). This suggest that deploying the genotypes under those environments would provide silmilar results. Therefore the genotypes G9, G10, G12, and G13 and all others that fell in that sector are adapted to that environments and are expected to produce good and similar grain yield.

5.4.4.1 Ranking environments based on the performance of the genotypes

Ranking of the test environments based on the relative performance of genotypes is important in studying specific adaptation of a genotype. It is done by drawing the axis line passing through the bi-plot origin and the genotype (Yan and Tinker, 2006). The axis line for each genotype run along its ranking (Figure 5.3). In this case G9 followed by G10 and G12 were the best and performed above average yield in the direction of E6 and E3, and lower than average in other environments such as E5 (Figure 5.3).

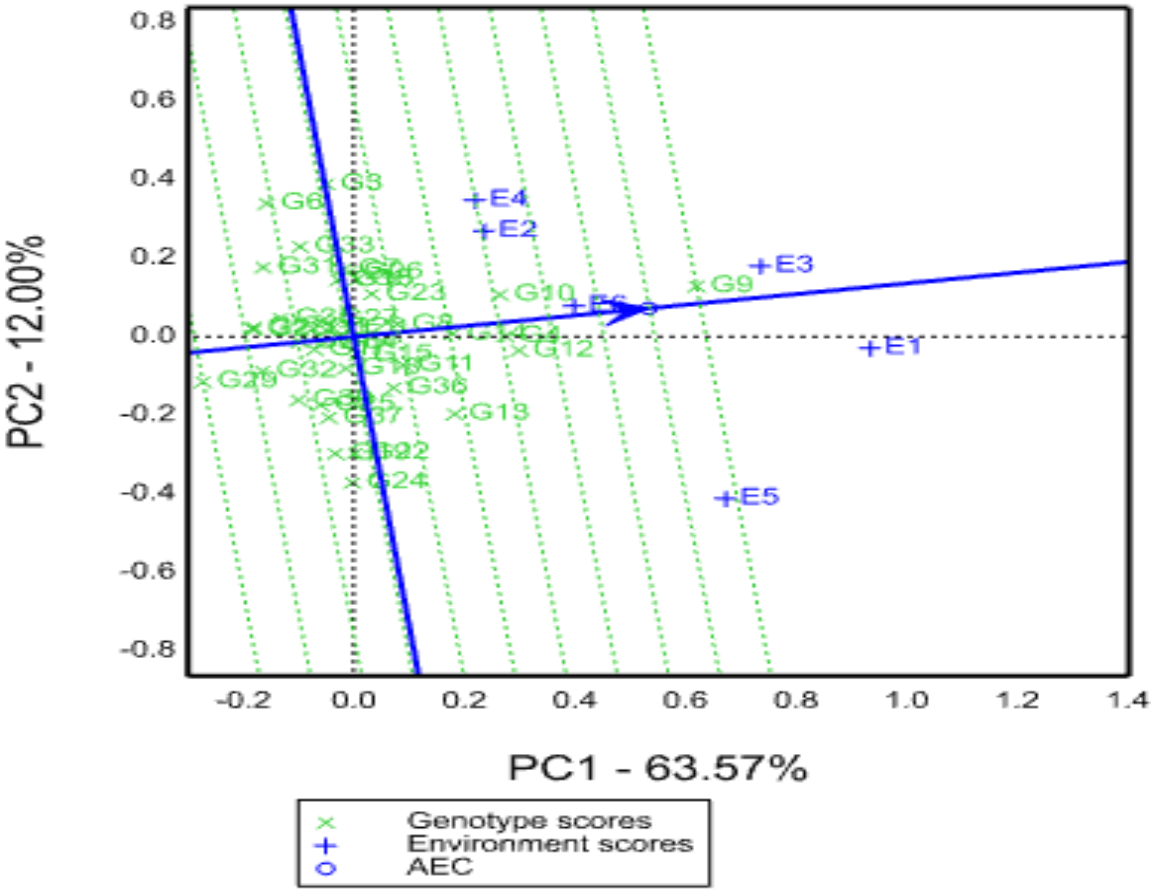

Figure 5.3 The average-environment coordination (AEC) ranking test environments in terms of the relative performance of a genotypes. See codes of genotypes and environments in Table 5.1 and Table 5.2 respecetively.

5.4.4.2 Ranking environments based on the ideal environment

According to Kaya et al. (2006), the ideal test environment is the one with the larger PC1 scores. This environment should have more power to discriminate genotypes main effects. The ideal environment is defined by the arrow pointing to it. The concentric circles are drawn in order to aid easy visualization of the distances between the ideal environment and each environment (Yan and Wu, 2008). The ideal environments could be used as benchmark for genotype selection in multi yield trials (MYTs). An environment located closer to an ideal environment is usually desirable. E3 and E1 are in the direction of ideal environment and also with larger PC1 score (Figure 5.4), therefore these were ideal and more representative among all the environments tested. These were also the most powerful in discrimination of genotypes (Kaya *et al.,* 2006). The ideal environments (E1 and E5) showed large IPC1 scores of 0.8 and 1.0 respectively while E2 and E4 displayed low IPC1 score of 0.25 simultaneously.

5.4.4.3 Ranking genotypes based on the ideal genotype

An ideal genotype is the one with the highest mean performance and is highly stable. This means that it should perform best across all the test environments (Kaya *et al.,* 2006). Ideal genotypes are associated with greatest vector length of the high yielding genotypes and zero (0) genotype environment interaction (GEI) as per arrow pointing to it (Figure 5.4 and Figure 5.5). Such ideal genotypes might not exist in real life, however, can be useful for genotype evaluation. A desirable genotype is one that is located closer to an ideal genotype which is usually at the centre of the concentric circles. The concentric circles were drawn to make visualization of the distance between ideal genotypes and genotypes under investigation. The genotype focused scaling uses the PC1 and PC2 as the original units of the genotypes yield in the same way as the average-environment coordination (AEC) unit. The origin of the yield is also used as the unit of the distance between genotypes and the ideal genotype. In this case genotype ranking take into consideration both the genotype mean yield and the genotype stability. In Figure 5.4, genotype G9 fell on the 3rd concentric circle closes to the ideal environments E3 and E1, while in (Figure 5.5), G9 fell at the centre of the concentric circle making it an ideal genotype in terms of high yielding and stability in relation to the rest of the genotypes. The desirable genotypes include G4, G10, G12 and G14 which were located on the 3rd and 4th centric circle (Figure 5.5), while the ideal environments identified were E1, E3 and E6. The rest of the genotypes includingincluding G8, G11, G13, G15 fell far from the centre of the concentric circle and thus referred to as unfavourable genotypes because they are unstable and lower yielding (Figure 5.5). Genotype G14 is one of the check variety,

Shindimba, which was known for high yielding and its large white grains but was less favoured by farmers because of its coiled pod shape. The newly developed derivatives of Shindimba (G3, G4, G9, G10, and G12) have straight pod shapes which is highly preferred by farmers (Figure 5.6).

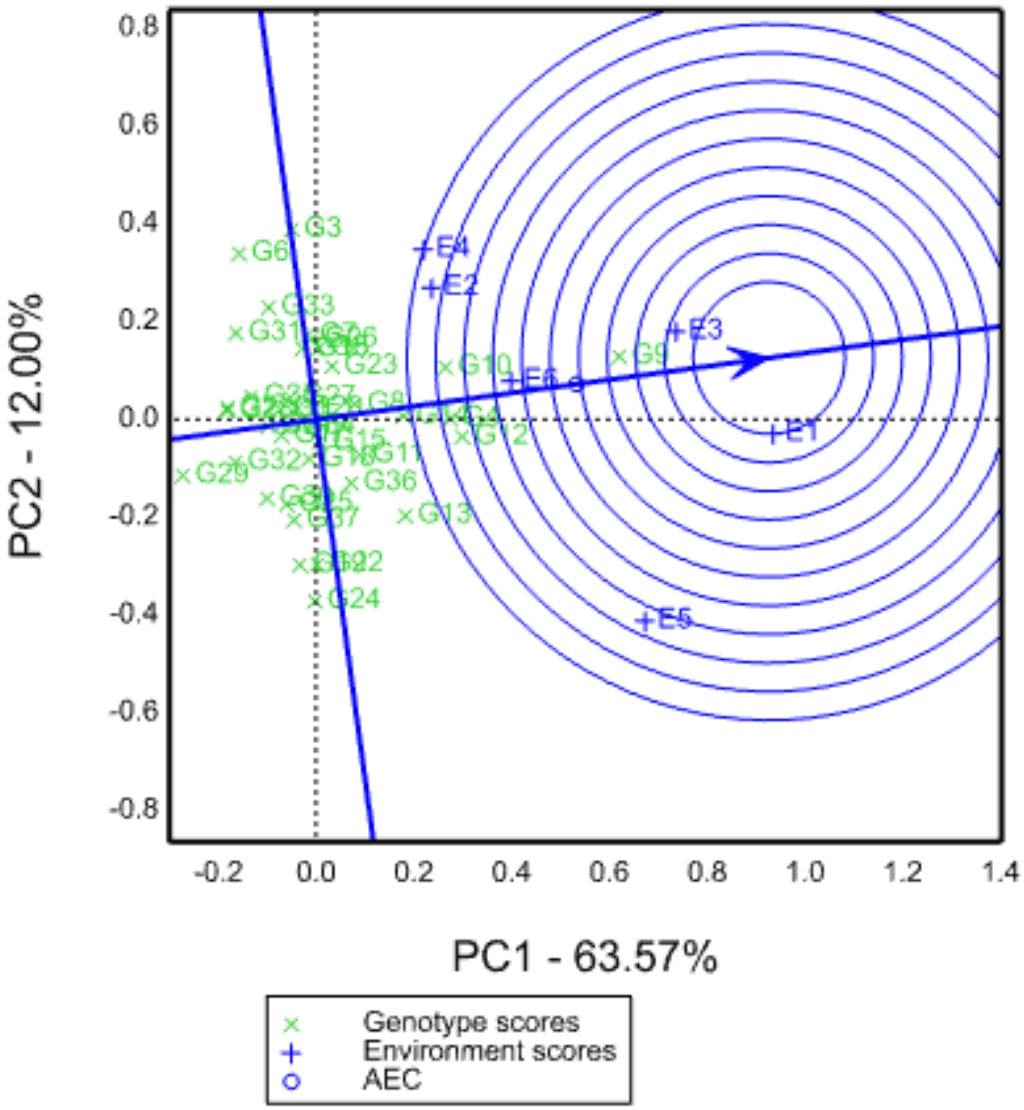

Figure 5.4 the average-environment coordination (AEC) view comparison biplot comparing all the environments relative to an ideal environment (the centre of the concentric circles). See codes of genotypes and environments in Table 5.1 and Table 5.2, respecetively.

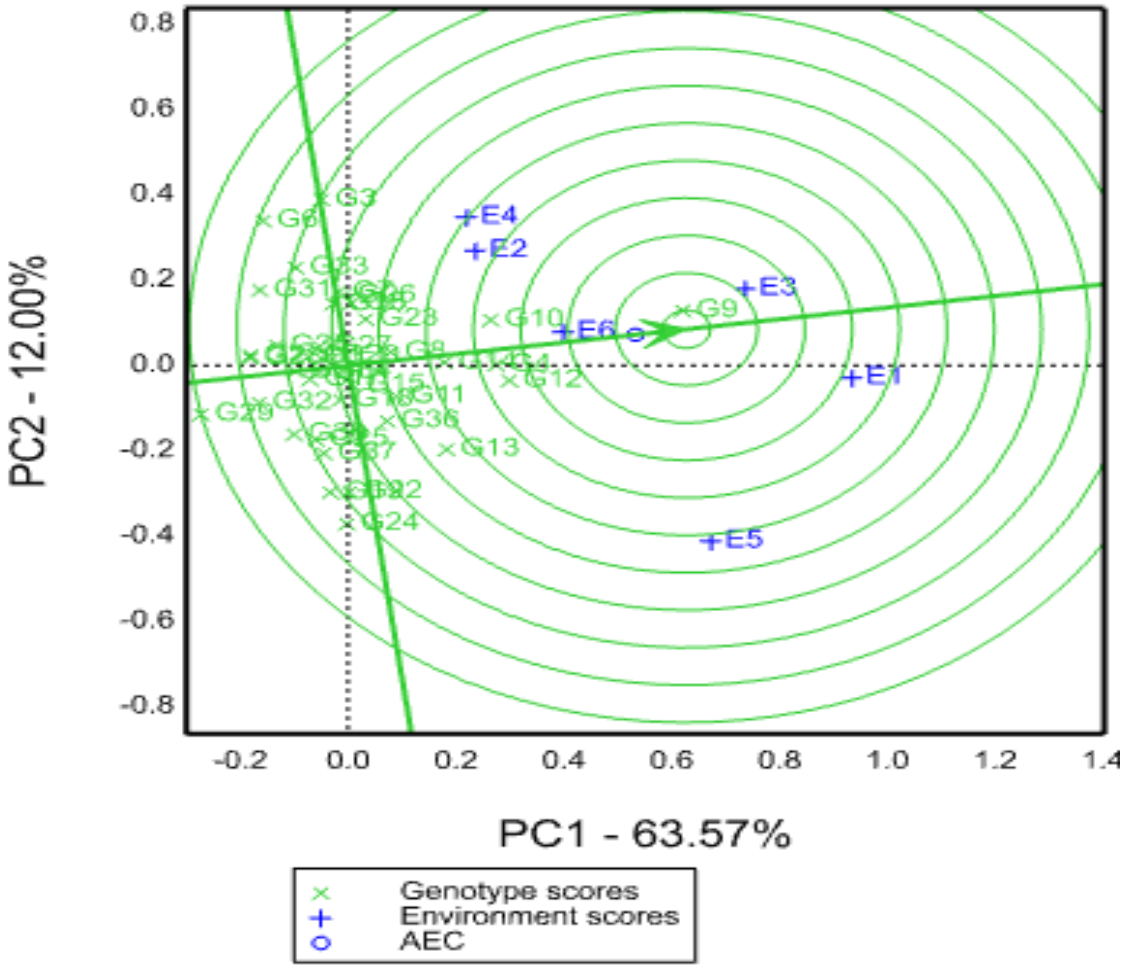

Figure 5.5 The average-environment coordination (AEC) view comparison bi-plot comparing all the genotypes relative to an ideal genotypes (the centre of the concentric circles). See codes of genotypes and environments in Table 5.1 and Table 5.2, respecetively.

Figure 5.6 Cowpea parental line Shindimba showing coiled pods (left) and its mutant derivative with straight pod shape (right) selected in the present study at Omahenene site of northern Namibia.

5.5 Discussion

The present results revealed that the environmental conditions accounted for most of the total variation among genotypes. This means that one cultivar may have the highest yield in one environment, while another cultivar may excel in other environments. Various studies (Yan and Tinker, 2006; Yan and Wu, 2008; Mujahid *et al.,* 2011; Zerihun, 2011; Anley *et al.,* 2013) reported on the environmental variations and its effects on genotype performance and stability. Due to high significant difference between G x E, the yield response of the 37 genotypes was different across all six test environments in this study (Table 5.5). Most of the environments were correlated for example environment E1 and E3 as well as E2, E4 and E6 were closely related based on the smaller angle between them, while comparing E4 and E5 were loosely correlated due to the wider angle between them (Figure 5.1).

The vector view of the AMMI bi-plot (Figure 5.1), provides information on the interrelationships among the environments. In this case, there were correlation and indirect selection can be applied where the same characters are measured on the same genotypes at different environment. On the other hand, when there is no correlations among the environments, the phenotypic correlation between environments can be used to study indirect response to selection (Cooper and Delacy, 1994). ASV, the AMMI analyses were able to pinpoint stable and less stable genotypes (Table 5.8). Furthermore, the AMMI model was able to discriminate and explain 77.49 % of the total variation in this experiment (Table 5.6).

Therefore, PCs can be used to predict the best-fit model for AMMI to explain interpretable interaction patterns (Yan and Tinker, 2006). The AMMI was also able to identify the first best four genotypes in each environment (Table 5.7). For example at E2 (Bagani during season 2015/16), G9, G3, G6 and G10 (ShL3P74, ShR10P10, ShL3P7-2, R3P4, in that order) were the best performers with overall mean yield of 1.37 $t.ha^{-1}$, while in E1 (Bagani season 2014/16) genotype G9, G12, G4 and G10 (Sh3P74, ShR9P5, ShL2P4, ShR3P4, in that order) were the best performers with the mean grain yield of 1.65 $t\ ha^{-1}$ (Table 5.7).

The results revealed that all the best and high yielding genotypes were derived from the parental line Shindimba with seeds irradiated at 100 and 200 Gy. These includes genotype G9 which performed best across all the six environment followed by G4, G10 and G12. The best mutant genotypes (G4, G9, G10 and G12) originated from Shindimba displayed straight pod shape which is preferred by farmers in the northern Namibia (Horn et al. 2015). Typically, Shindimba has coiled pod shape which is not favoured by farmers (Figure 5.6).

Ranking of some genotypes in certain environments by the AMMI model indicates that the genotypes performed differently in terms of grain yield. According to Mahmodi et al. (2011) genotypes with similar rankings across environments are classified as stable. Ranking method of genotypes was also suggested by Fox et al. (1990) as a nonparametric superiority measure for general adaptability. Stratified ranking of the cultivars was used in each environment to determine the proportion of sites in which each cultivar occurred in the top, middle, and bottom third of the ranks, forming the nonparametric measures (Fox et al. 1990). This situation results from a significant GEI and it is referred to as cross over GEI (Yan and Tinker, 2006; Mitrovia *et al.,* 2012). In cross over GEI, the significant change in ranks occurs from one environment to another while in non-cross over the ranking of genotypes remains constant across environments and the interaction is significant due to change in the magnitude of response.

Furthermore, the IPCA1 scores revealed some positive correlation between genotypes and the environments for example for G3, G6 and G7 while genotypes G5, G9 and G12 showed a high negative correlation with the environments (Table 5.8). Genotypes G15, G18 and G19 showed the lowest negative correlation with the environment while G4, G5 and G15 showed the lowest positive correlation with the environments (Table 5.8). According to Mahmodi et al. (2011) the larger the IPCA score, either negative or positive, the more specifically adapted a genotype is to certain environments while a smaller ASV scores indicate a more stable genotype across environments. Genotype G20 was the most stable with the ASV of 0.08 while G13 was the most unstable with the ASV of 0.83 (Table 5.8). This findings suggests that a breeder can chose G20 over G13 in terms of stability. The significant differences in the genotypes under study could be as a result of variation in their genetic makeup from induced mutation. Furthermore significant differences in the environments are indication of diverse type of environment of different locations or seasons.

The GGE bi-plot model was used in this experiment to deduce useful information from different bi-plots constructed from it. A scatter biplot depicting a which–won-where model was constructed to determine which genotype performed better where and which environment provide ideal conditions for the genotypes (Figure 5.2). Genotypes G3, G6, G9, G24 and G29 (ShR10P10, ShL3P7-12, ShL3P74, ShR8P9, ShR9P1, respetively) were refered to as responsive genotypes because of their location at the corners of the bi-plot and these are the genotypes with the longest vectors (Figure 5.1) and thus called vertex genotypes (Kaya *et al.,* 2006; Yan and Tinker, 2006; Mahmodi *et al.,* 2011). The bi-plot also revealed that there was only one major mega environment for cowpea genotypes since all the environments were grouped under one sector correponding to a mega environment (Table 5.8). On the contrary,

when different environments fall into different sectors, it indicates that they have different high yielding cultivars for different sectors which means there is a cross-over interaction. In this case the test environments could be divided into different mega environments (Mahmodi *et al.,* 2011). In this study 75.57% G x E variations were explained by the bi-plot (Figure 5.5). According to Yan and Tinker (2006), the cosine of the angle between the vectors of the two environments approximates the correlation coefficient between them. According to Kaya et al. (2006), the ideal test environment is the one with the larger PC1 scores. Based on the ranking biplot genotypes, G9 performed good across all the environments but had higher than average yield in the direction of E6 and E3, and lower than average in other environment such as E5 (Fig 5.3). G9 followed by G3 recorded the highest mean yield, and were located on the average environmental coordination (AEC or AEA) (Figure 5.3). According to Yan and Tinker (2006), the AEC absica points to higher mean yield across environments. Thus, G9 had the highest mean yield followed by G10, G12, G4, G14 and G13 (ShR3P4, Sh R9P5, ShL2P4, Shindimba parent and ShR2P11) in that order.

The bi-plot was able to explain 75.57% of the G x E variations (Fig 5.3). According to Jalata (2011) genotypes with PC1 scores greater than 0 are referred to as high yielding while those with PC1 less than 0 are referred as low yielding. These findings suggest that genotypes showing high correlation with the environments are able to exploit specific agro-ecological zones (Jalata, 2011; Mitrovia *et al.,* 2012). Based on the concept of ideal genotype based on the performance of a genotype, the concentric circles drawn in order to aid easy visualization of the distances between the ideal environment and each environment (Yan and Wu, 2008) help to visualise and identify the ideal genotype for specific environments. The ideal environments could be used as benchmark for genotype selection in multi yield trials (MYTs). The desirable genotypes included G4, G10, G12 and G14 (ShL2P4, ShR3P4, ShR9P5 and Shindimba) which were located on the 3rd and 4th centric circle respectively (Figure 5.5). The rest of the genotypes including G8, G11, G13, G15 (ShR1P4, ShR10P12, ShR2P11 and NkR1P12) fell far from the centre of the concentric circle and thus referred to as unfavourable genotypes.

5.6 Conclusions

The present study was able to produce and isolate promising cowpea mutant genotypes for different agro-ecological conditions in Namibia. Both AMMI and GGE bi-plot methods were able to discriminate between the ideal and non-performing genotypes and environments for cowpea genotypes. It was concluded that most of the test environment were similar in terms of the results produced, even though variations were also detected. It is therefore suggested that one of the six environments could be used to obtain sufficient information on the performance of the genotypes. This is advisable as it could also reduce the cost while at the same time increase efficiency. Genotype x environment (G x E) was best implemented in this experiment to select broadly adapted genotypes.

The following four promising mutant genotypes: G9 (ShL3P74), G10 (ShR3P4), G12 (ShR9P5) and G4 (ShL2P4) were identified with better grain yields of 2.83, 2.06, 1.99 and 1.95, $t.ha^{-1,}$ in that order. The parental lines designated as G14 (Shindimba), G26 (Nakare) and G37 (Bira) provided mean grain yields of 1.87, 1.48 and 1.30 $t.ha^{-1}$ respectively. Elite mutant selections (G4, G9, G10 and G12), all derived from the parental line Shindimba were best grain yielders with straight pod shape desired by cowpea farmers in the northern Namibia.

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Chapte 6 Participatory varietal selection among elite cowpea genotypes in northern Namibia

Abstract

Participatory varietal selection (PVS) enables identification of farmers-preferred crop genotypes for large-scale production or targeted breeding. The objective of this study was to select elite cowpea varieties that meet farmers' needs and preferences and using farmers' participation and indigenous knowledge. Participatory cowpea varietal selection was undertaken in the northern Namibia using a set of newly developed and elite varieties developed through gamma irradiation and continuous selection from the M_2 through the M_6 generations. Thirteen, 10, and 11 candidate mutant cowpea varieties derived from three local varieties Shindimba, Bira and Nakare, respectively were evaluated along with the parents. Field evaluations were conducted across three selected villages in Omusati Region of northern Namibia where the crop is predominantly cultivated. Test varieties were independently assessed and scored using nine agronomic traits involving 114 participating farmers. All the new genotypes descended from Bira were favourably selected by all participating farmers for their best above ground biomass. The genotype L1P12 (Bi450) was preferred by 81% of farmers for its higher pod setting ability. The genotypes R4P5 (Nk150) with longer pod size and R3P1 (Bi600%) with early maturity were ideal candidates preferred by 84% and 82% of famers, in that order. All participating farmers selected genotypes L2P4 (Sh100), L2P7 (Sh100), L3P7-2 (Sh100), L3P74 (Sh100), L7P1 (Sh100), P6R2 (Nk100), R10P10 (Sh100), R1P3 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R2P9 (Nk150), R4P1 (Sh100), R9P5 (Sh200) and R9P9 (Nk100) for their desirable white grain colour. L3P74 (Sh100), P6R2 (Nk100), R1P3 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R2P9 (Nk150), R4P1 (Sh100), and R9P9 (Nk100) were selected by all respondents for their bigger grain size. Genotypes L1P12 (Bi450) and L9P7 (NK150) were rated very good providing higher pod yield. Experimental mutants were rated as very good performers in terms of pest tolerance when compared to the check variety Shindimba. Furthermore, participating farmers selected the following genotypes: L3P7-2 (Sh100), L7P1 (Sh100), L9P7 (NK150), P6R2 (Nk100), R10P10 (Sh100), R10P12 (Sh200), R10P5 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R3P4 (Sh100), R4P1 (Sh100), R5P1 (Nk150), R8P9 (Nk150) showing a relatively better drought tolerance than the local checks. Overall, the present study selected the following ten farmers-preferred cowpea varieties: R9P5 (Sh200), R3P4 (Sh100), R4P1 (Sh100), L3P74 (Sh100), R1P12 (Nk100), R8P9 (Nk150),

R5P1 (Nk150), R2P9 (Nk150), R10P5 (Nk150) and R11P2 (Bi600) for their larger seed size, white grain colour, high pod setting ability, insect pest tolerance, early maturity, longer pod size, drought tolerance, high biomass and pod yields. The selected candidate lines will be subjected to distinct, uniformity and stability trials for varietal registration and release in northern Namibia.

Keywords: cowpea, genotypes, farmer-preferences, participatory varietal selection

6.1 Introduction

Cowpea is one of the important food legume crops in the hot-dry tropics and subtropics and sub-Saharan Africa (SSA). In the northern Namibia, cowpea is grown by the majority of farmers for food, feed, cash incomes and soil ferity improvement (Hillyer *et al.,* 2006; Zegada-Lizarazu *et al.,* 2006; Horn *et al.,* 2015).

Important agronomic traits of cowpea preferred by farmers include early maturity, insect pest resistance, drought tolerance, better above ground biomass, higher seed yield and cooking quality (Abadassi, 2015; Horn *et al.,* 2015). In Namibia, landraces varieties of cowpea are widely cultivated by smallholder farmers. In the country only three landrace varieties area available and widely grown. These varieties are Nakare (IT81D-985), Shindimba (IT89KD-245-1), and Bira (IT87D-453-2). The varieties were initially acquired from the International Institute for Tropical Agriculture in Nigeria. They are widely cultivated in the northern communal areas of the country as well as in Southern Angola and Zambia (Ng and Marachel, 1985). In Namibia, the three varieties have become generally low yielders and prone to drought stress, and pest and diseases. Therefore, there is a need to broaden the genetic bases of the crop and to develop improved and locally adapted cowpea varieties.

Breeding cowpea for biotic and abiotic stress tolerance and improved yield are the overriding considerations in Namibia. Hence seeds of the three cowpea varieties were gamma irradiated with varied doses for targeted selection (see Chapter 3, Section 3.2.2). Subsequently, 34 elite lines were selected through rigorous evaluations from the M_2 through M_6 generations (see Chapter 4, Section 4.2.3). These elite lines were selected for their desirable agronomic characteristics including flowering ability, early maturity, high biomass production, desirable grain colour and improved grain yields. Furthermore, the elite lines were phenotypically stable and recommended for further evaluation by farmers for large-scale production or breeding in Namibia (Horn *et al.,* 2016).

Farmers' knowledge, preference and acceptance of newly developed crop varieties and production technologies is important for their ultimate adoption and use. A participatory varietal selection (PVS) is an important tool to involve farmers in the selection of newly developed varieties at the target production environments. This will enable identification of farmers'-preferred varieties for release and wide adoption (vom Brocke *et al.,* 2010). Participatory varietal selection allows farmers to evaluate and select from a range of candidate varieties that had not been previously recommended or released in the prevailing agro-ecologies. Often PVS trials are conducted under farmers' own fields based on their own management

conditions (Tiwari *et al.,* 2009). Because of its advantage in providing detailed information about the needs and requirements of the farmers on the newly developed varieties, this technique has been widely used by various research groups (Witcombe *et al.,* 2001; Dorward *et al.,* 2007; Thapa *et al.,* 2009). Therefore, the objective of this study was to select elite cowpea varieties that meet farmers' needs and preferences and using farmers' participation and indigenous knowledge. The selected candidate lines will be subjected to distinct, uniformity and stability trials for varietal registration and release in northern Namibia.

6.2 Materials and methods

6.2.1 Plant materials

Thirty four elite mutant genotypes and three local check cowpea varieties were used in this study. The details of the genotypes are presented in Chapter 5, Table 5.2. The lines were derived through gamma irradiation and continuous selection as outlined in Chapter 3, 3.2.2. The lines were selected based on their suitable agronomic performance (Chapter 4, section 4.2.3) and yield stability when evaluated during 2014/2015 and 2015/2016 at the M_6 and M_7 generations, in that order. Therefore the candidate varieties were advanced for PVS.

6.2.2 Participatory variety evaluation

6.2.2.1 Sampling procedure

A purposive sampling procedure was followed for this study. Consequently two constituencies (Outapi and Ruacan) and three villages (Onavivi, Onelao and Etunda) situated in Omusati Region were selected for the study. Onavivi and Onelao villages are located in Outapi Constituency, while Etuda is located in Ruacana Constituency. The study areas were purposefully selected because of their known high cowpea production. In each village 38 farmers were selected based on their willingness to participate in the PVS providing a total of 114 participant farmers in evaluation of 37 genotypes.

6.2.2.2 Field establishment

Trials were established using 37 cowpea genotypes (34 elite lines and 3 local checks) under selected farmers' fields. Non-replicated plots were prepared and each variety was sown using 4 rows. The spacing between plants within a row was 20 cm, while the inter-row spacing was 75 cm. The three villages were treated as replications. Plot layout and planting at farmer's fields were done by the staff of the Ministry of Agriculture, Water and Forestry (MAWF). Farmers were responsible for the general agronomic management of the trials under their own

fields. The on-farm trials were monitored by the research and technical personnel of MAWF with selected farmers every second week until the crops were harvested.

Figure 0.1 Participating farmers and researchers during cowpea varietal selection under farmers' fields in northern Namibia (left photo taken at Onelao village and right at Onavivi village).

6.2.3 Data collection and analysis

Participating farmers' assessed the elite varieties and checks established across the three villages (Figure 0.1). The trials were conducted during January 2015 to April 2016. Data were collected through farmers' scores as described by previous workers (Thapa et al., 2009; Virk *et al.,* 2009). A scoring form was designed in a local language (Oshiwambo). Participants scored the varieties based on their preference and overall observation of each variety. Nine agronomic attributes or scoring criteria were chosen to capture farmers' preferences of each tested variety. The traits observed included: above ground biomass number of pods per plant (NPP), pod size (PS), early maturity (EM), grain colour (GC), grain size (GS), pod yield (PY), pest tolerance (PT) and drought tolerance (DT). These are farmers-preferred traits of cowpea in northern Namibia (Horn *et al.,* 2015). Each attribute was rated by the farmers using a scale of 0 to 5, where 0 = no rate, 1= very poor, 2= poor, 3= average, 4= good and 5= very good. Data were subjected to statistical analyses using the cross-tabulation procedure and contingency chi-square values were calculated for significant tests using SPSS (Release 16.0) computer package (SPSS Inc., 2007).

6.3 Results

6.3.1 Comparison of farmers' trait preferences across villages

Results showed that trait preferences of the elite cowpea lines across the three villages did not show significant differences except for pest and drought tolerance (Table 0.1). Therefore, data of the three villages were pooled to compare farmers' trait preferences of the new varieties.

Table 0.1 Summary of chi-square tests comparing association of farmers' traits preferences among 37 cowpea genotypes each evaluated by 38 farmers using participatory variety selection in three villages of the Omusati Region of northern Namibia during 2015/16.

		Villages										
		Onavivi		Onelao		Etunda		Total		Chi-square tests		
Traits	Response	C	EC	C	EC	C	EC	C	EC	X^2	df	Pvalue
PC	1	494.0	502.7	519.0	502.7	495.0	502.7	1508.0	1508.0	9.96	8	0.268
	2	183.0	159.0	141.0	159.0	153.0	159.0	477.0	477.0			
	3	123.0	118.0	124.0	118.0	107.0	118.0	354.0	354.0			
	4	95.0	98.3	96.0	98.3	104.0	98.3	295.0	295.0			
	5	511.0	528.0	526.0	528.0	547.0	528.0	1584.0	1584.0			
NPP	1	433.0	470.3	472.0	470.3	506.0	470.3	1411.0	1411.0	13.14	8	0.107
	2	203.0	186.7	196.0	186.7	161.0	186.7	560.0	560.0			
	3	181.0	176.3	170.0	176.3	178.0	176.3	529.0	529.0			
	4	192.0	187.0	178.0	187.0	191.0	187.0	561.0	561.0			
	5	397.0	385.7	390.0	385.7	370.0	385.7	1157.0	1157.0			
PS	1	129.0	121.0	118.0	121.0	116.0	121.0	363.0	363.0	7.37	8	0.497
	2	160.0	170.7	172.0	170.7	180.0	170.7	512.0	512.0			
	3	174.0	193.0	192.0	193.0	213.0	193.0	579.0	579.0			
	4	227.0	222.0	218.0	222.0	221.0	222.0	666.0	666.0			
	5	716.0	699.3	706.0	699.3	676.0	699.3	2098.0	2098.0			
EM	1	151.0	151.3	167.0	151.3	136.0	151.3	454.0	454.0	4.86	8	0.773
	2	177.0	175.7	174.0	175.7	176.0	175.7	527.0	527.0			
	3	184.0	187.7	178.0	187.7	201.0	187.7	563.0	563.0			
	4	267.0	266.3	262.0	266.3	270.0	266.3	799.0	799.0			
	5	627.0	625.0	625.0	625.0	623.0	625.0	1875.0	1875.0			
GC	1	61.0	59.0	55.0	59.0	61.0	59.0	177.0	177.0	13.80	8	0.087
	2	45.0	46.0	45.0	46.0	48.0	46.0	138.0	138.0			
	3	72.0	71.7	63.0	71.7	80.0	71.7	215.0	215.0			
	4	108.0	136.7	145.0	136.7	157.0	136.7	410.0	410.0			
	5	1120.0	1092.7	1098.0	1092.7	1060.0	1092.7	3278.0	3278.0			
GS	1	164.0	179.0	188.0	179.0	185.0	179.0	537.0	537.0	12.60	8	0.126
	2	22.0	25.0	28.0	25.0	25.0	25.0	75.0	75.0			
	3	66.0	82.0	88.0	82.0	92.0	82.0	246.0	246.0			
	4	213.0	192.3	193.0	192.3	171.0	192.3	577.0	577.0			
	5	941.0	927.7	909.0	927.7	933.0	927.7	2783.0	2783.0			
PY	1	136.0	133.0	132.0	133.0	131.0	133.0	399.0	399.0	2.62	8	0.956
	2	166.0	163.3	157.0	163.3	167.0	163.3	490.0	490.0			
	3	191.0	200.3	204.0	200.3	206.0	200.3	601.0	601.0			
	4	243.0	251.7	250.0	251.7	262.0	251.7	755.0	755.0			
	5	670.0	657.7	663.0	657.7	640.0	657.7	1973.0	1973.0			
PT	1	0.0	12.7	38.0	12.7	0.0	12.7	38.0	38.0	511.32	8	0.00
	2	266.0	101.3	0.0	101.3	38.0	101.3	304.0	304.0			
	3	1140.0	1292.0	1368.0	1292.0	1368.0	1292.0	3876.0	3876.0			
	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
DT	1	13.0	29.7	36.0	29.7	40.0	29.7	89.0	89.0	23.14	8	0.001
	2	31.0	43.0	49.0	43.0	49.0	43.0	129.0	129.0			
	3	14.0	14.3	19.0	14.3	10.0	14.3	43.0	43.0			
	4	1348.0	1319.0	1302.0	1319.0	1307.0	1319.0	3957.0	3957.0			
	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			

PC= above ground biomass, NPP=number of pods per plant, PS= pod size, PY=pod yield, EM= early maturity, GC= grain colour, GS=grain size, PT=pest tolerance, DT= drought tolerance, X^2 = Chi-Square, 1= very poor, 2= poor, 3=average, 4=good and 5=very good, df = degrees of freedom given by (v1-1)(v2-1) where v1 and v2 correspond to class/number of responses and villages, respectively and EC= expected count and C= actual Count.

6.3.2 Participatory varietal selection of cowpea varieties

Results from the participatory evaluation of nine agronomic attributes of 37 cowpea varieties are presented from Table 0.2 through to Table 0.10. The main findings are briefly described in the following sections for each selection criteria.

Table 0.2 Summary of chi-square tests associating rating of cowpea genotypes for above ground biomass during participatory variety selection conducted in three villages (Onavivi, Onlao and Etunda) of Omusati Region in northern Namibia.

		Above ground biomass				
Genotypes	**Class**	**Very Poor**	**Poor**	**Average**	**Good**	**Very Good**
L10P7 (Sh100)	Count	64.0	19.0	11.0	12.0	8.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
L1P12 (Bi450)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
L2P4 (Sh100)	Count	59.0	13.0	21.0	11.0	10.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
L2P7 (Sh100)	Count	58.0	18.0	18.0	12.0	8.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
L3P7-2 (Sh100)	Count	62.0	24.0	12.0	8.0	8.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
L3P74 (Sh100)	Count	26.0	11.0	21.0	26.0	30.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
L7P1 (Sh100)	Count	69.0	15.0	8.0	10.0	12.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
L9P7 (NK150)	Count	65.0	15.0	10.0	14.0	10.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
Nakare (0)	Count	65.0	17.0	11.0	6.0	15.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
P6R2 (Nk100)	Count	55.0	12.0	14.0	19.0	14.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
Parent (Bi0)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R10P10 (Sh100)	Count	57.0	20.0	11.0	16.0	10.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R10P12 (Sh200)	Count	59.0	25.0	16.0	6.0	8.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R10P15(Nk150)	Count	53.0	21.0	17.0	9.0	14.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R10P5 (Nk150)	Count	56.0	15.0	12.0	13.0	18.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R11P11(Bi450)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R11P2 (Bi600)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R1P12 (Nk100)	Count	59.0	19.0	13.0	12.0	11.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R1P3 (Nk150)	Count	62.0	17.0	13.0	8.0	14.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R1P3-2 (Bi300)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R1P4 (Sh100)	Count	56.0	23.0	15.0	11.0	9.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R2P11(Sh200)	Count	48.0	39.0	11.0	6.0	10.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R2P9 (Nk150)	Count	62.0	9.0	15.0	10.0	18.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R3P1 (Bi600)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8

Genotypes	Class	Above ground biomass				
		Very Poor	Poor	Average	Good	Very Good
R3P4 (Sh100)	Count	61.0	20.0	11.0	15.0	7.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R4P1(Sh100)	Count	63.0	21.0	17.0	4.0	9.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R4P11 (Bi600)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R4P5 (Nk150)	Count	53.0	16.0	20.0	11.0	14.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R5P1(Nk150)	Count	56.0	19.0	11.0	11.0	17.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R5P4 (Bi300)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R7P12 (Bi450)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R8P1 (Bi350)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R8P9 (Nk150)	Count	54.0	13.0	13.0	10.0	24.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R9P1 (Bi450)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R9P5 (Sh200)	Count	55.0	29.0	12.0	9.0	9.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
R9P9 (Nk100)	Count	67.0	10.0	12.0	11.0	14.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
Shindimba (0)	Count	64.0	17.0	9.0	15.0	9.0
	Expected Count	40.8	12.9	9.6	8.0	42.8
Chi-square test		X^2		df		P-value
		3201.698		144		0.000

df = degrees of freedom given by (v1-1)(v2-1) where v1 and v2 correspond to class/number of genotypes and scales, respectively and X^2 = Chi-Square.

5.7.2.1 Above ground biomass

There was highly significant difference (P<0.001; X^2=3201.698; df =144) in the selection of the new varieties for their above ground biomass (Table 0.2). The following genotypes: L1P12 (Bi450), R8P1 (Bi350), R9P1 (Bi450), R3P1 (Bi600), R5P4 (Bi300), R1P3-2 (Bi300), R7P12 (Bi450), R4P11 (Bi600), R11P11 (Bi450) and R11P2 (Bi600) were selected for their very good above ground biomass (PC) by all participating farmers of the three villages.

6.3.2.1 Number of pods per plant

Table 0.3 summarises the preference of farmers with regards to the number of pods per plant. Farmers preferences varied significantly (P<0.001; X^2=1834.007; df =144) in selecting tested genotypes for the number of pods per plant. The genotype L1P12 (Bi450) was rated as very good for the number of pods per plant by 81% of participating farmer followed by the genotypes R11P2 (Bi600), R7P12 (Bi450) and R11P11 (Bi450).

Table 0.3 Summary of chi-square tests associating rating of cowpea genotypes for number of Pods per plant during participatory variety selection conducted in three villages (Onavivi, Onlao and Etunda) of Omusati Region in northern Namibia.

		Number of pods per plant				
Genotypes	**Class**	**Very Poor**	**Poor**	**Average**	**Good**	**Very Good**
L10P7 (Sh100)	Count	47.0	18.0	16.0	20.0	13.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
L1P12 (Bi450)	Count	6.0	6.0	9.0	12.0	81.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
L2P4 (Sh100)	Count	31.0	18.0	24.0	26.0	15.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
L2P7 (Sh100)	Count	39.0	19.0	19.0	23.0	14.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
L3P7-2 (Sh100)	Count	51.0	27.0	14.0	12.0	10.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
L3P74 (Sh100)	Count	16.0	10.0	20.0	35.0	33.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
L7P1 (Sh100)	Count	43.0	17.0	17.0	15.0	22.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
L9P7 (NK150)	Count	65.0	13.0	11.0	13.0	12.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
Nakare (0)	Count	71.0	17.0	9.0	13.0	4.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
P6R2 (Nk100)	Count	66.0	11.0	13.0	17.0	7.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
Parent (Bi0)	Count	3.0	9.0	9.0	11.0	82.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R10P10 (Sh100)	Count	45.0	21.0	16.0	19.0	13.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R10P12 (Sh200)	Count	35.0	28.0	23.0	15.0	13.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R10P15(Nk150)	Count	67.0	14.0	11.0	13.0	9.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R10P5 (Nk150)	Count	67.0	15.0	12.0	10.0	10.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R11P11(Bi450)	Count	6.0	7.0	11.0	12.0	78.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R11P2 (Bi600)	Count	6.0	4.0	11.0	13.0	80.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R1P12 (Nk100)	Count	63.0	18.0	7.0	11.0	15.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R1P3 (Nk150)	Count	66.0	15.0	10.0	13.0	10.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R1P3-2 (Bi300)	Count	9.0	11.0	9.0	13.0	72.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R1P4 (Sh100)	Count	55.0	25.0	13.0	10.0	11.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R2P11(Sh200)	Count	33.0	32.0	18.0	19.0	12.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R2P9 (Nk150)	Count	65.0	8.0	15.0	15.0	11.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R3P1 (Bi600)	Count	12.0	6.0	15.0	13.0	68.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R3P4 (Sh100)	Count	16.0	24.0	31.0	24.0	19.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R4P1(Sh100)	Count	40.0	24.0	19.0	21.0	10.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R4P11 (Bi600)	Count	13.0	8.0	9.0	15.0	69.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R4P5 (Nk150)	Count	66.0	18.0	13.0	6.0	11.0
	Expected Count	38.1	15.1	14.3	15.2	31.3

Genotypes	Class	Number of pods per plant				
		Very Poor	Poor	Average	Good	Very Good
R5P1(Nk150)	Count	70.0	13.0	9.0	10.0	12.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R5P4 (Bi300)	Count	6.0	5.0	12.0	16.0	75.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R7P12 (Bi450)	Count	6.0	6.0	9.0	14.0	79.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R8P1 (Bi350)	Count	18.0	8.0	12.0	11.0	65.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R8P9 (Nk150)	Count	67.0	12.0	8.0	14.0	13.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R9P1 (Bi450)	Count	15.0	8.0	12.0	11.0	68.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R9P5 (Sh200)	Count	16.0	34.0	35.0	15.0	14.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
R9P9 (Nk100)	Count	65.0	10.0	15.0	15.0	9.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
Shindimba (0)	Count	46.0	21.0	13.0	16.0	18.0
	Expected Count	38.1	15.1	14.3	15.2	31.3
Chi-square test		Pearson X^2	df		P-value	
		1834.0	144.0		0.000	

df = degrees of freedom given by (v1-1)(v2-1) where v1 and v2 correspond to class/number of genotypes and scales, respectively and X^2 = Chi-Square.

6.3.2.2 Pod sizes

Highly significant differences ($P<0.001$; $X^2=1228.592$; df =144) were detected in the selection of the new varieties for their pod sizes (Table 0.4). The genotype R4P5 (Nk150) had better acceptance for its longer pod size and selected by 84% participating farmers. This was followed by the genotypes L1P12, R4P11, and R8P1.

6.3.2.3 Early maturity

Early maturity is a drought escape mechanism. Consequently, this trait is an important farmers' preferred attribute of cowpea varieties in northern Namibia. Farmers rating varied significantly ($P<0.001$; $X^2=593.820$; df =144) in their selection of the new varieties for early maturity (Table 0.5). The genotype R3P1 (Bi600) was rated as very good and early maturing by 82% of the participating farmers. Other early maturing and farmers selected varieties included R1P12 (Nk100), R8P1 (Bi350), and R2P9 (Nk100).

6.3.2.4 Grain colour

Grain colour is an important quality parameter in cowpea varietal selection. In the study areas farmers preferences of the varieties varied significantly ($P<0.001$; $X^2=1425.352$; df =144) for grain colour (Table 0.6). The following genotypes: L2P4 (Sh100), L2P7 (Sh100), L3P7-2

(Sh100), L3P74 (Sh100), L7P1 (Sh100), Nakare, P6R2 (Nk100), R10P10 (Sh100), R1P3 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R2P9 (Nk150), R4P1 (Sh100), R9P5 (Sh200), and

Table 0.4 Summary of chi-square tests associating rating of cowpea genotypes for pod size during participatory variety selection conducted in three villages (Onavivi, Onlao and Etunda) of Omusati Region in northern Namibia.

		Pod size				
Genotypes	**Class**	**Very Poor**	**Poor**	**Average**	**Good**	**Very Good**
L10P7 (Sh100)	Count	18.0	22.0	24.0	24.0	26.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
L1P12 (Bi450)	Count	0.0	9.0	12.0	11.0	82.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
L2P4 (Sh100)	Count	21.0	18.0	26.0	25.0	24.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
L2P7 (Sh100)	Count	15.0	26.0	20.0	27.0	26.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
L3P7-2 (Sh100)	Count	10.0	25.0	31.0	26.0	22.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
L3P74 (Sh100)	Count	12.0	20.0	21.0	29.0	32.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
L7P1 (Sh100)	Count	15.0	16.0	21.0	39.0	23.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
L9P7 (NK150)	Count	7.0	15.0	13.0	12.0	67.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
Nakare (0)	Count	2.0	0.0	15.0	15.0	82.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
P6R2 (Nk100)	Count	7.0	6.0	8.0	21.0	72.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
Parent (Bi0)	Count	0.0	16.0	16.0	9.0	73.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R10P10 (Sh100)	Count	18.0	24.0	17.0	28.0	27.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R10P12 (Sh200)	Count	12.0	24.0	34.0	23.0	21.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R10P15(Nk150)	Count	7.0	12.0	11.0	15.0	69.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R10P5 (Nk150)	Count	9.0	9.0	8.0	13.0	75.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R11P11(Bi450)	Count	0.0	9.0	6.0	8.0	91.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R11P2 (Bi600)	Count	0.0	9.0	10.0	15.0	80.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R1P12 (Nk100)	Count	7.0	9.0	7.0	13.0	78.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R1P3 (Nk150)	Count	12.0	9.0	9.0	16.0	68.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R1P3-2 (Bi300)	Count	2.0	11.0	13.0	16.0	72.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R1P4 (Sh100)	Count	14.0	26.0	30.0	31.0	13.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R2P11(Sh200)	Count	21.0	16.0	20.0	17.0	40.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R2P9 (Nk150)	Count	15.0	8.0	8.0	17.0	66.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R3P1 (Bi600)	Count	0.0	10.0	15.0	13.0	76.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R3P4 (Sh100)	Count	14.0	31.0	23.0	22.0	24.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R4P1(Sh100)	Count	11.0	23.0	27.0	32.0	21.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
R4P11 (Bi600)	Count	0.0	6.0	17.0	10.0	81.0

Genotypes	Class	Pod size				
		Very Poor	Poor	Average	Good	Very Good
R4P5 (Nk150)	Expected Count	9.8	13.8	15.6	18.0	56.7
	Count	9.0	9.0	6.0	6.0	84.0
R5P1(Nk150)	Expected Count	9.8	13.8	15.6	18.0	56.7
	Count	13.0	6.0	10.0	13.0	72.0
R5P4 (Bi300)	Expected Count	9.8	13.8	15.6	18.0	56.7
	Count	0.0	8.0	10.0	7.0	89.0
R7P12 (Bi450)	Expected Count	9.8	13.8	15.6	18.0	56.7
	Count	0.0	10.0	15.0	11.0	78.0
R8P1 (Bi350)	Expected Count	9.8	13.8	15.6	18.0	56.7
	Count	0.0	9.0	11.0	14.0	80.0
R8P9 (Nk150)	Expected Count	9.8	13.8	15.6	18.0	56.7
	Count	7.0	7.0	7.0	18.0	75.0
R9P1 (Bi450)	Expected Count	9.8	13.8	15.6	18.0	56.7
	Count	0.0	10.0	12.0	17.0	75.0
R9P5 (Sh200)	Expected Count	9.8	13.8	15.6	18.0	56.7
	Count	15.0	18.0	24.0	25.0	32.0
R9P9 (Nk100)	Expected Count	9.8	13.8	15.6	18.0	56.7
	Count	13.0	8.0	11.0	16.0	66.0
Shindimba (0)	Expected Count	9.8	13.8	15.6	18.0	56.7
	Count	57.0	18.0	11.0	12.0	16.0
	Expected Count	9.8	13.8	15.6	18.0	56.7
Chi-square test		Pearson X^2	df		P-value	
		1228.592	144		0.000	

df = degrees of freedom given by (v1-1)(v2-1) where v1 and v2 correspond to class/number of genotypes and scales, respectively and X^2 = Chi-Square.

R9P9 (Nk100) were rated as very good by all participating farmers. These genotypes had white grain colour. Farmers also selected genotypes R10P5 (Nk150), R1P12 (Nk100) and R3P1 (Bi600) which showed cream, chocolate and red colour, respectively (Table 0.6).

6.3.2.5 Grain size

There was highly significant difference ($P<0.001$; $X^2=561.090$; df =144) in the rating of the test genotypes for grain size (Table 0.7). The genotypes L3P74 (Sh100), P6R2 (Nk100), R1P3 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R2P9 (Nk150), R4P1 (Sh100) and R9P9 (Nk100) were rated as very good by all participating farmers due to the larger grain sizes.

6.3.2.6 Pod yield

Pod yield is an important selection criterion of cowpea varieties for their immature and tender pods or for final seed yield. There was a clear statistical difference ($P<0.001$; $X^2=1834.007$; df =144) among farmers selection of the new varieties for pod yield per plant (Table 0.8). The genotypes L1P12 (Bi450), L9P7 (Nk150) and R7P12 (Bi450) were promising pod yielders and rated very good by 72% participating farmers. Other important varieties bearing higher number of pods and selected by growers were R11P2 (Bi600), R5P1 (Nk150), and R10P15 (Nk150) (Table 0.3).

6.3.2.7 Pest tolerance

Cowpea is susceptible to a number of insect pests such *Aphis craccivora* (Koch) and *Callosobruchus maculatus* in northern Namibia. Farmers evaluation and selection showed significant difference ($P<0.001$; $X^2=2998.088$; df =144) towards insect pest tolerance of the new varieties Table 0.9). Interestingly, all the new cowpea varieties were rated as very good for their pest tolerance by all participating farmers compared to the local checks (Table 0.9).

6.3.2.8 Drought tolerance

Drought tolerant and productive cowpea varieties are the leading farmers' preferences in the farming systems of Namibia. Chi-square test suggested the presence of highly significant difference ($P<0.001$; $X^2=647.471$; df =144) in the selection of the test varieties for tolerance to drought (Table 0.10). The following genotypes: L3P7-2 (Sh100), L7P1 (Sh100), L9P7 (NK150), P6R2 (Nk100), R10P10 (Sh100), R10P12 (Sh200), R10P5 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R3P4 (Sh100), R4P1 (Sh100), R5P1 (Nk150) and R8P9 (Nk150) were rated to be drought tolerant by all participating farmers (Table 0.10).

Table 0.5 Summary of chi-square tests associating rating of cowpea genotypes for early maturity during participatory variety selection conducted in three villages (Onavivi, Onlao and Etunda) of Omusati Region in northern Namibia.

		Early maturity				
Genotypes	**Class**	**Very Poor**	**Poor**	**Average**	**Good**	**Very Good**
L10P7 (Sh100)	Count	17.0	23.0	22.0	26.0	26.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
L1P12 (Bi450)	Count	15.0	6.0	8.0	19.0	66.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
L2P4 (Sh100)	Count	22.0	22.0	19.0	27.0	24.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
L2P7 (Sh100)	Count	12.0	22.0	27.0	25.0	28.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
L3P7-2 (Sh100)	Count	13.0	24.0	30.0	29.0	18.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
L3P74 (Sh100)	Count	11.0	21.0	23.0	25.0	34.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
L7P1 (Sh100)	Count	21.0	15.0	16.0	21.0	41.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
L9P7 (NK150)	Count	6.0	15.0	11.0	16.0	66.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
Nakare (0)	Count	3.0	0.0	12.0	27.0	72.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
P6R2 (Nk100)	Count	10.0	15.0	12.0	15.0	62.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
Parent (Bi0)	Count	6.0	15.0	9.0	19.0	65.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R10P10 (Sh100)	Count	16.0	27.0	19.0	26.0	26.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R10P12 (Sh200)	Count	15.0	28.0	27.0	21.0	23.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R10P15(Nk150)	Count	6.0	12.0	13.0	20.0	63.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R10P5 (Nk150)	Count	12.0	10.0	12.0	14.0	66.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R11P11(Bi450)	Count	10.0	8.0	8.0	25.0	63.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R11P2 (Bi600)	Count	11.0	11.0	9.0	20.0	63.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R1P12 (Nk100)	Count	11.0	10.0	10.0	15.0	68.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R1P3 (Nk150)	Count	14.0	9.0	14.0	17.0	60.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R1P3-2 (Bi300)	Count	10.0	13.0	9.0	19.0	63.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R1P4 (Sh100)	Count	18.0	18.0	30.0	30.0	18.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R2P11(Sh200)	Count	16.0	17.0	16.0	25.0	40.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R2P9 (Nk150)	Count	15.0	7.0	7.0	21.0	64.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R3P1 (Bi600)	Count	8.0	4.0	6.0	14.0	82.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R3P4 (Sh100)	Count	12.0	27.0	25.0	25.0	25.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R4P1(Sh100)	Count	14.0	17.0	26.0	30.0	27.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R4P11 (Bi600)	Count	11.0	8.0	10.0	21.0	64.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R4P5 (Nk150)	Count	10.0	10.0	11.0	23.0	60.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R5P1(Nk150)	Count	16.0	9.0	15.0	23.0	51.0

Genotypes	Class	Early maturity				
		Very Poor	Poor	Average	Good	Very Good
	Expected Count	12.3	14.2	15.2	21.6	50.7
R5P4 (Bi300)	Count	12.0	8.0	12.0	20.0	62.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R7P12 (Bi450)	Count	9.0	13.0	9.0	19.0	64.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R8P1 (Bi350)	Count	12.0	12.0	6.0	18.0	66.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R8P9 (Nk150)	Count	8.0	13.0	13.0	18.0	62.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R9P1 (Bi450)	Count	9.0	9.0	12.0	17.0	67.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R9P5 (Sh200)	Count	18.0	23.0	30.0	25.0	18.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
R9P9 (Nk100)	Count	14.0	6.0	12.0	21.0	61.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
Shindimba (0)	Count	11.0	20.0	13.0	23.0	47.0
	Expected Count	12.3	14.2	15.2	21.6	50.7
Chi-square test		Pearson X^2	df		P-value	
		593.82	144		0.000	

df = degrees of freedom given by (v1-1)(v2-1) where v1 and v2 correspond to class/number of genotypes and scales, respectively and X^2 = Chi-Square.

Table 0.6 Summary of chi-square tests associating rating of cowpea genotypes for grain colour during participatory variety selection conducted in three villages (Onavivi, Onlao and Etunda) of Omusati Region in northern Namibia.

Genotypes	Class	Grain colour				
		Very Poor	Poor	Average	Good	Very Good
L10P7 (Sh100)	Count	0.0	0.0	21.0	18.0	75.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
L1P12 (Bi450)	Count	9.0	0.0	17.0	22.0	66.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
L2P4 (Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
L2P7 (Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
L3P7-2 (Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
L3P74 (Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
L7P1 (Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
L9P7 (NK150)	Count	14.0	9.0	14.0	25.0	52.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
Nakare (0)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
P6R2 (Nk100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
Parent (Bi0)	Count	11.0	9.0	12.0	18.0	64.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R10P10 (Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R10P12 (Sh200)	Count	15.0	9.0	11.0	20.0	59.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R10P15(Nk150)	Count	4.0	10.0	15.0	18.0	67.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R10P5 (Nk150)	Count	0.0	0.0	3.0	16.0	95.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R11P11(Bi450)	Count	6.0	14.0	18.0	22.0	54.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R11P2 (Bi600)	Count	12.0	11.0	10.0	25.0	56.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R1P12 (Nk100)	Count	0.0	0.0	12.0	14.0	88.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R1P3 (Nk150)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R1P3-2 (Bi300)	Count	16.0	0.0	2.0	25.0	71.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R1P4 (Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R2P11(Sh200)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R2P9 (Nk150)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R3P1 (Bi600)	Count	12.0	0.0	2.0	21.0	79.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R3P4 (Sh100)	Count	12.0	13.0	9.0	14.0	66.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R4P1(Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R4P11 (Bi600)	Count	6.0	15.0	8.0	21.0	64.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R4P5 (Nk150)	Count	6.0	11.0	15.0	8.0	74.0
	Expected Count	4.8	3.7	5.8	11.1	88.6
R5P1(Nk150)	Count	4.0	13.0	10.0	18.0	69.0

Genotypes	Class	Grain colour				
		Very Poor	Poor	Average	Good	Very Good
R5P4 (Bi300)	Expected Count	4.8	3.7	5.8	11.1	88.6
	Count	6.0	0.0	3.0	23.0	82.0
R7P12 (Bi450)	Expected Count	4.8	3.7	5.8	11.1	88.6
	Count	6.0	12.0	17.0	28.0	51.0
R8P1 (Bi350)	Expected Count	4.8	3.7	5.8	11.1	88.6
	Count	12.0	0.0	6.0	20.0.0	76.0
R8P9 (Nk150)	Expected Count	4.8	3.7	5.8	11.1	88.6
	Count	17.0	12.0	10.0	12.0	63.0
R9P1 (Bi450)	Expected Count	4.8	3.7	5.8	11.1	88.6
	Count	9.0	0.0	0.0	22.0	83.0
R9P5 (Sh200)	Expected Count	4.8	3.7	5.8	11.1	88.6
	Count	0.0	0.0	0.0	0.0	114.0
R9P9 (Nk100)	Expected Count	4.8	3.7	5.8	11.1	88.6
	Count	0.0	0.0	0.0	0.0	114.0
Shindimba (0)	Expected Count	4.8	3.7	5.8	11.1	88.6
	Count	0.0	0.0	0.0	0.0	114.0
Chi-square test		Pearson X^2	df		P-value	
		1425.352	144		0	

df = degrees of freedom given by (v1-1)(v2-1) where v1 and v2 correspond to class/number of genotypes and scales, respectively and X^2 = Chi-Square.

Table 0.7 Summary of chi-square tests associating rating of cowpea genotypes for grain size during participatory variety selection conducted in three villages (Onavivi, Onlao and Etunda) of Omusati Region in northern Namibia.

Genotypes	Class	Grain size				
		Very Poor	Poor	Average	Good	Very Good
L10P7 (Sh100)	Count	0.0	9.0	12.0	33.0	60.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
L1P12 (Bi450)	Count	48.0	0.0	17.0	13.0	36.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
L2P4 (Sh100)	Count	0.0	4.0	2.0	20.0	88.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
L2P7 (Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
L3P7-2 (Sh100)	Count	0.0	3.0	2.0	1.0	108.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
L3P74 (Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
L7P1 (Sh100)	Count	0.0	8.0	12.0	34.0	60.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
L9P7 (NK150)	Count	0.0	0.0	7.0	47.0	60.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
Nakare (0)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
P6R2 (Nk100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
Parent (Bi0)	Count	57.0	0.0	12.0	18.0	27.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R10P10 (Sh100)	Count	0.0	0.0	0.0	4.0	110.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R10P12 (Sh200)	Count	15.0	12.0	11.0	25.0	51.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R10P15(Nk150)	Count	6.0	0.0	8.0	36.0	64.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R10P5 (Nk150)	Count	0.0	0.0	8.0	15.0	91.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R11P11(Bi450)	Count	54.0	0.0	12.0	13.0	35.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R11P2 (Bi600)	Count	54.0	0.0	6.0	15.0	39.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R1P12 (Nk100)	Count	0.0	0.0	1.0	24.0	89.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R1P3 (Nk150)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R1P3-2 (Bi300)	Count	26.0	3.0	13.0	31.0	41.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R1P4 (Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R2P11(Sh200)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R2P9 (Nk150)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R3P1 (Bi600)	Count	39.0	0.0	14.0	17.0	44.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R3P4 (Sh100)	Count	18.0	20.0	10.0	12.0	54.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R4P1(Sh100)	Count	0.0	0.0	0.0	0.0	114.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R4P11 (Bi600)	Count	51.0	0.0	11.0	18.0	34.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R4P5 (Nk150)	Count	6.0	0.0	6.0	38.0	64.0
	Expected Count	14.5	2.0	6.6	15.6	75.2
R5P1(Nk150)	Count	7.0	0.0	5.0	36.0	66.0

Genotypes	Class	Grain size				
		Very Poor	Poor	Average	Good	Very Good
R5P4 (Bi300)	Expected Count	14.5	2.0	6.6	15.6	75.2
	Count	33.0	0.0	6.0	27.0	48.0
R7P12 (Bi450)	Expected Count	14.5	2.0	6.6	15.6	75.2
	Count	36.0	0.0	9.0	12.0	57.0
R8P1 (Bi350)	Expected Count	14.5	2.0	6.6	15.6	75.2
	Count	39.0	6.0	16.0	14.0	39.0
R8P9 (Nk150)	Expected Count	14.5	2.0	6.6	15.6	75.2
	Count	6.0	0.0	4.0	38.0	66.0
R9P1 (Bi450)	Expected Count	14.5	2.0	6.6	15.6	75.2
	Count	42.0	6.0	7.0	15.0	44.0
R9P5 (Sh200)	Expected Count	14.5	2.0	6.6	15.6	75.2
	Count	0.0	4.0	35.0	21.0	54.0
R9P9 (Nk100)	Expected Count	14.5	2.0	6.6	15.6	75.2
	Count	0.0	0.0	0.0	0.0	114.0
Shindimba (0)	Expected Count	14.5	2.0	6.6	15.6	75.2
	Count	0.0	0.0	0.0	0.0	114.0
Chi-square test		Pearson X^2	df		P-value	
		2561.09	144		0.000	

df = degrees of freedom given by (v1-1)(v2-1) where v1 and v2 correspond to class/number of genotypes and scales, respectively and X^2 = Chi-Square.

Table 0.8 Summary of chi-square tests associating rating of cowpea genotypes for pod yield during participatory variety selection conducted in three villages (Onavivi, Onlao and Etunda) of Omusati Region in northern Namibia.

		Pod yield				
Genotypes	**Class**	**Very Poor**	**Poor**	**Average**	**Good**	**Very Good**
L10P7 (Sh100)	Count	15.0	18.0	18.0	33.0	30.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
L1P12 (Bi450)	Count	6.0	7.0	13.0	16.0	72.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
L2P4 (Sh100)	Count	9.0	15.0	23.0	31.0	36.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
L2P7 (Sh100)	Count	6.0	18.0	25.0	32.0	33.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
L3P7-2 (Sh100)	Count	18.0	27.0	19.0	16.0	34.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
L3P74 (Sh100)	Count	3.0	10.0	12.0	32.0	57.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
L7P1 (Sh100)	Count	13.0	14.0	29.0	23.0	35.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
L9P7 (NK150)	Count	6.0	6.0	9.0	21.0	72.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
Nakare (0)	Count	0.0	0.0	20.0	27.0	67.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
P6R2 (Nk100)	Count	9.0	10.0	8.0	23.0	64.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
Parent (Bi0)	Count	6.0	7.0	12.0	27.0	62.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R10P10 (Sh100)	Count	16.0	24.0	20.0	21.0	33.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R10P12 (Sh200)	Count	15.0	26.0	28.0	21.0	24.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R10P15(Nk150)	Count	3.0	9.0	16.0	18.0	68.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R10P5 (Nk150)	Count	12.0	12.0	15.0	17.0	58.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R11P11(Bi450)	Count	6.0	7.0	12.0	27.0	62.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R11P2 (Bi600)	Count	9.0	10.0	12.0	12.0	71.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R1P12 (Nk100)	Count	8.0	11.0	14.0	19.0	62.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R1P3 (Nk150)	Count	15.0	9.0	12.0	17.0	61.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R1P3-2 (Bi300)	Count	9.0	8.0	12.0	17.0	68.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R1P4 (Sh100)	Count	12.0	24.0	15.0	22.0	41.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R2P11(Sh200)	Count	18.0	26.0	23.0	23.0	24.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R2P9 (Nk150)	Count	17.0	6.0	6.0	20.0	65.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R3P1 (Bi600)	Count	12.0	6.0	18.0	10.0	68.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R3P4 (Sh100)	Count	12.0	25.0	28.0	24.0	25.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R4P1(Sh100)	Count	9.0	27.0	30.0	25.0	23.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R4P11 (Bi600)	Count	12.0	7.0	12.0	18.0	65.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R4P5 (Nk150)	Count	6.0	7.0	8.0	22.0	71.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
R5P1(Nk150)	Count	12.0	6.0	6.0	21.0	69.0

Genotypes	Class	Pod yield				
		Very Poor	Poor	Average	Good	Very Good
R5P4 (Bi300)	Expected Count	10.8	13.2	16.2	20.4	53.3
	Count	15.0	8.0	15.0	15.0	61.0
R7P12 (Bi450)	Expected Count	10.8	13.2	16.2	20.4	53.3
	Count	3.0	9.0	18.0	12.0	72.0
R8P1 (Bi350)	Expected Count	10.8	13.2	16.2	20.4	53.3
	Count	12.0	12.0	15.0	7.0	68.0
R8P9 (Nk150)	Expected Count	10.8	13.2	16.2	20.4	53.3
	Count	9.0	10.0	8.0	14.0	73.0
R9P1 (Bi450)	Expected Count	10.8	13.2	16.2	20.4	53.3
	Count	15.0	6.0	7.0	21.0	65.0
R9P5 (Sh200)	Expected Count	10.8	13.2	16.2	20.4	53.3
	Count	15.0	30.0	31.0	24.0	14.0
R9P9 (Nk100)	Expected Count	10.8	13.2	16.2	20.4	53.3
	Count	14.0	6.0	16.0	12.0	66.0
Shindimba (0)	Expected Count	10.8	13.2	16.2	20.4	53.3
	Count	22.0	27.0	16.0	15.0	34.0
	Expected Count	10.8	13.2	16.2	20.4	53.3
Chi-square tests		Pearson X^2	df		P-value	
		675.781	144		0.000	

df = degrees of freedom given by (v1-1)(v2-1) where v1 and v2 correspond to class/number of genotypes and scales, respectively and X^2 = Chi-Square.

Table 0.9 Summary of chi-square tests associating rating of cowpea genotypes for insect pest tolerance during participatory variety selection conducted in three villages (Onavivi, Onlao and Etunda) of Omusati Region in northern Namibia.

Genotypes	Class	Pest tolerance		
		Poor	Average	Very Good
L10P7 (Sh100)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
L1P12 (Bi450)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
L2P4 (Sh100)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
L2P7 (Sh100)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
L3P7-2 (Sh100)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
L3P74 (Sh100)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
L7P1 (Sh100)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
L9P7 (NK150)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
Nakare (0)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
P6R2 (Nk100)	Count	0.0	38.0	76.0
	Expected Count	1.0	8.2	104.8
Parent (Bi0)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R10P10 (Sh100)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R10P12 (Sh200)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R10P15(Nk150)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R10P5 (Nk150)	Count	0.0	38.0	76.0
	Expected Count	1.0	8.2	104.8
R11P11(Bi450)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R11P2 (Bi600)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R1P12 (Nk100)	Count	0.0	38.0	76.0
	Expected Count	1.0	8.2	104.8
R1P3 (Nk150)	Count	0.0	38.0	76.0
	Expected Count	1.0	8.2	104.8
R1P3-2 (Bi300)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R1P4 (Sh100)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R2P11(Sh200)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R2P9 (Nk150)	Count	0.0	38.0	76.0
	Expected Count	1.0	8.2	104.8
R3P1 (Bi600)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R3P4 (Sh100)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
.0R4P1(Sh100)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R4P11 (Bi600)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R4P5 (Nk150)	Count	0.0	0.0	114.0
	Expected Count	1.0	8.2	104.8
R5P1(Nk150)	Count	0.0	0.0	114.0

Genotypes	Class	Pest tolerance		
		Poor	Average	Very Good
R5P4 (Bi300)	Expected Count	1.0	8.2	104.8
	Count	0.0	0.0	114.0
R7P12 (Bi450)	Expected Count	1.0	8.2	104.8
	Count	0.0	0.0	114.0
R8P1 (Bi350)	Expected Count	1.0	8.2	104.8
	Count	0.0	0.0	114.0
R8P9 (Nk150)	Expected Count	1.0	8.2	104.8
	Count	0.0	0.0	114.0
R9P1 (Bi450)	Expected Count	1.0	8.2	104.8
	Count	0.0	0.0	114.0
R9P5 (Sh200)	Expected Count	1.0	8.2	104.8
	Count	0.0	0.0	114.0
R9P9 (Nk100)	Expected Count	1.0	8.2	104.8
	Count	0.0	38.0	76.0
Shindimba (0)	Expected Count	1.0	8.2	104.8
	Count	38.0	76.0	0.0
	Expected Count	1.0	8.2	104.8
Chi-square tests		Pearson X^2	df	P-value
		2998.088	144	0.000

df = degrees of freedom given by (v1-1)(v2-1) where v1 and v2 correspond to class/number of genotypes and scales, respectively and X^2 = Chi-Square.

Table 0.10 Summary of chi-square tests associating rating of cowpea genotypes for drought tolerance during participatory variety selection conducted in three villages (Onavivi, Onlao and Etunda) of Omusati Region in northern Namibia.

		Drought tolerance			
Genotypes	**Class**	**Poor**	**Average**	**Good**	**Very Good**
L10P7 (Sh100)	Count	0.0	8.0	3.0	103.0
	Expected Count	2.4	3.5	1.2	106.9
L1P12 (Bi450)	Count	6.0	6.0	0.0	102.0
	Expected Count	2.4	3.5	1.2	106.9
L2P4 (Sh100)	Count	0.0	0.0	5.0	109.0
	Expected Count	2.4	3.5	1.2	106.9
L2P7 (Sh100)	Count	12.0	0.0	0.0	102.0
	Expected Count	2.4	3.5	1.2	106.9
L3P7-2 (Sh100)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
L3P74 (Sh100)	Count	1.0	10.0	4.0	99.0
	Expected Count	2.4	3.5	1.2	106.9
L7P1 (Sh100)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
L9P7 (NK150)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
Nakare (0)	Count	0.0	12.0	6.0	96.0
	Expected Count	2.4	3.5	1.2	106.9
P6R2 (Nk100)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
Parent (Bi0)	Count	6.0	0.0	0.0	108.0
	Expected Count	2.4	3.5	1.2	106.9
R10P10 (Sh100)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
R10P12 (Sh200)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
R10P15(Nk150)	Count	0.0	12.0	0.0	102.0
	Expected Count	2.4	3.5	1.2	106.9
R10P5 (Nk150)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
R11P11(Bi450)	Count	2.0	18.0	6.0	88.0
	Expected Count	2.4	3.5	1.2	106.9
R11P2 (Bi600)	Count	4.0	0.0	0.0	110.0
	Expected Count	2.4	3.5	1.2	106.9
R1P12 (Nk100)	Count	6.0	6.0	0.0	102.0
	Expected Count	2.4	3.5	1.2	106.9
R1P3 (Nk150)	Count	2.0	6.0	6.0	100.0
	Expected Count	2.4	3.5	1.2	106.9
R1P3-2 (Bi300)	Count	4.0	0.0	0.0	110.0
	Expected Count	2.4	3.5	1.2	106.9
R1P4 (Sh100)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
R2P11(Sh200)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
R2P9 (Nk150)	Count	2.0	0.0	0.0	112.0
	Expected Count	2.4	3.5	1.2	106.9
R3P1 (Bi600)	Count	5.0	0.0	0.0	109.0
	Expected Count	2.4	3.5	1.2	106.9
R3P4 (Sh100)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
R4P1(Sh100)	Count	0.0	0.0	0.0	114.0
	Expected Count	2.4	3.5	1.2	106.9
R4P11 (Bi600)	Count	2.0	0.0	0.0	112.0
	Expected Count	2.4	3.5	1.2	106.9
R4P5 (Nk150)	Count	0.0	12.0	4.0	98.0
	Expected Count	2.4	3.5	1.2	106.9
R5P1(Nk150)	Count	0.0	0.0	0.0	114.0

Genotypes	Class	Drought tolerance			
		Poor	Average	Good	Very Good
R5P4 (Bi300)	Expected Count	2.4	3.5	1.2	106.9
	Count	6.0	6.0	6.0	96.0
R7P12 (Bi450)	Expected Count	2.4	3.5	1.2	106.9
	Count	4.0	18.0	0.0	92.0
R8P1 (Bi350)	Expected Count	2.4	3.5	1.2	106.9
	Count	8.0	0.0	0.0	106.0
R8P9 (Nk150)	Expected Count	2.4	3.5	1.2	106.9
	Count	0.0	0.0	0.0	114.0
R9P1 (Bi450)	Expected Count	2.4	3.5	1.2	106.9
	Count	6.0	0.0	0.0	108.0
R9P5 (Sh200)	Expected Count	2.4	3.5	1.2	106.9
	Count	12.0	3.0	3.0	96.0
R9P9 (Nk100)	Expected Count	2.4	3.5	1.2	106.9
	Count	1.0	0.0	0.0	113.0
Shindimba (0)	Expected Count	2.4	3.5	1.2	106.9
	Count	0.0	12.0	0.0	102.0
	Expected Count	2.4	3.5	1.2	106.9
Chi-square test	Pearson X^2		df		P-value
	647.471		108		0.000

df = degrees of freedom given by (v1-1)(v2-1) where v1 and v2 correspond to class/number of genotypes and scales, respectively and X^2 = Chi-Square.

6.4 Discussion

The present study assessed field performance of 37 cowpea genotypes (34 elite mutants and 3 local checks) involving 114 farmers selected from three villages in Omusati Region of northern Namibia. The study determined farmers' preferred cowpea varieties using nine agronomic attributes through farmers' participation and indigenous knowledge. The results of this study are presented in Table 0.1 to Table 0.10.

6.4.1 Above ground biomass

The present study found that the test genotypes descended from the local variety Bira were favourably selected by all farmers (Table 0.2). The selected varieties showed very good above ground biomass which is a direct indicator of high biomass production. The importance of cowpea in the farming systems is well documented (Hillyer *et al.,* 2006; Kimiti and Odee, 2010; Horn *et al.,* 2015). Cowpea fresh biomass is an important farmers'-preferred agronomic trait in Namibia. Young and succulent leaves and stems of the crop is used as leaf vegetable. The dried biomass is used for livestock feed or left in the soil to enrich soil organic matter content (Nielsen *et al.,* 1997; Kapewangolo et al., 2007; Horn *et al.,*, 2015). Also in sub-African countries including Tanzania, Kenya and Nigeria cowpeas are used as leaf vegetable (Maredia *et al.,* 2000).

6.4.2 Number of pods per plant

The study identified the genotype R8P9 (Nk150) being very good for its higher pod setting ability which was selected by 64% of participants (Table 0.3). During this study, farmers indicated that the number of pod per plant was an important parameter when determining grain yield in cowpea. Grain yield was reported to be positively correlated with the number of pods per plant (Abadassi, 2015; Matikiti, 2015). Also, grain yield is believed to be determined by various components including the number of pods per plant, number of seeds per pod, and grain weight (Abadassi, 2015). However, during this study it was noted that farmers would opt for high above ground biomass and bushy genotypes instead of erect types even the latter had high pod setting ability. High biomass production of a cowpea variety is desired for food as leaf vegetable, feed as well as in soil organic matter improvement (Hillyer *et al.,* 2006; Kimiti and Odee, 2010).

6.4.3 Pod size

Most participating farmers (79%) selected the genotype R11P11 (Bi450) as the most suitable candidate with very good and larger pod size (Table 0.4). In Namibia, farmers prefer larger pod size bearing > 10 grains per pod (Horn *et al.,* 2015). Pod size is an important yield component determining the number of grains per pod and ultimately grain yield per plant (Abadassi, 2015; Matikiti, 2015).

6.4.4 Early maturity

In the current study the genotype R1P12 (Nk100) was found to be the most promising early maturing variety favourably rated by 59.7% of participating farmers Table 0.5). In Namibia and other arid and semi-arid countries, farmers prefer short cycle duration cowpea varieties which mature within 55 to 60 days after planting (Abadassi, 2015; Horn *et al.,* 2015).

6.4.5 Grain colour

Farmers selected a fairly large number of genotypes such as L2P4 (Sh100), L2P7 (Sh100), L3P7-2 (Sh100), L3P74 (Sh100), L7P1 (Sh100), Nakare (0), P6R2 (Nk100), R10P10 (Sh100), R1P3 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R2P9 (Nk150), R4P1 (Sh100), R9P5 (Sh200), and R9P9 (Nk100) for their white grain colour (Table 0.6). A wide range of grain colour such as white, red, black or speckled were recorded among the test genotypes. Farmers in Namibia have varied preferences to grain colour of cowpea varieties (Horn *et al.,* 2015). The majority of participating farmers indicated their preference to white grain. However, some famers indicated their willingness to adopt any new cowpea variety of other grain colour provided it has high yielding potential. In other African countries where marketing of cowpea is well established, farmers regarded grain colour as an important selection criterion affecting market potential and consumer preference (Cisse and Hall, 2003; Langyintuo *et al.,* 2003; Timko *et al.,* 2007; Matikiti, 2015).

6.4.6 Grain size

Grain size is an important trait considered by cowpea producers or consumers. In general, farmers prefer medium to large grain sizes of cowpea. Consequently, the following genotypes: L3P74 (Sh100), P6R2 (Nk100), R1P3 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R2P9 (Nk150), R4P1 (Sh100) and R9P9 (Nk100) were selected by all farmers for their larger grain size (Table 0.7). Most farmers in the study areas preferred larger grain sizes of cowpea (Horn *et al.,* 2015). Studies in West Africa also indicated that large grain black eye pea would fetch

premium price in the market than small sized grains (Langyintuo *et al.,* 2003; Timko *et al.,* 2007).

6.4.7 Pest tolerance

Insect pest infestation is one of the major constraints to cowpea production in Namibia (Horn *et al.,* 2015) and sub-Saharan Africa (Gbaguidi *et al.,* 2013). Yield losses reaching 100% are reported due to field and storage pests of cowpea (Cisse and Hall, 2003; Nabirye *et al.,* 2003; Dugje *et al.,* 2009; Gbaguidi *et al.,* 2013; Horn *et al.,* 2015). During the present study major pest incidences were not observed except in the local genotype Shindimba which succumbed to aphid infestation. Hence all the experimental genotypes were rated as very good in terms of pest tolerance by all participating farmers (Table 0.9). Variety Shindimba has coiled pod shape and susceptible to major pests, which are the major impediments to its production in Namibia. Therefore, the present study attempted to select varieties with long pod, white grain and insect pest tolerance.

6.4.8 Drought tolerance

The present study identified drought tolerant cowpea genotypes including L3P7-2 (Sh100), L7P1 (Sh100), L9P7 (NK150), P6R2 (Nk100), R10P10 (Sh100), R10P12 (Sh200), R10P5 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R3P4 (Sh100), R4P1 (Sh100), R5P1 (Nk150) and R8P9 (Nk150) (Table 0.10). Farmers indicated that they have lost landrace varieties of cowpeas due to low and erratic rainfall and prolonged droughts during the past years. A loss of landrace varieties and a lack of improved seeds has led most farmers to abandon cowpea cultivation (Horn *et al.,* 2015). Therefore, there is a need to breed improved cowpea cultivars for economic traits including drought tolerance.

6.5 Conclusions

Through participatory evaluation the present study selected the following ten farmers-preferred cowpea varieties: R9P5 (Sh200), R3P4 (Sh100), R4P1 (Sh100), L3P74 (Sh100), R1P12 (Nk100), R8P9 (Nk150), R5P1 (Nk150), R2P9 (Nk150), R10P5 (Nk150) and R11P2 (Bi600) for their larger seed size, white grain colour, high pod setting ability, insect pest tolerance, early maturity, longer pod size, drought tolerance and high above ground biomass and pod yields. The selected candidate lines will be subjected to distinct, uniformity and stability trials for varietal registration and release in northern Namibia.

6.6 References

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General overview of the thesis

7.1 Introduction and research objectives

In Namibia, cowpea (*Vigna unguiculata*) is the second most important crop next to pearl millet. About 95% of the smallholder farmers in the northern part of the country grow cowpea for food, feed and cash incomes. There is a lack of high yielding, drought and pest tolerant varieties of major food crops including cowpea in Namibia. Therefore, improved cowpea varieties are required for sustainable production and to ensure food security in the country. The value of newly bred cowpea variety depends on farmers' and end users' preferences. Cowpea varieties with enhanced yield, pest and disease resistance, drought tolerance and other desirable agronomic attributes would be possibly adopted by farmers and consequently by consumers and processors. Therefore, development of improved cowpea varieties is an overriding consideration for sustainable production and productivity in Namibia. This section presents the thesis overview and summarizes the research objectives and key findings of the study.

The objectives of this study were:

1. To assess farmers'-perceived production constraints, preferred traits, the farming system of cowpea, and their combined implications for breeding cowpea for northern Namibia.
2. To determine the ideal dose of gamma radiation to induce genetic variation in selected cowpea genotypes.
3. To identify desirable cowpea genotypes after gamma irradiation of three traditional cowpea varieties widely grown in Namibia including Nakare (IT81D-985), Shindimba (IT89KD-245-1) and Bira (IT87D-453-2) through continuous selection and selfing from M_2 through M_6 generations.
4. To determine G x E interaction and yield stability of elite mutant cowpea selections and to identify promising genotypes and representative test and production environments.
5. To select elite cowpea varieties that meet farmers' needs and preferences through farmers' participation and indigenous knowledge.

7.2 Summary of major findings

The first study focused on a survey using participatory rural appraisal tools. This was conducted across four selected regions of northern Namibia including Kavango East, Kavango West, Oshikoto and Omusati. Data was collected using structured interviews involving 171 households. The following were the main outcomes:

- About 70.2 % farmers grow local unimproved cowpea varieties.
- About 62.6% farmers reported low yields of cowpea varying from 100-599 kg/ha, while 6% achieved good grain harvests of 1500-1999 kg/ha.
- Most farmers (59.1%) produced cowpea for home consumption, while 23.4% indicated its food and market value'.
- Field pests such as aphids (reported by 77.8% respondents), leaf beetles (53.2%) and pod borers *(*60%) and bruchids (100%) were the major constraints to cowpea production.
- *Striga gesnerioides* and *Alectra vogelii* (Benth) were the principal parasitic weeds reported by 79.5% respondent farmers affecting cowpea production.
- Soil fertility level were very low across the study regions and all farmers did not apply any fertilizers on cowpea.
- Farmers-preferred traits of cowpea included a straight pod shape (reported by 61.4% respondents), a long pod size bearing at least 10 seeds (68.4%), white grain colour (22.2%) and higher above ground biomass (42.1%).
- Inter-cropping of cowpea with sorghum or pearl millet was the dominant cowpea farming system in northern Namibia.

The second study determined the ideal dose of gamma radiation to induce genetic variation in selected cowpea genotypes. Seeds of three introduced and released cowpea genotypes (Nakare [IT 81D-985], Shindimba [IT89KD-245-1] and Bira [IT87D-453-2] were gamma irradiated using seven doses (0, 100, 200, 300, 400, 500 and 600 Gy) at the International Atomic Energy Agency (IAEA), Austria. The following were the main outcomes:

- The optimum doses at LD_{50} for genotypes Nakare and Shindimba were at 150 and 200 Gy, respectively. Genotype Bira could tolerate increased dose of 600 Gy.
- Using simple linear regression model the LD_{50} based on percentage reduction of germination for genotypes Nakare, Shindimba and Bira were established to be 168.54, 194.88 and 671.37 Gy, respectively.

The third study identified desirable cowpea genotypes after gamma irradiation of three traditional cowpea varieties widely grown in Namibia including Nakare (IT81D-985), Shindimba (IT89KD-245-1) and Bira (IT87D-453-2) through continuous selections. Desirable mutants were selected from M_2 through M_6 generations. The following were the main outcomes:

- Substantial genetic variability was detected among cowpea genotypes after mutagenesis across generations including flowering ability, maturity, flower and seed colours and grain yields.
- Thirty four phenotypically and agronomically stable novel cowpea mutants were isolated at the M_6 derived from the above three parents. The selected promising lines were recommended for adaptability and stability tests across representative agro-ecologies for large-scale production or breeding in Namibia.

The fourth study determined the G x E interaction and yield stability of elite mutant cowpea selections and identified promising genotypes and representative test and production environments. In this study 34 selected and elite genotypes and three check varieties were evaluated at three testing sites (Bagani, Mannheim and Omahenene) over two cropping seasons providing six environments. The following were the main outcomes:

- Four promising mutant genotypes: G9 (ShL3P74), G10 (ShR3P4), G12 (ShR9P5) and G4 (ShL2P4) with better grain yields of 2.83, 2.06, 1.99 and 1.95, $t.ha^{-1,}$ in that order were identified.
- The following elite mutant selections designated as G4, G9, G10 and G12, all derived from the parental line Shindimba, were best grain yielders with straight pod shape desired by cowpea farmers in northern Namibia.
- The best environments in discriminating the test genotypes were Bagani during 2014/15 and Omahenene during 2014/15 production season.

The last study focused on participatory varietal selection using candidate cowpea genotypes in the northern Namibia. Field evaluations were conducted across three selected villages in Omusati Region of northern Namibia involving 114 participating farmers. The following were the main outcomes:

- The new genotypes derived from Bira (L1P12 (Bi450), R8P1 (Bi350), R9P1 (Bi450), R3P1 (Bi600), R5P4 (Bi300), R1P3-2 (Bi300), R7P12 (Bi450), R4P11 (Bi600), R11P11 (Bi450) and R11P2 (Bi600) were favourably selected by all participating farmers for their best above ground biomass.

- The genotype L1P12 (Bi450) was preferred by 81% of farmers for its higher pod setting ability. The genotypes R4P5 (Nk150) with longer pod size and R3P1 (Bi600%) with early maturity were ideal candidates preferred by 84% and 82% of famers, in that order.
- All participating farmers selected genotypes L2P4 (Sh100), L2P7 (Sh100), L3P7-2 (Sh100), L3P74 (Sh100), L7P1 (Sh100), P6R2 (Nk100), R10P10 (Sh100), R1P3 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R2P9 (Nk150), R4P1 (Sh100), R9P5 (Sh200) and R9P9 (Nk100) for their desirable white grain colour.
- L3P74 (Sh100), P6R2 (Nk100), R1P3 (Nk150), R1P4 (Sh100), R2P11 (Sh200), R2P9 (Nk150), R4P1 (Sh100), and R9P9 (Nk100) were selected by all respondents for their bigger grain size.
- Genotypes L1P12 (Bi450) and L9P7 (NK150) were rated very good providing higher pod yield.
- Overall, the following ten farmers-preferred cowpea varieties: R9P5 (Sh200), R3P4 (Sh100), R4P1 (Sh100), L3P74 (Sh100), R1P12 (Nk100), R8P9 (Nk150), R5P1 (Nk150), R2P9 (Nk150), R10P5 (Nk150) and R11P2 (Bi600) were selected with desirable traits such as larger seed size, white grain colour, high pod setting ability, insect pest tolerance, early maturity, longer pod size, drought tolerance, high biomass and pod yields.

7.3 Implications of the research findings to cowpea breeding for improved yield and related traits using gamma irradiation

The following implications for breeding were noted:

- Involving farmers in identification of their perceived production constraints, preferred traits and farming system of cowpea is very important to better enhance and speed the adoption process of improved varieties in the country. Therefore, farmers' views and priorities will be considered in cowpea breeding programme in Namibia.
- The findings on appropriate irradiation doses may assist as reference base to undertake large-scale mutagenesis of the selected cowpea genotypes to induce genetic variation for breeding.
- The selected novel cowpea genotypes are valuable genetic resources for future genetic enhancement and breeding.
- The selected promising genotypes can be recommended for adaptability and stability tests across representative agro-ecologies for large-scale production in Namibia or similar environments.