BREEDING FOR DROUGHT TOLERANCE IN COWPEA [*Vigna unguiculata* (L.) Walp.] USING MARKER ASSISTED BACKCROSSING

By

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DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

The potential of cowpea to address food security in Burkina Faso in particular is well established. However, there is limited information on drought tolerance and diversity in the germplasm in Burkina Faso and farmers' perceptions on the effects of drought and their varietal preferences are not known. The present study was, therefore, conducted to: (1) identify farmers' perceptions on the impact of drought on cowpea production and identify their preferences regarding cultivars and traits, (2) identify drought tolerant varieties in cowpea germplasm, (3) determine single nucleotide polymorphisms (SNPs) based genetic diversity in the cowpea germplasm, and (4) implement marker-assisted backcrossing to transfer yield and stay-green QTLs into Moussa local, a farmer preferred landrace. A participatory rural appraisal (PRA) was conducted to identify farmers' perceptions on the impact of drought on cowpea production. This study established that farmers have a deep knowledge about cowpea production constraints. Limited access to seed of improved variety was ranked as the most important constraint in all the areas where the study was conducted. Drought was classified among the four most important constraints to cowpea production in the three districts where the PRA was conducted. The preferred grain traits for all regions were white colour, large seeds with a rough texture for food and market purposes, except for the northern region where brown grain colour was preferred for food. The identification of drought-tolerant varieties in cowpea germplasm through field screening of fifty genotypes and the use of selection indices revealed wide genotypic variability among the tested germplasm. Biplot displays indicated that the genotypes could be grouped into four categories according to their drought tolerance and yielding ability as indicated below: high yielding-drought tolerant (group A), high yielding-drought susceptible (group B), low yielding-drought tolerant (group C), and low yielding-drought susceptible (group D). Genotypes like Djouroum local, KVx404-8-1, IT98K-1111-1, Gorom local, CB27, IT93K-693-2, Mouride, and KVx61-1 were clustered in group A, that is they were high yielding and drought tolerant. The stress tolerance index was the best criterion

for assessing genotypes for variability to drought tolerance because it enabled the identification of high yielding and drought tolerant genotypes. Genetic diversity was assessed using 181 SNP markers on 50 cowpea lines. The phylogenetic pattern of this germplam revealed seven clusters. The lines were almost grouped based on their geographical origin, and the breeding background. Thus, materials which originated from Burkina Faso were clustered in the same group while those from IITA/Nigeria were also almost all clustered in the same group. The genetic distance was low (≤0.29) suggesting a narrow genetic base in the cowpea germplasm used in this study. SNPs were efficient in the study of the diversity and a core collection of 20 lines was generated for further use in the breeding program. Marker-assisted backcrossing (MABC) was used to transfer QTLs for yield under drought and stay-green into Moussa local, a farmer preferred landrace. Two backcrosses assisted by SNP markers in foreground and background selections were sufficient to select for QTLs presence and to recover the background of Moussa local, the recurrent parent. The BC_3F_1s were selfed and six BC_3F_{2s} were evaluated for preliminary yield under drought stress and non-stress conditions. Out of the six, three MABC selected lines were promising and yielded better than the check and the parents. From these recombinant lines, several high yielding lines are likely to be developed for release in the near future. Most of them could be used in intercropping which will make great impact on cowpea production in Burkina Faso. In general, potential parents for genetic improvement for yield and drought tolerance were identified. However, further studies for assessing yield stability of cowpea genotypes are necessary and could be achieved by including more seasons and sites to get a better understanding of the genotype × environment interaction and yield stability of cowpea in Burkina Faso for all the materials identified including the MABC lines.

Key words: Burkina Faso, farmers, drought tolerance, cowpea, genotypes, genetic distance, SNPs, marker-assisted backcrossing, Participatory Rural Appraisal.

DEDICATION

I dedicate this thesis to:

Alice, my lovely wife I am grateful for all love, care, and advices you have been providing to Yiyé Yanis Ebenezer and Yissainè Jorès, our children.

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LIST OF ABBREVIATIONS

ABA: Abscisic Acid

AFLP: Amplified Fragment Length Polymorphism

AGRA: Alliance for Green Revolution in Africa

cDNA: complementary Deoxyribonucleic Acid

cM: Centimorgan

CRSP: Collaborative Research Support Program

DLS: Delayed leaf senescence

DM: Dry matter

DNA: DeoxyriboNucleic Acid

EST: Expressed Sequence Tag

EUW: Efficient Use of Water

FAO: Food and Agriculture Organization of the United Nations

FFF: Farmer Field Fora

FFS: Farmer Field School

FGD: Focus Group Discussion

GBS: Genotyping By Sequencing

GCP: Generation Challenge Programme

GGT: Graphical GenoType

GMP: Geometric Mean Productivity

He: Expected heterozygosity

HTTP: High-Throughput

IITA: International Institute of Tropical Agriculture

Indel: Insertion deletion

INERA: Institut de l'Environnement et de Recherches Agricoles

KASP/KASPAR: Kompetitive Allele Specific PCR

MAB: Marker-Assisted Breeding

MABC: Marker-Assisted Backcrossing

MAF: Major Allele Frequency

MARS: Marker-Assisted Recurrent Selection

MAS: Marker-Assisted Selection

MB: Molecular Breeding

MP: Mean Productivity

mRNA: messenger Ribonucleic Acid

NaClO: Sodium hypochlorite

OA: Osmotic Adjustment

PCA: Principal Component Analysis

PCR: Polymerase Chain Reaction

PIC: Polymorphic Information Content

PRA: Participatory Rural Appraisal

PVS: Participatory Variety Selection

QTL: Quantitative Trait Loci

RAPD: Random Amplified Polymorphism DNA

REML: Residual Maximum Likelihood

RFLP: Restricted Fragment Length Polymorphism

SAMPL: Sequence Amplified Microsatellite Polymorphism Locus

SCARs: Sequence Characterized Amplified Regions

SI: Stress Intensity

SNP: Single Nucleotide Polymorphism

SSI: Stress Susceptibility Index

SSR: Simple Sequence Repeat

STI: Stress tolerance index

TE: Transpiration Efficiency

TOL: Tolerance index

UCR: University of California Riverside

UK: United Kingdom

UPGMA: Unweighted Pair-wise Group Method

USA: United State of America

WUE: Water Use Efficiency

Ys: Yield under stressed conditions

Yw or Yp: Yield under non-stressed conditions

$\bar{Y}^2w$: Mean yield under non-stressed conditions

CHAPTER ONE

1.0. GENERAL INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most important grain legumes grown in the semi-arid regions of Africa. Out of the world's total production area of 14 million hectares, West Africa accounts for about 9 million ha (Singh *et al.*, 2003). Cowpea is mostly grown in the semi-arid region of West Africa (Ehlers and Hall, 1997) because of its large adaptation to climatic conditions. It is grown in over 9.5 million ha with a production of 2.9 million tons (Omo-Ikerodah *et al.*, 2005). In the USA, under optimum conditions, the average yield of cowpea is 7000 $kgha^{-1}$ whereas in Africa, average yield of the resource poor farmer is 300 $kgha^{-1}$ (Ehlers and Hall, 1997; Tignegre, 2010).

Burkina Faso is ranked amongst the top three cowpea producers in West Africa with a production of 580,000 tons in 2013 after Nigeria (2.50 million tons) and Niger (1.30 million tons) (Faostat, 2014). Cowpea production has increased from 276,349 tons in 2004 to 580,000 tons in 2013 with rapid decline in some years as a result of drought occurrence. Similarly, area under cultivation increased from 588,000 ha (2004) to 1,200,500 ha (2013) indicating that increase in production was associated with increase in area under cultivation. However, cowpea has contributed about 20 billion CFA (32,542,604 USD) to Burkina Faso's net domestic product for the last ten years (Statistika, 2002). It has recently been recognized by the government of Burkina Faso as a strategic crop that would contribute to achieving food security and alleviating poverty, due to its market potential.

Cowpea production is suitable for subsistence farming systems in which low inputs are involved due to its ability to thrive on relatively poor soil (Pasquet, 1999; Pronaf, 2003). It has high level of adaptation due to its inherent ability to withstand drought, tolerate shade, and fix atmospheric nitrogen (Singh, 1997). It is the first crop harvested during the cropping season before staple cereals crops (largely pearl millet and sorghum) and, therefore, referred to as a ‘‘hungry-season crop’’(Agbicodo *et al.*, 2009). It is an important cash-crop and source of nutrients (protein 23-25%) to the rural communities in tropical Africa (Ehlers and Hall, 1997; Ouedraogo, 2001). After harvesting the pods, the fodder is also harvested and used as feed for livestock. In terms of utilization, the diversity of diets based on cowpea, and the short cooking time renders cowpea popular for rural people and low income workers in towns (Tignegre, 2010). Leaves, fresh peas and fresh pods are also consumed (Ehlers and Hall, 1997). However, production is affected by several biotic and abiotic factors that lead to severe yield reduction at the smallholder farmer level (Ehlers and Hall, 1997).

Drought is one of the most important constraints threatening the food security of the world (Barters and Nelson, 1994). Cowpea production in Burkina Faso is hampered by recurrent drought. The rainfall patterns have been irregular and below normal throughout the semi-arid zones of West Africa including Burkina Faso. In the Sudan and Sahelian semi-arid regions, the frequency and intensity of drought have increased over the last 30 years (Hall *et al.*, 2003) due to climatic changes and human activities (Wittig *et al.*, 2007). Estimates on yield reduction due to terminal drought range from 21-30% between stressed and non-stressed conditions (Chiulele, 2010). However, yield losses in plant production depend on geographical region and length of cropping season (Sabaghpour *et al.*, 2006). Drought spells in farmers’ field has results in reduction of yields of

available genotypes. Most of these genotypes are susceptible to drought. Drought can strike at anytime, anywhere. Plants are most prone to damage due to limited water during flowering and pod setting stages (Bahar and Yildirim, 2010). Recently drought episodes (1984, 1991, 2004, and 2011) resulted in important crop yield losses and famine in Burkina Faso. The most recent drought was in 2011. In that year, a deficit of about 154462 tons was recorded in crop production (http://fr.allafrica.com/stories/) in Burkina Faso. Therefore, it is desirable to improve these adapted genotypes for tolerance to drought in order to obtain high and stable yields.

Until recently, the strategy for improving cowpea varieties in Burkina Faso for drought depended on the use of drought avoidance mechanism through the use of early maturing varieties. These varieties are meant to attain physiological maturity before drought occurs. However, the erratic rainfalls at the beginning and towards the end of the rainy season affect the early maturing varieties resulting in a substantial reduction in grain yield and total biomass production (Agbicodo *et al.*, 2009). Therefore, the use genetic tolerance would be the best alternative for reducing the effects of drought in cowpea. Despite the genetic variability existing within wild *Vigna* species, improved varieties, and landraces in Burkina Faso (Tignegre, unpublished data), no in-depth investigation has been done to determine their tolerance to drought. Studies on genetic variability and diversity in drought tolerance need to be conducted to assist in the identification of suitable parents to improved cowpea for drought tolerance.

Drought tolerance in cowpea is governed by multiple genes whose effects are often masked by or interact with the environment (Timko and Singh, 2008). As such, breeding for drought using conventional methodologies is not easy to achieve. Molecular markers can be used to identify

regions of the genome that harbor the genes that contribute to drought tolerance (Timko and Singh, 2008). If the most important genes can be tagged with molecular markers; they could be reliably introgressed into highly desirable cultivars that are susceptible to drought therefore, improving their tolerance to drought. The availability of high throughput genotyping platforms provides new opportunities for improvement of complex traits like drought tolerance through marker-assisted breeding (MAB).

Cowpea remained among "orphan crops" with limited genomic resources for long but significant genomic resources have recently been developed and made available to the public. These new development include high quality consensus genetic map (Muchero *et al.*, 2009a; Lucas *et al.*, 2011), high-throughput genotyping systems based on the Illumina GoldenGate and KBiosciences KASPAR systems, fingerprints of more than 600 potential parent lines, and a physical map (Http://Phymap.Ucdavis.Edu/Cowpea/, 2013). With the map density now available in cowpea (average marker density of 0.6 cM and no gap > 4 cM), in many cases it will be possible to identify flanking markers. By using markers that flank the target drought tolerance QTLs, linkage drag can be minimized. These advances in plant molecular genetics have provided plant breeders with powerful tools to identify and select Mendelian components underlying both simple and complex agronomic traits (Ribaut and Hoisington, 1998). SNP markers tightly linked to drought tolerance QTLs have been identified in cowpea (Muchero *et al.*, 2010). These markers will be useful in the introgression of drought tolerant genes into desirable cowpea genotypes using MAB.

The overall research goal of the study was to identify promising cowpea genotypes that are drought tolerant and high yielding that would contribute to food security.

The specific objectives were to:

- Determine farmer perceptions on the impact of drought in cowpea
- Identify sources of tolerance to drought stress in the cowpea germplasm
- Determine the SNP-based genetic diversity of a set of cowpea germplasm
- Introgress drought tolerant (stay green and yield) QTLs into Moussa local, a farmer preferred cowpea genotypes using marker-assisted backcrossing.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Introduction

This review focuses on tolerance of cowpea (*Vigna unguiculata* (L.) Walp.) to drought in the semi-arid areas of Africa. The importance of cowpea, drought as a constraint to cowpea production, conventional breeding approaches and the potentials of molecular breeding tools in developing drought tolerant cowpea varieties are also discussed.

2.1.1. Cowpea: Origin and domestication

The origin of the cultivated cowpea has been a matter of speculation and discussion for many years. Cowpea has been mentioned since the antiquity by Dioscoride, has been described by Linne from a cultivated species from Antilles as *Dolichos unguiculata*, then, *Vigna sinensis* and later became *Vigna unguiculata* (Faris, 1963; Pasquet and Baudoin, 1997). Early observations in cowpea revealed that cowpeas in Asia are different from cowpeas grown in Africa, suggesting that both Asia and Africa could be independent centers of origin for the crop (Timko and Singh, 2008). Based on cytological and morphological studies, it has been reported that Nigeria is the center of domestication of cowpea in West Africa (Faris, 1963). Some other studies confirmed that statement and indicated that *V. unguiculata* is from West Africa where some wild relatives are found at the edge of the forest (Pernes, 1984). Controversially, some studies using DNA markers, especially the amplified fragment length polymorphism (AFLP) profiles, led to propose domestication in North eastern Africa (Coulibaly *et al.*, 2002).

V. unguiculata has 22 chromosomes (2n= 2x= 22). The genus *Vigna* is pantropical and highly variable (Timko and Singh, 2008). This genus contains, in addition to cowpea, other members like mungbean (*V. radiata*), and the bambara groundnut (*V. subterranea*). The genus was initially divided into several subgenera based upon morphological characteristics, extent of genetic hybridization/reproductive isolation, and geographic distribution of species (Marechal *et al.*, 1978).

In contrast to many other important world crops, relatively little is understood about the domestication history, worldwide dispersal and distribution of genetic variation of cowpea (Huynh *et al*., 2013). The location of cowpea domestication in Africa is still uncertain. Different centers of origin and diversity have been proposed (Ba *et al.*, 2004). Evidence were provided based on molecular markers that early domestication occurred in northeastern Africa (Coulibaly et al., 2002). For Steele (1976), cowpea in these regions could have been domesticated together with sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum typhoides*) in the third millennium Before Christ. Some speculations support that cowpea may have followed the same route out of Africa as sorghum, moving first from eastern Africa to the Arabian Peninsula and then onto the Asian subcontinent (Faris, 1965; Pant et al., 1982) and to East Asia. For Tosti and Negri (2002), cowpea may have also moved to Europe from the Middle East because cowpea was known in southern Europe during Roman times. Therefore, it is plausible that cowpea first moved from western Africa to the New World with African people during the slave-trading period (Huynh *et al*., 2013), but little or no documentation exists to support the extent of this movement. Huynh et al. (2013) used SNP makers to examine the gene pool structure of African wild annual cowpea *V. unguiculata subsp. dekindtiana* from both East and West Africa and to determine their relatedness

to African wild cowpeas and non-African domesticated cowpeas. These authors found out the genetic materials into two gene pools. The two gene pools were distributed in two distinct geographical zones separated by the dense and vast rainforests of the Congo River basin (Figure 2.1). This region is too wet and not

Figure 2.1: Worldwide distribution and gene pool structure of cowpea landraces. Relative proportions of blue and red colors for each symbol represent the likelihood of an accession assigned to gene pools 1 and 2, respectively. (Source: Huynh *et al.* 2013).

2.1.2. Botany of cowpea

Cowpea is a self-pollinated crop with a little rate of outcrossing attributed to insect activities (Rachie and Roberts, 1974). The floral structure of cowpea is characterized by a symmetric flower with a style and a short beak (stigma) (Marechal *et al.*, 1978) (Figure 2.2). One flower contains ten stamens and each stamen carries one anther sac which contains pollen grains for pollination and fertilization. Flower opening occurs after pollination and fertilization, which reduces chances for out-crossings due to foreign pollen (Marechal *et al.*, 1978). The plant has large flower buds that facilitate the emasculation during the process of crosses. This makes crosses easier in cowpea as compared to other grain legume crops.

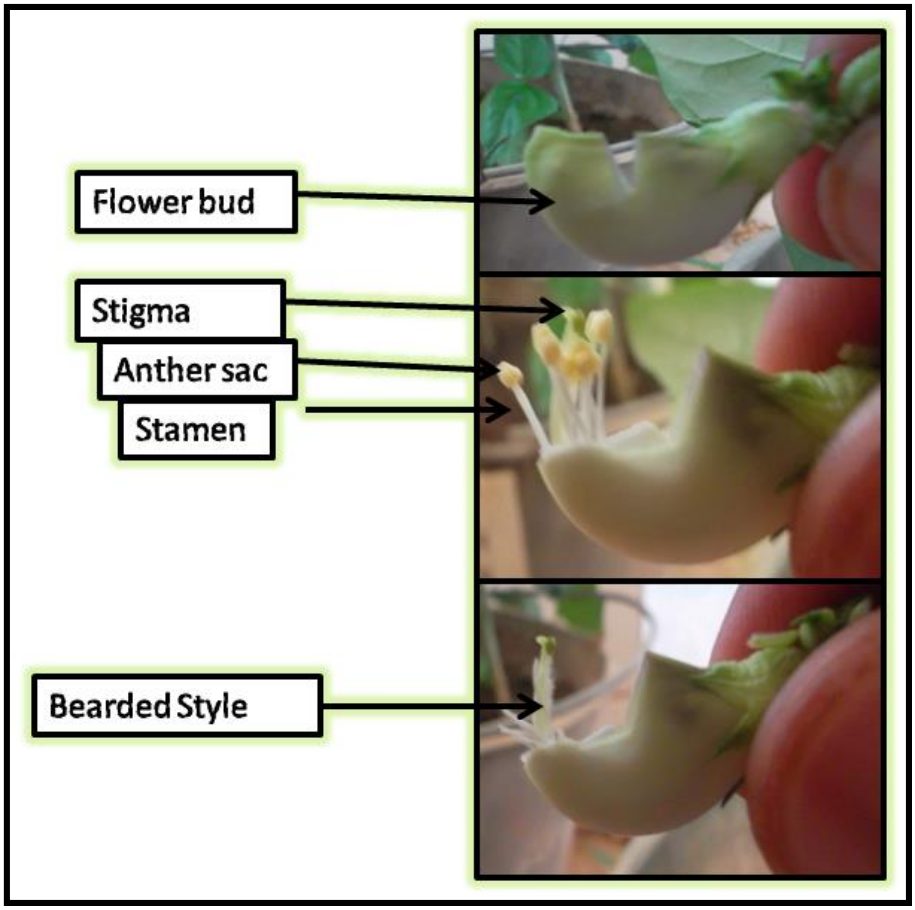

Figure 2.2: A cowpea flower showing female (stigma) and male (anthers) sex

The cowpea plant is a herbaceous, warm-season annual requiring temperature of at least 18°C throughout all stages of its development and having an optimal growing temperature of about 28°C (Craufurd *et al.*, 1997).

2.1.3. Cultivation and utilization of cowpea in Africa

Cowpea is of major importance to the livelihoods of millions of relatively poor people in less developed countries of the tropics (Quin, 1997). From the fresh leaves to the dry grain, the whole plant is utilized either for human consumption or animal feed. The production provides an important source of income to most rural families and is, therefore, considered as a cash crop for

rural people (Quin, 1997; Ouedraogo, 2001). The fresh young leaves and immature pods are used as vegetables, while various meal dishes are prepared from the dry grain. All the plant parts that are used for food are nutritious, providing protein, vitamins, and minerals (Quin, 1997; Timko and Singh, 2008). Cowpea grain contains, on average 23-25% protein, and 50-67% starch (Bressani, 1985; Carnovale *et al.*, 1990). The digestibility of protein is higher in cowpea than other legumes (Carnovale *et al.*, 1990; Marconi *et al.*, 1992). For these authors the protein digestibility ranges from 75-84% in wild relatives, with available protein ranging from 17 - 22%. After harvesting the pods, the aboveground parts of the plant are harvested and the haulms are used as a nutritious fodder for the livestock.

Cowpea consumption and processing in Burkina Faso has really evolved. A survey reported that cowpea was mentioned in 84% of household as a staple food (Konkobo *et al.*, 2002). In the same study, the authors reported that cowpea meals were known in a local tongue as "*yamleog ribo*" which means food of desire but have now evolved to the level of common food because all the households questioned in the survey eat cowpea in one or another way. Seventy eight percent (78%) of the household prepares cowpea mixed with rice or couscous (maize semolina) (Konkobo *et al.*, 2002). A lot of processors are using cowpea to enrich children's food and to make cakes, couscous etc. (Figure 2.3).

Figure 2.3: Different types of products made of cowpea or enriched with cowpea.

Going beyond its importance for food and feed, cowpea is important in farming systems in semiarid lands. That is because of its ability to fix nitrogen from the atmosphere and to withstand hard climatic conditions such as heat and drought (Hall *et al.*, 2002). One hectare of cowpea can bring to the soil about 40-80 kg of nitrogen (Quin, 1997).

2.1.4. Marketing and economics of cowpea in West Africa

Cowpea is the most economically important indigenous African legume crop (Langyintuo *et al.*, 2003). Examination of data from the FAO, national statistics, and the Bean/Cowpea Collaborative Research Support Program (CRSP), allowed these authors consolidate information on cowpea production, marketing and trade in West and Central Africa. The study showed that in the 1990s,

West and Central Africa produced about 70% of the world's cowpea in 80% of the area allocated to this crop in the world. During the same time period and specifically in 1998, Burkina Faso imported about 8000 tons of cowpea from Niger and exported a total of 5500 tons to Togo, Cote d'Ivoire, Ghana and Benin (Langyintuo *et al.*, 2003). At that period, the estimated cowpea production in Burkina Faso was higher than the demand with a surplus of about 146 000 tons (Langyintuo *et al.*, 2003) making the country a net exporter of cowpea. The available markets for the exports are Ghana and Cote d'Ivoire (Statistika, 2002; Langyintuo *et al.*, 2003).

2.1.5. Constraints to cowpea production

The most important causes of yield loss in cowpea are (i) insect pests, (ii) virus and fungal pests that attack the foliage and stems, including *Cowpea Aphid-Borne Mosaic Virus*, charcoal rot or ashy stem blight due to *Macrophomina phaseolina*, and pod and seedling diseases, (iii) drought and heat, (iv) the parasitic weed *Striga gesnerioides*, (v) low inherent yield potential of landraces, (vi) the unavailability of seed of improved varieties, and (vii) the inaccessibility to inputs such as pesticides (Singh and Tarawali, 1997; Tignegre, 2010). These constraints are classified as biotic and abiotic constraints for cowpea production (Singh and Jackai, 1985). The biotic constraints are:

- the pre-flowering insects (aphids)
- the post-flowering insects (thrips, maruca,)
- the storage insects (bruchids)
- The cryptogrammic, bacterial, and viral diseases
- *Striga gesneroides* and *Alectra vogeolii.*

The main abiotic constraints are:

- drought
- heat
- low soil fertility.

Drought is one of the most important constraints threatening the food security of the world (Barters and Nelson, 1994). Linsley *et al.* (1959) have defined drought as a sustained period of time without significant rainfall. Katz and Glantz (1977) differentiate between meteorological and agricultural drought. For these authors, a meteorological drought could be defined as a time period when the amount of precipitation is less than some designated percentage of the long term mean in one hand and in the other hand, an agricultural drought could be defined in terms of seasonal vegetation development. Agricultural drought occurs when there is not enough moisture available at the right time for the growth and development of crops through to harvest of the economic part of the plant (Magloire, 2005). Some genotypes escape drought (drought avoidance) while some others are drought tolerant (Mitra, 2001). Plant resistance to drought stress can be improved through drought avoidance or drought tolerance.

Drought avoidance is the ability of a plant to escape periods of drought, particularly during the most sensitive periods of its development due to earliness. Drought avoidance mechanisms tend to conserve water by promoting water use efficiency (WUE) (Blum, 2005, 2009; Amudha and Balasubramani, 2011). Although some genotypes can escape drought as a result of it being early this, however, is not the best because drought is not predictable. If such genotypes are subjected to drought they could be susceptible, therefore, it is important for genotypes should also

incorporate drought tolerance genes. The combination of drought avoidance and tolerance would be an effective strategy to improve cowpea for tolerance to drought. Drought tolerance as defined by Magloire (2005) is the ability of the plant to endure or withstand a dry period by maintaining a favorable internal water balance under drought conditions.

2.2. Challenges in breeding for drought tolerance

Success in breeding for drought tolerance in cowpea has not been as pronounced as for many other traits partly due to the lack of simple, cheap, and reliable screening methods to select drought tolerant plants and progenies from the segregating populations (Singh *et al.*, 1997). Drought tolerance is physiologically and genetically a complex trait whose expression depends on the magnitude and timing of stress in relation to plant-growth stage (Blum, 1996). It is by far the most important environmental stress in agriculture and many efforts have been made to improve crop productivity under water-limiting conditions (Cattivelli *et al.*, 2008). Drought studies typically distinguish between early-, mid- and late-season drought stress, all of which present unique challenges to plant growth and productivity. Late-season or post-flowering drought stress occurs during the grain filling stage causing depressed photosynthesis capacity leading to poor source development and diminished ability to allocate carbon toward the developing grain (Blum, 1996). One of the challenges in breeding for drought tolerance is the screening methodology. Different workers used different methods to evaluate genetic differences in drought tolerance (Bidinger *et al.*, 1982). These methods ranged from wooden boxes (Mai-Kodomi *et al.*, 1999a; Muchero *et al.*, 2008), pots and hydroponic (Ogbonnaya *et al.*, 2003) to simple field screenings (Singh *et al.*, 1999; Chiulele, 2010; Ishiyaku and Aliyu, 2013). However, progress in breeding cultivars for dry

environments has been slow, and selection for yield has been mainly achieved by testing advanced lines over several locations and years (Hall *et al.*, 1997; Cattivelli *et al.*, 2008). This is because of the need to assess the yield of large numbers of lines across several locations and years, and the substantial variation from the effects of environment, error and genotype x environment interactions. Therefore, breeding for drought tolerance is a challenging task because of the complexity of drought responses, environmental factors, and their interactions.

2.2.1. Mechanisms of drought tolerance in cowpea

All plant species respond to water stress by reducing transpiration by closing of its stomata and the reduction of leaf area (Amigues *et al.*, 2006). When plants are subjected to drought stress, a number of physiological biochemical and morphological responses have been observed and the magnitude of the response varies among species and between varieties within a crop species (Kramer, 1980). To avoid dehydration, cowpea ensures the maintenance of water absorption and the ramification of its roots system. Sarr *et al.* (2001) showed that water deficit in the soil is responsible for root elongation and ramification of the secondary roots in the deep humid parts of the soil allowing the optimization of water absorption. An important strategy of drought resistance in cowpea seems to rely on its capacity to regulate the expression of enzymes responsible for the degradation of membrane lipids (El Maarouf *et al.*, 1999). Roy-Macauley (1999) reported four genes controlling these mechanisms as two phospholipases C and D, one ascorbate peroxydase, and one other protein. Mai-Kodomi *et al*. (1999a) observed two types of drought tolerance response in cowpea screened in boxes. Type 1 tolerant line stopped growth and conserved moisture in all the plant tissues, stayed alive for over two weeks, and died gradually. The Type 2 line

continued slow growth of the trifoliate leaves and showed early senescence on unifoliates which dropped off with continued water stress but the growing tip remained turgid.

2.2.2. Physiological responses to drought

Plants respond by a number of physiological responses at the molecular, cellular, and whole-plant levels when subjected to water stress. Plant water use efficiency and osmotic adjustment are two major physiology mechanisms used by plants to respond to drought stress.

2.2.2.1.Plant water use efficiency

Water use efficiency (WUE) is a key factor determining plant productivity under limited water supply (Blum, 2009; Amudha and Balasubramani, 2011). This parameter is often used to imply that rainfed plant production can be increased per unit water used, resulting in 'more crop per drop'. Agronomically, WUE is defined as the ratio between total dry matter (DM) produced and water used (Jones, 1993). However, it is defined physiologically as the ratio between the rate of carbon fixed and the rate of water transpired. Water use efficiency (WUE), measured as the biomass produced per unit transpiration, describes the relationship between water use and crop production (Blum, 2009; Amudha and Balasubramani, 2011). In water-stress conditions, it would be important to produce a high amount of biomass, which contributes to crop yield, using a low or limited amount of water (Amudha and Balasubramani, 2011).

There are some concerns about WUE. Blum (2009), argues that selection for high WUE in breeding for water-limited conditions will most likely lead, under most conditions, to reduced yield

and reduced drought resistance. For this author, as long as the biochemistry of photosynthesis cannot be improved genetically, greater genotypic transpiration efficiency (TE) and WUE are driven mainly by plant traits that reduce transpiration and crop water-use. On the other hand, efficient use of water (EUW) implies maximal soil moisture capture for transpiration which also involves reduced non-stomatal transpiration and minimal water loss by soil evaporation (Blum, 2009). Therefore, EUW is a major target for yield improvement in water-limited environments and it is not just a coincidence that EUW is an inverse acronym of WUE because very often high WUE is achieved at the expense of reduced EUW.

2.2.2.2. Osmotic adjustment

Morgan (1984) defined the Osmotic adjustment (OA) as the net increase in intercellular solutes in response to water stress. The OA allows turgor maintenance at lower water potential. It has been considered as one of the crucial processes in plant adaptation to drought, because it sustains tissue metabolic activity and enables re-growth upon rewetting but varies greatly among genotypes. Plant productivity under arid conditions has been associated with OA in a number of species such as sorghum (Tangpremsri *et al.*, 1995).

2.2.3. Breeding for drought tolerance in cowpea

Plants response to drought stress could be assessed at the morphological, biochemical and physiological level (Hamidou *et al.*, 2007). Traits such as leaf area index, chlorophyll stability index, relative water content, diffusion pressure deficit (Yadava and Patil, 1984; Singh *et al.*, 1999), osmotic adjustment (Flower and Ludlow, 1987), carbon isotope discrimination, water use

efficiency, and root/shoot ratio (Hall *et al.*, 1990; Hall *et al.*, 1992; Ismail and Hall, 1992, 1993; Matsui and Singh, 2003) have been used as indices for drought tolerance. There are several screening methods available for selecting drought tolerant genotypes in cowpea. The most common are wooden boxes, pots, hydroponic and field screenings (Singh *et al.*, 1999; Ogbonnaya *et al.*, 2003). Agbicodo *et al.* (2009) reported that the wooden box is effective for screening cowpea at the seedling stage for drought tolerance when large number of lines are used. The authors reported that traits such as delayed leaf senescence (DLS), free proline levels, abscisic acid (ABA), stomatal conductance and chlorophyll florescence could be used as indices for drought tolerance.

Significant progress has been made at the International Institute of Tropical Agriculture (IITA) in the development of drought tolerant cowpea genotypes. Crosses made from Suvita 2 (Gorom local) in Kamboinse and Pobe-Mengao (Iita-Safgrad, 1984, 1985, 1986, 1987) showed transgressive segregation in the F_2 population but variety was released from these crosses. Singh and Matsui (2002) identified IT89KD-374-57, IT88DM-867-11, IT98D-1399, IT98K-131-1, IT97K-568-19, IT98K-452-1, and IT98K-241-2 as best drought-tolerant lines. Other drought tolerant genotypes identified include Gorom local and TN88-63 by Hamidou *et al.* (2007), and IT93K-503-1 and INIA-41 (Chiulele, 2010).

2.2.4. Genetics of drought tolerance in cowpea

Mating design is a term usually applied to schemes used in plant breeding programs to design crosses for specific purpose (Acquaah, 2007). In cowpea breeding the most commonly used mating designs are the diallel (Chiulele, 2010), North Carolina design II (Orawu, 2007; Alidu *et al.*, 2013), biparental mating design and generation mean analysis. (Mai-Kodomi *et al.*, 1999b) studied the

inheritance of drought tolerance in cowpea lines (TVu11986) with Type 1 drought tolerance, Dan Ila with Type 2 drought tolerance, and TVu7778 (susceptible) using a diallel. Drought screening was carried out using the wooden box method. The results revealed that both Type 1 (Rds1) and Type 2 (Rds2) reactions are controlled by a single dominant gene that is independent. Tests of allelism indicated that Type 1 is dominant over Type 2 and the F_2 population between the two types segregated 3 Type 1 : 1 Type 2 indicating that the genes Rds1 and Rds2 are either closely linked or are allelic at the same locus. Chiulele (2010) found that both additive and non-additive gene action were important for yield and yield components under drought. However, additive gene action was more important than non-additive gene action for days to flowering, number of pods per plant, number of seeds per pod and hundred seed weight. Chozin *et al.* (2006) reported delayed leaf senescence, stem diameter and leaf temperature were controlled by additive effects. These studies showed that variability exist in cowpea as far as drought is concerned.

2.3. Application of molecular markers in cowpea

Recent efforts have focused on the genetic dissection of drought tolerance through identification of markers defining quantitative trait loci (QTLs) with effects on specific traits related to drought tolerance (Agbicodo *et al.*, 2009). Advances in molecular genetics have led to the identification of multiple genes or genetic markers associated with genes that affect traits of interest in crop plants, including genes for single-gene traits and QTLs or genomic regions that affect quantitative traits. According to Dekkers (2004), this has provided opportunities to enhance response to selection, especially for traits that are difficult to improve by conventional selection due to their low heritability or traits for which measurement of phenotype is difficult and expensive. Biochemical

markers have been used to identify markers that are linked to the gene for resistance to *Aphis craccivora* in cowpea (Githiri *et al.*, 1996). Vaillancourt and Weeden (1992) used them to demonstrate the lack of isozyme similarity between *Vigna unguiculata* and other species of subgenus *Vigna*. Isozyme analysis has also been used by Pamella and Gepts (1992) to study the genetic relationships within *Vigna unguiculata.* Pasquet (1999) used allozyme variation in a study of the genetic relationships among subspecies of *V. unguiculata.*

Genetic maps have also been constructed for cowpea (Fatokun *et al.*, 1993; Fatokun *et al.*, 1997; Menendez *et al.*, 1997; Ouedraogo, 2001; Ouedraogo *et al.*, 2002) using DNA markers combined with morphological markers. Ouedraogo *et al.* (2012a) have also identified AFLP markers linked to some *Striga* resistant genes and converted them into SCARs for easy use in marker assisted selection. DNA markers have also been used for germplasm characterization (Coulibaly *et al.*, 2002) and assessing the genetic diversity in cowpea (Ba *et al.*, 2004). By comparing AFLPs, RAPD, and SAMPL (Sequence Amplified Microsatellite Polymorphic Locus) markers, Tosti and Negri (2002) have been able to study the diversity amongst farmer varieties of cowpea. Li *et al.* (2001) have used SSRs markers to study the genetic similarities and relationship between improved cowpea varieties and cultivars. SSRs used by Li *et al.* (2001) have also been used by (Batieno, 2008) to screen cowpea RILs to identify linkage between the markers and aphid resistance genes in cowpea. A modern consensus map has been developed (Muchero *et al.*, 2009a) and updated in April 2011 (Lucas *et al.*, 2011). The new consensus map contains 1,107 EST-derived SNP markers (856 bins) on 11 linkage groups (680cM).

Molecular markers have been extensively used in the study of drought tolerance. cDNAs homologous to alcohol dehydrogenase, dehydrin, NADPH-dependent aldehyde reductase, 12-oxo-phytodienoic acid reductase, 9-cis-epoxycarotenoid dioxygenase, and lipoxygenase were isolated (Luchi *et al.*, 1996a; Luchi *et al.*, 1996b) from a drought tolerant cowpea genotype. In the same way, Badiane *et al.* (2004) screened cowpea varieties by inducing water deficit and running RAPD analyses. QTLs have been mapped by Muchero *et al.* (2009b) for drought stress-induced premature senescence and maturity in cowpea. A restriction site polymorphism-based has also been used to investigate the co-location of candidate genes with QTLs for drought stress-induced premature senescence previously identified in cowpea (Muchero *et al.*, 2010). All these works reported here demonstrate the fast evolution of molecular methods used in cowpea. It is, therefore, possible to conclude that cowpea is no longer an orphan crop in the domain of molecular breeding.

2.4. Overview of DNA markers, QTLs mapping, and marker-assisted selection

The goal of plant breeding is to assemble desirable combinations of genes in new varieties. In the commonly used pedigree breeding method, selecting desirable plants begins in early generations for traits of high heritability. However, for traits of low heritability, selection is often postponed until the lines become more homozygous in later generations (F_5 or F_6) (Collard and Mackill, 2008). Selection of superior plants involves visual assessment for agronomic traits or resistance to stresses, as well as laboratory tests for quality or other traits. Based on the extent and complexity of selection required in breeding programs and the number and size of populations to be handled, one can easily appreciate the usefulness of new tools that may assist breeders in plant selection.

DNA marker technology, derived from research in molecular genetics and genomics, offers great promise for plant breeding. Reliability, quantity and quality of DNA, technical procedure for marker assay, level of polymorphism and cost are important factors of consideration in marker-assisted breeding (Collard and Mackill, 2008).

Simple sequence repeats (SSRs) or microsatellites were the most commonly used markers (Gupta *et al.*, 1999; Gupta and Varshney, 2000) because they are highly reproducible, co-dominant in inheritance, relatively simple and cheap to use and generally highly polymorphic. But, they require polyacrylamide gel electrophoresis and generally give information only about a single locus per assay, although multiplexing of several markers is possible (Collard and Mackill, 2008). In recent years, a novel class of markers named single nucleotide polymorphism (SNPs) has emerged as an important tool in plant genomics and it is increasingly used as molecular markers for various applications in several laboratories (Jehan and Lakhanpaul, 2006). SNPs are markers that have a single base pair position in the genomic DNA in which different alleles exist in normal individuals in some populations. SNP markers derived from specific DNA sequences quantitative trait loci (QTLs) are cheaper and more useful for marker-assisted selection (MAS) and have become the marker of choice when high-throughput genotyping assays have been developed. The last two decades witnessed the widespread use of molecular markers to study complex quantitative traits in different crop species (Bernardo, 2008). Thus, the aggressive use of marker-based selection in a breeding program will eventually lead to large amounts of marker and phenotypic data.

MAS and marker-assisted backcrossing (MABC) have been the first successful wide-scale implementations of marker technology in breeding programs (Delannay, 2009). These

methodologies work by using markers linked to specific traits to quickly and effectively select plants carrying those traits in segregating populations. The main benefit of MAS is to provide quick and accurate genotyping of progenies for traits that are typically hard or laborious to phenotype accurately. MABC aims at introgressing a mapped trait from a donor parent to a recurrent parent via multiple backcrossing steps in order to convert the recurrent parent with the new trait. The use of markers in such case allows a clean transfer of the trait with a minimum amount of donor parent genetic material being carried along (foreground selection), and facilitates the quick recovery of the recurrent parent background in a reduced number of backcrossing generations (background selection). Unlike the conventional backcross breeding, the MAB method can be viewed as a four-step selection process to quickly recover the recurrent parent genotype (Frisch *et al.*, 1999). This includes (1) selecting individuals carrying the targeted alleles, (2) selecting individuals homozygous for the recurrent parent genotype at loci flanking the target locus, (3) selecting individuals homozygous for recurrent parent genotype at remaining loci on the same chromosome comprising the targeted allele, and (4) selecting individuals that are homozygous for the recurrent parent genotype at most loci (across the whole genome) among those that remain. A MABC project consists of two main parts being performed in tandem (Delannay, 2009):

(i) Selection of plants carrying the target trait from the donor parent through the successive backcrossing generations (foreground selection)

(ii) Among those plants carrying the target trait, identification at each generation of individuals carrying the largest representation of the genome of the recurrent parent (background selection).

Plant breeders are using molecular markers extensively to increase the efficiency of their backcross breeding programs (Fatmi, 1999). The use of molecular markers reduces the number of backcross generations and the time required to recover a very high level of similarity to the recurrent parent, and insures that no large, unwanted segments of donor parent genome remain intact. MABC has proven especially effective for incorporating genes into commercially desirable lines and varieties. Zhao *et al.* (2012) reported that *qHSR1*, a major quantitative trait locus for resistance to head smut in maize was successfully integrated into ten high-yielding inbred lines that were susceptible to head smut. Each of the ten high-yielding lines was crossed with a donor parent Ji1037 that contains *qHSR1* and is completely resistant to head smut, followed by five generations of backcrossing to the respective recurrent parents. A marker linked at 0.7 cM to the *Yd2* gene for resistance to barley yellow dwarf virus was successfully used to select for resistance in a marker- assisted backcrossing methods in barley (Jefferies *et al.*, 2003). In maize, MABC was also successfully employed to improve complex traits such as grain yield. By using MABC, six chromosomal segments each in two elite lines (Tx303 and Oh43), were transferred into two inbred lines (B73 and Mo17) with three generations of backcrossing followed by two generations of selfing (Stuber *et al.*, 1999). Then, the improved lines with better performance were selected based on initial evaluations of testcross hybrids. The single-cross hybrids between improved B73 x improved Mo17 yielded better than the check hybrids by 12-15% (Stuber *et al.*, 1999). Semagn *et al.* (2006) provided a detailed review on the progress and prospects of MABC in crop breeding. Based on the successful use of MABC in maize and in some other crops not reported here, it is, possible to use similar method in cowpea to introgress drought-tolerant genes into farmer preferred cowpea genotypes in Burkina Faso that are susceptible to drought.

CHAPTER THREE

3.0. FARMERS' PERCEPTION OF DROUGHT IMPACT ON COWPEA

3.1. Introduction

In the past, plant breeders have not engaged farmers in the development of new varieties. Consequently, a lot of work was done and the process of adoption of many new varieties failed. These failures were imputable to the fact that those farmers' and consumers' preferences were not taken into account in the process of varietal development. In Burkina Faso, the current extension system is based on the classical training and visit approach (Tignegre, 2010). In this system, the agricultural extension agents are linked to the farmers and the researchers for the release of new varieties. The failure to meet farmers' and consumers' needs by researchers might be the reason for which food security is still a major problem in developing countries. It is a waste of time and effort when farmers reject a variety at the end of the selection process, because their own criteria have not been taken into account (Chiulele, 2010; Tignegre, 2010). Farmers' needs and preferences must be taken into account in the whole process of variety development. A better understanding of such issues could help researchers to define their own role in the research process, acknowledge the strengths and weaknesses of their own and farmers' approaches, overcome communication gaps, and find novel solutions for problems that typically occur in the process of participatory technology development (Efisue, 2006; Hoffmann *et al.*, 2006; Chiulele, 2010). To cope with these needs and preferences, the information must be collected with farmers using bottom to top approaches. Farmer participatory evaluation can produce valuable feedback for breeders and

agronomists for the perception of research results and open new research areas (Kitch *et al.*, 1997; Saidou *et al.*, 2011).

Recently, in Burkina Faso, some participatory rural appraisal (PRA) studies were conducted to identify cowpea production constraints (Pronaf, 2003). The objectives of these studies were to evaluate the social and economic impacts of cowpea technologies and this showed that the income generated by cowpea at Donsin had increased from 0.0% (1990) to 14.1% (2001). Later on, another PRA was conducted to study cowpea production system, the importance and production constraints of cowpea, farmers' perception of *Striga gesnerioides* (Willd)Vatke and farmers' preferred traits (Tignegre, 2010). The results showed that drought was always mentioned as an important constraint to cowpea production. As such, drought was ranked third at Donsin. A new participatory method has been implemented with success. This approach is known as farmer field schools (FFS) or fora (FFF) (Braun and Duveskog, 2008). The FFS technology has been used as a means for technology transfer in farmers' fields (Nathaniels, 2005).

The crop is of major importance to the livelihoods of millions of relatively poor people in less developed countries of the tropics (Bressani, 1985; Carnovale *et al.*, 1990; Marconi *et al.*, 1992; Quin, 1997). The production has really doubled between 2001 (300 000 T) and 2010 (600 000 T) with same trends for areas covered during the same period (Countrystat, 2012).

In terms of cowpea exports, Burkina Faso is a net exporter, which generates income to cowpea farmers (Statistika, 2002; Langyintuo *et al.*, 2003). Ghana and Cote d'Ivoire are the major markets for cowpea exports (Statistika, 2002; Langyintuo, 2004).

Regarding drought management in Burkina Faso, some local techniques have been improved by researchers. Some of these techniques are the *zai*, the half-moon-shaped, and the rock-fenced techniques. All these techniques are used to allow a good infiltration of water from rains into the soil and reduce soil erosion. In addition, the government of Burkina Faso has implemented a cloud seeding program to induce artificial rains and to ensure that sufficient water is retained in dams for off-season cropping.

In view of the importance of the farmers' needs and preferences, farmers' perceptions of drought and drought tolerant varieties have to be captured and included in variety development. In addition to drought tolerance, sometimes, farmers have certain preferences that breeders may ignore. In the most important cowpea growing regions of Burkina Faso, farmers are aware of variety's traits and have peculiar preferences based on these traits. They adopt production practices that preserve these traits. Therefore, a participatory rural appraisal sets as a way for learning farmers' knowledge in order to include them in the breeding objectives. Therefore, in order to capture farmers' preferences and incorporate their views into a cowpea breeding program, it was necessary to conduct a participatory rural appraisal. This is expected to increase the adoption of the developed cowpea varieties. The objective of this study was to determine farmers' perceptions about the effects of drought in cowpea production, to identify and ranked the main constraints to cowpea production, and to identify farmer preferred varieties based on traits like yield, tolerance to drought and income generation. The identification of farmers' preferred traits were not part of this study. They are already known from the studies conducted by Pronaf (2003) and Tignegre (2010).

3.2. Materials and Methods

3.2.1. Study sites

Information on the participatory rural appraisal (PRA) research sites are presented in Table 3.1.The study was made in the most important cowpea production areas of Burkina Faso. The PRA was conducted in the villages of Donsin in the district of Ziniare, Pobe-Mengao in the district of Djibo, and Pissila in the district of Kaya. These three villages are distributed across two agro-ecological zones (Sahel and Sudan savanna) of the country where drought problem is recurrent.

Table 3.1: General information on the PRA research sites.

	Villages		
	Pobé	**Pissila**	**Donsin**
Region	Sahel	Centre-Nord	Plateau Central
Province	Soum	Sanematenga	Oubritenga
District	Djibo	Kaya	Ziniare
Latitude	N $13^0$54.190'	N $13^0$09.829'	N $12^0$35.426'
Longitude	W $001^0$45.691'	W $000^0$49.549'	W$001^0$25.048
Altitude (m)	334	321	297
Rainfall (mm)	400-600	500-700	600-900

3.2.2. Focus group discussion

Focus group discussions, pair-wise ranking, and participatory variety selection were used as PRA methodologies in this study for data collection. For the focus group discussions (FGDs), a random sample of one to five villages represented here by the name of the major village was used for the FGDs in each district. In each village, the FGDs were conducted with cowpea farmers (between 20 and 25 per group). When the number of participants exceeded 25 in a district, it was split into two groups. Where it was possible, about five to seven women were included in the groups to get the perceptions from both men and women. The need to have both women and men contacted was to allow the researcher to obtain perceptions on the characteristics of the cowpea from production to storage and marketing. A total of 87 cowpea farmers were involved in this PRA focus group discussion (25, 22 and 40 farmers from Pobe-Mengao, Pissila, and Donsin, respectively). Two extension specialists from the targeted zones were included to make a link with developers. The group of participants was guided by a moderator (or group facilitator), who introduced the topics for discussion and helped the group to participate in a lively and natural discussion amongst themselves.

3.2.3. Pair-wise ranking

The pair wise ranking technique was used to rank cowpea production constraints. In this study, the different constraints encountered by farmers in cowpea production in each village were first listed. This list was made by farmers themselves and then, each constraint was compared to the others.

3.2.4. Participatory variety selection

Participatory variety selection (PVS) sessions were held with the same groups as in the FGDS. Therefore, 25, 22 and 40 farmers were involved in the PVS in the village of Pobe-Mengao, Pissila, and Donsin, respectively. Two koligrammes of cowpea seed samples per variety were presented to farmers for the purpose of selection. Matured fruits of *neem* plant *(Azadirachta indica) or* stones were used to help farmers for quantifying varieties and traits they preferred. The ranking of cowpea genotypes for desirable selection criteria were done by giving hundred fruit of *neem* plant *(Azadirachta indica)* or stones to one farmer (Figure 3.3). The preferred variety or trait was given the highest number of fruits or stones, while the rejected variety or trait was given zero or few number of fruits or stones. The rank (percentage) was obtained by counting the number of fruits with regards to the variety or trait. High number of allocated fruits meant that farmers approved the option. Data on different traits were also recorded individually for farmers' preference (yield "productivity", storage ability, tolerance to drought, susceptibility to *Macrophomina*, and marketability of cowpea grain) and the reasons they were preferred. Percentages for favorable cases for all traits were calculated for each site.

3.3. Results

3.3.1. Constraints to cowpea production

Constraints to cowpea production were identified and ranked by farmers at the different sites. The unavailability of cowpea improved variety seed was the first problem mentioned in cowpea production at all sites (Table 3.2). At Pobe-Mengao, the unavailability of improved variety seeds was immediately followed by drought due to the infrequent rains and short rainy season. In this

area, farmers namely also described four drought components occurring during the cropping season, planting period, flowering period, pod setting period, and the unpredictable drought or intermittent drought. At Donsin and Pissila, the limited access to improved variety seeds was rather followed by soil fertility issues but drought was always mentioned as a principal constraint for cowpea production in all the sites. Farmers at Donsin indicated that cowpea storage was no longer an issue due to the use of the triple bagging technology provided by INERA and the University of Purdue. When asked about the kind of breeding materials farmers are waiting at all sites they unanimously pointed to materials that are tolerant to terminal drought. For them, they can manage the other intermittent drought components by multiple plantings.

Table 3.2: Cowpea production constraints ranked by farmers at three districts.

RANKING	DONSIN	POBE-MENGAO	PISSILA
1	LIMITED ACCESS TO IMPROVED VARIETY SEEDS	LIMITED ACCESS TO IMPROVED VARIETY SEEDS	LIMITED ACCESS TO IMPROVED VARIETY SEEDS
2	SOIL FERTILITY	DROUGHT	SOIL FERTILITY
3	FERTILEZER/INSECTICIDE	SOIL FERTILITY	DROUGHT
4	DROUGHT	FERTILEZER/INSECTICIDE	EQUIPMENTS
5	EQUIPMENTS	STRIGA	STRIGA
6	MACROPHOMINA	EQUIPMENTS	INSECTS
7	MARKET	CABMV	FERTILEZER/INSECTICIDE
8	STRIGA	MARKET (for extra production)	MARKET (for extra production)
9	INSECTS	INSECTS	CABMV
10	PYTHIUM	PYTHIUM	
11	CABMV		-
12	FIELD (new airport under construction)	-	-

3.3.2. Drought symptoms and impact on cowpea production

Perceptions on the symptoms induced by drought on cowpea plants, the impact of drought on cowpea production, and the consequences on cowpea farmers are shown in Table 3.3. The symptoms as well as the consequences and the impact were brainstormed by the farmers during the focus group discussions (FGDs) at the three site. Table 3.3 shows the summary of the three studied sites (Pobe-Mengao, Donsin, and Pissila).

The main symptoms identified flower abortion, poor pod and grain filling, plant death, and early senescence that affected the production by reducing grain and fodder yield, disturbed the planning of the cropping seasons and cause genetic erosion of landraces. The consequences of drought manifest in reduced income levels, poverty and famine which limits farmers' capacity to take care of their families.

Table 3.3: Perceptions of farmers on impacts of drought on cowpea production in three districts[a].

Symptoms in cowpea field	Impacts on cowpea production	Consequences on cowpea farmers
Poor pod filling	Grain yield reduction	Famine
		Poverty
	Fodder yield reduction	Indebtedness
Poor grain filling	Total loss of crop and landraces	Loss of income
		Children schooling
	Difficulties in planning cropping	compromised
Reduction in branching	season	Rural exodus
Reduction in grain weight	Loss of arable land	Begging
Green seeds		
Leaf yellowing		
wilting of plants		
Plant death		
Dropping of leaves (senescence) and flowers		
Shortening of plant cycle		
Breaking of grains during threshing		

[a]: Donsin, Pobe-Mengao, and Pissila

3.3.3. Cowpea participatory variety selection

Four to five criteria were used in the cowpea participatory variety selection. These criteria are the productivity of the variety, the marketability, the ability in drought tolerance and/or susceptibility to *Macrophomina*, and the storage ability. Grains of cowpea varieties that were already grown by farmers and known by them and new varieties that are about to be released were presented to farmers for the purpose of variety selection at all three sites.

The PRA results at this site are shown in Figure 3.1. In terms of drought tolerance, variety KVx61-1 was ranked first at Pobe-Mengao. The most drought-susceptible variety was variety KVx414-22-2 at Pobe-Mengao. For marketability, the same variety KVx61-1 ranked well at Pobe-Mengao. It was ranked first by women's group with a score of 56% and second by men's group with a score of 28%. In the men's group, variety KVx396-4-5-2D was ranked first with a score of 54% and third in the women's group (12%). The marketability of variety KVx745-11P, a dual purpose variety was the lowest among the ranked varieties with a score of 4% in men's group and it was not even ranked by women's group.

In terms of productivity, variety KVx61-1 was ranked first by the men's group with a score of 50% and was the only productive variety selected ranked by the women's group and scored 100%. It was followed in the men's group by varieties KVx396-4-5-2D (30%) and KVx414-22-2 (14%).

For storage, variety Gorom local (42%) was ranked first in the women's group followed by varieties KVx61-1 (30%), KVx745-11P (18%), and KVx396-4-5-2D (10%). The three remaining varieties were not classified by women farmers. The men's group ranked KVx396-4-5-2D (36%)

as first variety followed by varieties KVx396-4-4 (24%), Gorom local (16%), KVx61-1 (12%), and KVx414-22-2 (12%).

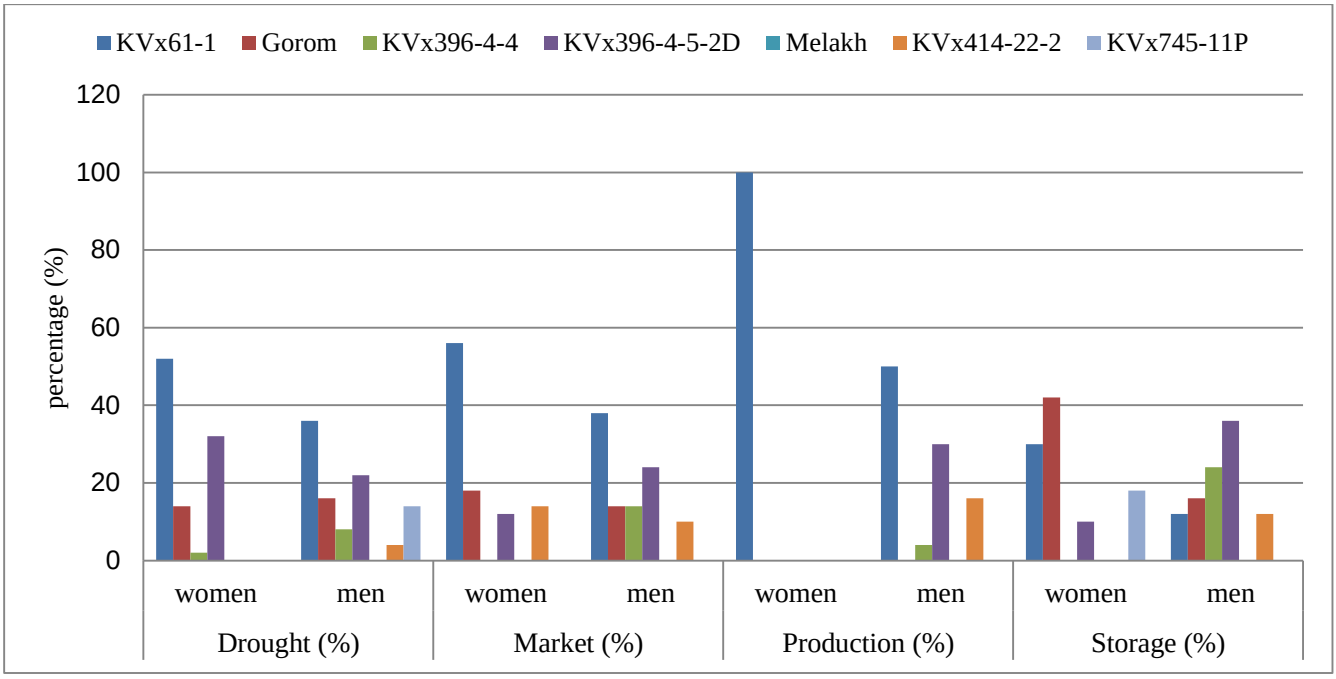

Figure 3.1: Cowpea variety acceptability (%) based drought and on farmers' preferred characteristics at Pobe-Mengao (Djibo), 2012.

The PRA results at this site are shown in Figure 3.2. In terms of drought tolerance, the women's group ranked variety KVx396-4-5-2D (47%) first at Pissila followed by varieties IT98K-205-8 (24%), KVx61-1 (17%) and KVx414-22-2 (11%). In the men's group, variety KVx61-1 (30%) got the highest score followed by varieties KVx414-22-2 (28%), IT98K-205-8 (22%) and KVx396-4-5-2D (20%). Three varieties have not been chosen by the two groups. For marketability, varieties KVx61-1 (29%) and KVx414-22-2 (28%) almost shared the lead in the women's group, while in the men's group, variety KVx414-22-2 (42%) was ranked first and variety IT98K-205-8 (29%) was second.

In terms of productivity, KVx61-1 (31%) was ranked first by the women's group and was also ranked first by the men's group with a score of 41%. It was followed in the women's group by varieties IT98K-205-8 (28%), KVx396-4-5-2D (21%), and KVx414-22-2 (20%). In the men's group variety KVx61-1 was followed by KVx396-4-5-2D (27%), IT98K-205-8 (23%) and KVx414-22-2 (8%).

For storage ability, variety KVx396-4-5-2D was ranked first in both men's and women's group with, respectively, a score of 38% and 29%. The second variety was IT98K-205-8 (23%) in the men's group and variety KVx414-22-2 (28%) in the women's group. Variety KVx61-1 was third in both men's and women's group with respectively, percentages of 21% and 17%.

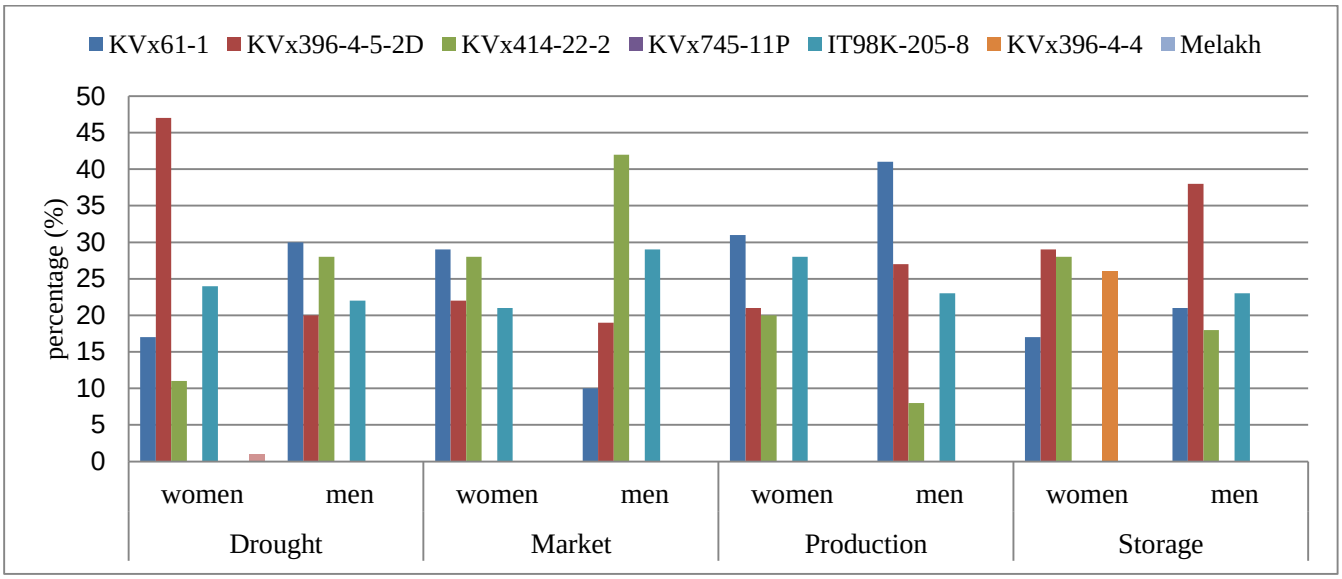

Figure 3.2: Cowpea variety acceptability (%) based drought and on farmers' preferred characteristics at Pissila (Kaya), in 2012.

The PRA results at this site are shown in Figure 3.3. In terms of drought tolerance, variety KVx61-1was ranked first by both men's group (59%) and women's group (49%) at Donsin followed by variety Moussa local in both cases with a score of 41% for men and 38% for women. The rest of the varieties were not scored by the two groups.

For marketability, variety KVx780-1 a new variety which is yet to be released and intentionally proposed to Donsin farmers was ranked first in women's group with the percentage of (56%) and the third place in men's group (15%) after another new variety KVx442-3-25 (16%). The landrace Moussa local got the highest score in the men's group with 45% and ranked the second place in women's group with 38%.

In terms of productivity, variety KVx61-1 was ranked first by both women's and men's groups with score of 45% and 39% respectively. This variety was followed in the men's group by Moussa local only (27%). Variety KVx61-1 was the only variety ranked for high productivity by the women's group.

For storage ability, the landrace Moussa local was ranked first in both men's and women's groups, with scores of 44% and 41% respectively. The second and last classified variety for storage ability was KVx61-1 in both groups with a score of 36% for women and 33% for men.

At Donsin the presence of *Macrophomina* was notified, and then, farmers were asked to rank this constraint based on the level of susceptibility of the different varieties to this fungal disease. Therefore, KVx61-1 was ranked in both groups as highly susceptible to *Macrophomina* followed by Moussa local in the men's group.

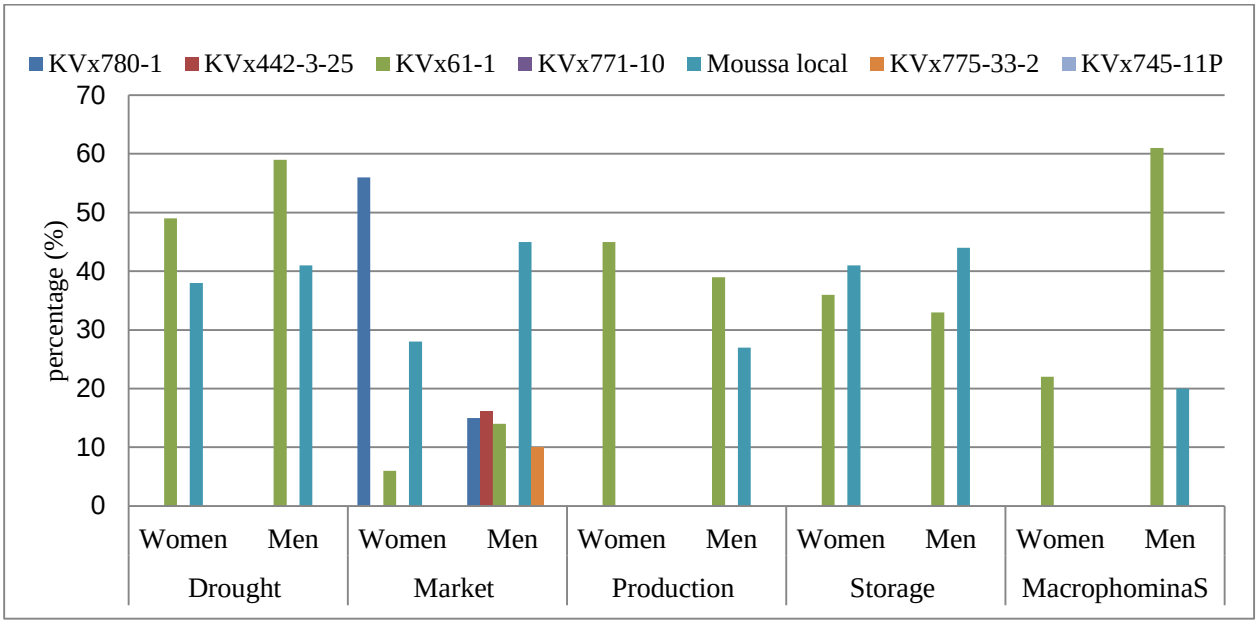

Figure 3.3: Cowpea variety acceptability (%) based drought and on farmers' preferred characteristics at Donsin (Ziniare) in 2012.

3.4. Discussion

In this study, focus group discussions (FGDs) and pair-wise ranking methods were used to engage farmers regarding their opinion on cowpea constraints mostly associated with drought and cowpea varieties. In general farmers demonstrated that they have a deep knowledge about cowpea production constraints.

Fertilizers, agro-chemicals were rarely used, especially for cowpea production. Farmers are aware of cowpea capacity to fix nitrogen and to enrich the soil for the next crop. Farmers were still practicing mixed-cropping because they perceived that it reduced high insect pressure on cowpea

than when cowpea was grown in sole cropping system. These perceptions about the inter-cropping were also reported (Tignegre, 2010).

None of the farmer-proposed control methods was effective in controlling drought in the field. However, a huge number of them are using soil and water control techniques to protect their soil from erosion and allow water to infiltrate the soil. These techniques keep the soil wet for long period and prevent plants from early dehydration. The techniques that are regularly used are: (1) the half-moon-shaped which is a kind of a hole dug to allow water to infiltrate the soil and keep the soil wet for plants to grow, (2) the *zaï* which is made of simple holes dug and filled with organic manure before the rains. It has almost the same role as the half-moon, and (3) the last technique is the rock-fenced techniques.

Several cowpea production constraints were highlighted by the farmers. There were poor access to agricultural inputs (fertilizers, pesticides). There was a general perception that rainfall had been declining over time and soils had been degraded over the past few years. As a result of that drought was mentioned between the first four constraints. KVx61-1 was the best variety for farmers in terms tolerance to drought and yield. Some of the constraints were linked to the lack of training for the extension system, the lack of infrastructure (equipment, access to a range of improved variety seeds); the other constraints were biotic stress, such as insects, diseases and *Striga*. These observations were also reported by other authors (Tignegre, 2010). Diseases such as seedling dumping due to *Pitium* and viral diseases were cited by the farmers as a constraint. This was mainly due to the fact that farmers did not know or differentiate disease symptoms from other constraints on the cop.

In this PRA study, the impact of drought in cowpea production was more or less general for all crops for farmers. For them, cowpea was better than other crops like maize, because cowpea can overcome some short water deficit and produce. The effects of drought in crop production were also notified in a study regarding climate change in Burkina Faso (Pana-Bf, 2006). This study showed that, because of recurrent drought, the area covered by crops like yam, maize, and cotton are being reduced year after year. The rural exodus reported by farmers during the PRA is confirmed (Pana-Bf, 2006), which showed that an important number of migrants were moving from the northern part of the country to the western part for those who want lands for agriculture up to big cities like Ouagadougou and Bobo-Dioulasso and even to the neighboring countries (Ghana, Cote d'Ivoire, etc.).

Productivity is a weighting factor for farmers, though yield is not the sole criterion upon which farmers always choose a variety. For instance, the choice of KVx61-1 by farmers at Donsin, Pobe-Mengao, and Pissila showed their preferences for more than one characteristic in the same variety (source of income, drought and *Striga* resistance). The choice of varieties KVx414-22-2 and the landrace Moussa local as income generating varieties was due to the quality of their grain: large-sized grain or whiteness of the testa. It was also chosen by farmers for the same characteristics (Tignegre, 2010). This is confirmed by the choice of the newly pre-released large-sized KVx780-1 proposed only for selection at Donsin, where the variety was part of the on-farm trials. This variation in the variety choices by farmers appeared to be influenced by the market demand. This observation confirmed the conclusion drawn by other authors (Coulibaly and Lowenberg-Deboer, 2002; Zannou *et al.*, 2004). In general, landraces were the most income-generating genotypes

because of their good agronomic and culinary characteristics (grain quality and taste) (Tignegre, 2010). These findings demonstrated that farmers' choices are mostly driven by market characteristics of the cowpea seeds. Most of their landraces have farmers' preferred traits. Unfortunately, their yields are very low in general and they are susceptible to multiple constraints. Therefore, there was needs to identify or improve cultivars including farmers' preferences in order to suite their demand (Yadaw *et al.*, 2006).

The survey made by Pronaf (2003) has shown that cowpea has increasingly become a source of income for farmers in Burkina Faso. In addition to providing food, cowpea can generate as much income (for people of Sahel and North Savanna zones) as cotton for people in more humid areas (Ouedraogo *et al.*, 1996). Farmers considered that the importance of cowpea was due to its roles as staple food, its adaptations (climate and local utilizations) and role as a source of income. The market demand can control farmers' preferences for grain characteristics (Coulibaly and Lowenberg-Deboer, 2002; Zannou *et al.*, 2004; Orawu, 2007). For example, in Sahel and Oudalan provinces, though farmers preferred the brown-coloured cowpea grain as their grown landraces, they also grow white-coloured grain of cowpea mostly for the market (Tignegre, 2010). Based on such characteristics, the breeding objectives in Burkina Faso could be selecting varieties with large-sized and white-coloured grain with local adaptation for food consumption and market demand at all sites. These findings confirmed those of Tignegre (2010) who reported the different constraints of cowpea production in Burkina Faso.

There is a potential to increase cowpea production if farmers have access to more agricultural inputs, including improved, *Striga*-resistant, and drought tolerant varieties, with the preferred grain

characteristics. The achievement of such potential would require that other abiotic constraints (soil degradation, rain declining over time), improved production systems (rotations, intercropping with cowpea) and market network be addressed. At the end of this PRA, farmers had the feeling that they had been involved and were excited because their views would be taken into account in cowpea variety selection process in order to meet their needs. They were hopeful that some urgent queries would be considered for the sake of their welfare: training, access to inputs (seeds, fertilizers, pesticides) and a better organized cowpea market network. They made a request to be involved in future research actions that would enable them to have access to seeds of improved varieties and need to be trained as cowpea producers.

3.5. Conclusion

Grain yield reduction, fodder yield reduction, total loss of crop and landraces, difficulties in planning cropping season, loss of arable land were most of the time during this study the main impact of drought on cowpea production in the areas were the study was conducted. The consequences of this, is the reduction of income levels, poverty, famine, indebtedness, difficulties to send children to school, rural exodus, and increase in number of people begging.

Farmers demonstrated that they have a deep knowledge about cowpea production constraints and difficulties to access improved variety seeds was the first constraint in all the areas where the study has been conducted. Other important constraints include drought tolerance, lack of equipment, soil fertility, and low access to fertilizer and pesticicdes.

The preferred grain traits for all regions identified in the past studies were white, large seeded, with a rough texture for food and market purposes, except for the northern region where brown grain was preferred for food.

Therefore, the development of new drought-tolerant cultivars for Burkina Faso will need a simultaneous selection for genotypes with resistance to the major abiotic and biotic constraints for farmers as well as for market preferred grain traits. These grain characteristics should be included in cowpea breeding programs to ease the adoption of improved varieties by Burkina Faso farmers. The participatory variety selection (PVS) during this participatory rural appraisal (PRA) paved the way for need-based selection by the farmers, and thereby could help promote quicker adoption of high yielding, large-seeded, and white coat colour varieties in the farming community.

CHAPTER FOUR

4.0. FIELD ASSESSMENT OF COWPEA GENOTYPES FOR DROUGHT TOLERANCE

4.1. Introduction

Despite the inherent capacity of cowpea to withstand drought, the erratic pattern of rainfalls exposes the crop to drought at the onset and at the end of the rainy season (Singh and Matsui, 2002). This can lead to yield losses ranging from 30% (Chiulele, 2010) to total crop failure.

To assess the tolerance to drought stress, a number of criteria have been used in crop plants. The stability analysis criterion for identifying environmentally sensitive and insensitive genotypes when they are evaluated under series of environments has been used (Finlay and Wilkinson, 1963). By using this approach, drought tolerance is assessed by the intercept of genotype yield regressed on environmental index. Other selection criteria also known as stress tolerance indices were proposed for selection of genotypes based on their performance in stressed and non-stressed conditions (Fisher and Maurer, 1978; Rosielle and Hamblin, 1981). Tolerance index (TOL), relative yield of genotypes under stressed (Ys) and non-stressed (Yp or Yw) conditions, stress susceptibility index (SSI), stress intensity (SI), and mean productivity (MP) were defined and used in selecting genotypes for drought tolerance (Fisher and Maurer, 1978; Rosielle and Hamblin, 1981). In addition, geometric mean productivity GMP) and stress tolerance index (STI) were proposed by Fernandez (1992). These stress tolerance indices were reported to be the most suitable for screening genotypes for drought tolerance because they enable the identification of high yielding and drought tolerant genotypes (Fernandez, 1992). A limited number of authors have used

these quantitative indices for stress tolerance to assess drought tolerant genotypes in cowpea (Chiulele, 2010; Ishiyaku and Aliyu, 2013).

Very few researchers have worked on drought stress in cowpea in Burkina Faso. These works were mainly on pots and proline accumulation screenings, different planting dates screening at drought prone areas. These works revealed sources of drought tolerant genotype in the germplasm KVx61-1 and Gorom local (Hamidou *et al.*, 2007) and KVx525 (Sawadogo, 2009). These studies mainly reported on biochemical and physiological traits. Until now, no in depth study was reported on the performance of Burkina Faso cowpea germplasm under drought imposed conditions in the field. Therefore, this study was conducted to assess cowpea genotypes for drought tolerance using some quantitative indices of stress tolerance such as stress intensity, mean productivity, tolerance index, stress susceptibility index, geometric mean productivity and stress tolerance index to classify the genotypes into different yielding and drought tolerance group.

4.2. Materials and Methods

4.2.1. Experimental materials, design and field layout

During the 2012 off-season from February to April, 50 cowpea genotypes (Table 4.1) were screened under run-off irrigation in the field conditions at Saria research station. Before planting, the land was ploughed and fertilized with organic manure (2.5t.ha^{-1}) to remove nutrient deficiency as a limiting factor. Two days before planting, the field was watered to field capacity. After sowing, the plants were maintained at near field water capacity until the emergence of flower buds (50% flower buds initiation). The plants were then, subjected to two soil moisture regimes (water-

stressed and well-watered or control). The experimental unit consisted of 2 row plots of 2 m long and about 10 plants per row. The spacing between rows was 0.8 m and the spacing between hills on the same row was 0.20 m with two plants per hill. The experimental design was an alpha lattice design with three replications, ten blocks per replication, and five genotypes per blocks. The field was regularly sprayed with insecticides (deltamethrin) at the dose 2 ml of insecticide per liter of water to avoid the effects on the insects' attacks on the plants.

Table 4.1: Cowpea genetic materials screened for tolerance to drought in field experiments in 2012

N^0	Genotypes	Origin	Seed colour	N^0	Genotypes	Origin	Seed colour
1	KVx404-8-1	Burkina Faso	White	26	Apagbaala	Ghana	White
2	Kaya local	Burkina Faso	White	27	IT96D-610	IITA/Nigeria	White
3	KVX525	Burkina Faso	White	28	IT95K-1479	IITA/Nigeria	White
4	F8/SR	Burkina Faso	White	29	IT00K-901-6	IITA/Nigeria	White
5	KVX421-2J	Burkina Faso	Brown	30	IT84S-2246	IITA/Nigeria	White
6	Djouroum local	Burkina Faso	White	31	IT99K-499-39	IITA/Nigeria	White
7	KVx780-3	Burkina Faso	White	32	IT98K-205-8	IITA/Nigeria	White
8	KVx780-6	Burkina Faso	White	33	IT98K-317-2	IITA/Nigeria	White
9	KVX396-4-5-2D	Burkina Faso	White	34	IT95M-190	IITA/Nigeria	White
10	KVX771-10	Burkina Faso	White	35	IT99K-573-2-1	IITA/Nigeria	White
11	KN1	Burkina Faso	Brown	36	IT93K-693-2	IITA/Nigeria	Brown
12	KVx780-1	Burkina Faso	White	37	IT98K-1111-1	IITA/Nigeria	White
13	Pobe local	Burkina Faso	White	38	IT93K-503-1	IITA/Nigeria	White
14	KVX61-1	Burkina Faso	White	39	IT84S-2049	IITA/Nigeria	White
15	Moussa Local	Burkina Faso	White	40	IT97K-207-15	IITA/Nigeria	White
16	KVX414-22-2	Burkina Faso	White	41	TN88-63	Niger	White
17	Donsin local	Burkina Faso	White	42	Bambey-21	Senegal	White
18	KVx780-4	Burkina Faso	White	43	Mouride	Senegal	White
19	BulkF7/SR	Burkina Faso	White	44	Melakh	Senegal	White
20	KVX775-33-2	Burkina Faso	White	45	58-57	Senegal	White
21	Komsare	Burkina Faso	Cream	46	UC-524B	UCR-USA	White
22	KVX30-309-6G	Burkina Faso	White	47	UCR-P-24	UCR-USA	White
23	KVX745-11P	Burkina Faso	White	48	CB46	UCR-USA	White
24	KVX442-3-25	Burkina Faso	White	49	CB27	UCR-USA	White
25	Gorom Local	Burkina Faso	Brown	50	Iron Clay	UCR-USA	White

4.2.2. Data collection

Data collected included grain yield, and yield components (pod yield, hundred seed weight, days to 50% flowering, and days to 95% maturity), total biomass, and fodder yield were recorded. Quantitative indices of stress tolerance were calculated using yield data. These stress tolerance indices were:

(i) Mean productivity (MP)

(ii) Tolerance index (TOL)

(iii) Stress susceptibility index (SSI)

(iv) Geometric mean productivity (GMP)

(v) Stress tolerance index (STI)

(vi) Stress intensity (SI)

The selection indices of stress tolerance for the mean productivity (MP), the tolerance index (TOL), the stress susceptibility index (SSI), the stress intensity (SI), the geometric mean productivity (GMP), and the stress tolerance index (STI) were calculated based on yield data in the two contrasting environments using the following formulae:

Mean productivity (MP)

$MP = (Ys+Yw)/2$

Tolerance index (TOL)

$TOL = Yw-Ys$

Stress susceptibility index (SSI)

$SSI = [1-(Ys/Yw)]/[1-(\bar{Y}s/\bar{Y}w)]$

Stress intensity (SI)

$SI = 1-(\bar{Y}s/\bar{Y}w)$

Geometric mean productivity (GMP)

$GM\ P = \sqrt{(Ys \times Yw)}$

Stress tolerance index (STI)

$ST\ I = (Ys \times Yw)/\bar{Y}^2w$

Where Ys and Yw (known as Yp (Fernandez, 1992)) are the yields of each genotype under drought-stressed and non-stressed conditions. $\bar{Y}s$ and $\bar{Y}w$ are respectively the mean yields of all genotypes under drought-stressed and non-stressed conditions.

The stress intensity (SI) score was classified into mild, moderate and severe. Stress intensity was mild when yield reduction was between 0 and 25%, moderate when yield reduction was situated between 25 and 50% and severe when yield reduction was between 50 and 100% (Chiulele, 2010).

4.2.3. Data analysis

Data on grain yield and yield components were analyzed using GenStat 14.0 computer software. The mixed models residual maximum likelihood (REML) was used for computing variance component. The REML analysis of collected data was done for the following environments: (1) irrigated throughout the experiment (well-watered) and (2) irrigated with imposed drought stress from during flower bud initiation period (water-stressed) and grain, pod yield and hundred seed weight reduction was calculated for the two contrasting environments.

Correlation analyses were made using yield and yield components data and calculated quantitative indices for stress tolerance. Principal component biplot analysis (PCA) was done using data on yield and the quantitative indices for stress tolerance to graphically display genetic relationships.

4.3. Results

4.3.1. Performance of genotypes yield and yield components

In the analysis, genotype x water regime interaction was not significant for all the parameters except for fodder yield (Table 4.2). Difference among genotypes and between water regime were highly significant ($p<0.01$) for all parameters studied (grain yield, hundred seed weight, total biomass, pod yield, fodder yield, and days to 95% Maturity) except days to 50% Flowering. In general, the genotypes flowered and matured earlier under non-stressed conditions than under stressed conditions. The mean for days to 50% flowering was almost the same for the two water regimes (44 days), but the genotypes under stressed matured (62 days) earlier that those under normal conditions (63 days).

The results of the analysis of variance per water regime (Table 4.3, 4.4, and 4.5) showed significant differences in grain yield and yield components between genotypes under non-stressed conditions while under stressed conditions only 100-seed weight showed significant difference (Table 4.3). The mean grain yields of genotypes under stressed and non-stressed conditions are respectively 542.59 $kg.ha^{-1}$ and 871.53 $kg.ha^{-1}$. The mean of genotypes ranged from 277.55 $kg.ha^{-1}$ to 1543.00 $kgha^{-1}$under non-stressed conditions and from 194.77 $kg.ha^{-1}$ to 729.19 $kgha^{-1}$ under stressed conditions. Under non-stressed conditions, genotypes KVX396-4-5-2D, KVX775-33-2, TN88-63, IT96D-610, Melakh, KVX61-1, BulkF7/SR, Mouride, KVX30-309-6G, KVx404-8-1, IT95K-

1479, IT98K-317-2, Apagbaala, KVx780-4, Iron Clay, IT93K-693-2, KVX442-3-25, Gorom Local, KVx780-1, and KN1 were high yielding and produced more than 900 kgha^{-1}while genotypes KVX414-22-2, Moussa Local, KVX525, IT98K-205-8, IT99K-499-39, KVX745-11P, and Bambey-21 were low yielding and produced less than 600 kg.ha^{-1}. Under stressed conditions, genotypes KVX745-11P, IT93K-693-2, KVx404-8-1, KVX61-1, KVX775-33-2, IT98K-1111-1, CB27, KVX30-309-6G, IT96D-610, TN88-63, Djouroum local, Mouride, Gorom Local, KVx780-3, BulkF7/SR, KVX396-4-5-2D, and KVX525 were moderate yielding and produced more than 600 kg.ha^{-1} while genotypes KVx780-4, UC-524B, Moussa Local, Kaya local, IT99K-573-2-1, IT98K-317-2, IT99K-499-39, F8/SR, and Bambey-21 were low yielding and produced less than 400 kg.ha^{-1} (Table 4.3).

Drought stress reduced grain yield but the genetic materials reacted differently to the stress. The grain yield of IT95M-190, TN88-63, KVx780-1, BulkF7/SR, KVX775-33-2, Kaya local, IT93K-503-1, IT95K-1479, Apagbaala, Iron Clay, F8/SR, UC-524B, IT99K-573-2-1, KVX396-4-5-2D, KVx780-4, Melakh, and IT98K-317-2 was reduced by more than 45% while that of KVX745-11P, KVX525, KVX414-22-2, Pobe local, and Djouroum local was not affected. Larger grain yield reductions were mostly recorded in high yielding genotypes under well-watered conditions under stress conditions. In general, genotypes with high performance under normal conditions yielded poorly under stress conditions. Low grain yielding genotypes were not severely affected by the yield reduction (Table 4.5).

Table 4.2: Analysis of variance for yield and yield components of 49 cowpea genotypes under stressed and non-stressed conditions

		Wald statistics						
Source	**DF**	**Grain Yield**	**100-seed weight**	**Total biomass**	**Pod Yield**	**Fod Yield**	**50%Flowering**	**95% Maturity**
Genotype	48	2.53**	7.83**	2.16**	2.08**	2.70**	6.72**	2.50**
Water Regime	1	125.96**	6.39**	150.24**	147.38**	68.90**	1.80ns	14.18**
Genotype x Water Regime	48	0.89ns	1.23ns	1.30ns	1.11ns	1.45*	0.50ns	1.15ns

**: significant at $p<0.01$

Table 4.3: Grain yield performance of 49 cowpea genotypes under water-stressed and well-watered conditions at Saria research station

		Grain Yield		
Yielding ability	Genotypes	Non-stressed	Stressed	Reduction (%)
HIGH	KVX396-4-5-2D	1543.00	620.04	59.82
	KVX775-33-2	1338.92	702.52	47.53
	TN88-63	1228.30	676.88	44.89
	IT96D-610	1217.98	679.25	44.23
	MELAKH	1184.97	459.08	61.26
	KVX61-1	1169.64	716.87	38.71
	BulkF7/SR	1161.49	623.11	46.35
	MOURIDE	1144.11	671.28	41.33
	KVX30-309-6G	1100.49	681.72	38.05
	KVx404-8-1	1091.28	722.22	33.82
	IT95K-1479	1074.62	517.22	51.87
	IT98K-317-2	1034.75	318.82	69.19
	APAGBAALA	1019.90	481.69	52.77
	KVx780-4	1014.55	394.45	61.12
	IRON CLAY	974.26	439.65	54.87
	IT93K-693-2	959.32	728.36	24.08
	KVX442-3-25	958.05	599.56	37.42
	GOROM LOCAL	931.15	648.77	30.33
	KVx780-1	907.94	496.86	45.28
	KN1	904.36	522.70	42.20
MODERATE	UC-524B	897.89	386.61	56.94
	IT98K-1111-1	892.71	694.80	22.17
	CB27	878.53	684.08	22.13
	58-57	874.68	579.78	33.72
	KVX771-10	863.69	502.17	41.86
	IT84S-2246	858.95	598.94	30.27
	ITOOK-901-6	841.02	597.76	28.92
	IT95M-190	837.19	462.54	44.75
	KVx780-3	836.67	645.11	22.90
	IT93K-503-1	832.09	401.38	51.76
	KVx780-6	810.88	501.65	38.14
	Donsin local	788.86	521.94	33.84
	Djouroum local	785.43	672.37	14.39
	IT84S-2049	784.25	553.79	29.39
	IT99K-573-2-1	771.71	328.74	57.40
	IT97K-207-15	740.85	573.45	22.59
	CB46	695.92	506.29	27.25
	Kaya local	636.66	329.07	48.31
	UCR-P-24	635.60	489.21	23.03
	F8/SR	634.77	275.50	56.60
	KVX421-2J	613.60	489.37	20.25
	Pobe local	610.47	589.16	3.49
LOW	KVX414-22-2	589.87	582.18	1.30
	MOUSSA LOCAL	584.37	374.41	35.93
	KVX525	578.47	612.11	-5.81
	IT98K-205-8	568.13	454.97	19.92
	IT99K-499-39	557.67	309.51	44.50
	KVX745-11P	472.17	729.19	-54.44
	Bambey-21	277.55	194.77	29.83
MEAN		**871.53**	**542.59**	
CV(%)		**29.34**	**32.34**	
Significance		***	ns	

***: significant at p<0.001 and ns: not significant

Table 4.4: Pod yield performance of 49 cowpea genotypes under water-stressed and well-watered conditions at Saria research station

		Pod Yield		
Yielding ability	Genotypes	Non-stressed	Stressed	Reduction (%)
HIGH	KVX396-4-5-2D	1999.67	770.70	-75.13
	KVX775-33-2	1832.97	958.82	28.42
	TN88-63	1408.37	811.58	16.00
	IT96D-610	1513.04	834.09	-3.82
	MELAKH	1567.04	645.58	14.28
	KVX61-1	1561.37	645.66	11.78
	BulkF7/SR	1301.84	765.79	22.57
	MOURIDE	1596.78	680.65	23.52
	KVX30-309-6G	1455.64	856.86	15.35
	KVx404-8-1	1396.37	703.79	27.15
	IT95K-1479	1375.57	679.51	16.18
	IT98K-317-2	1341.91	464.67	29.19
	APAGBAALA	1652.41	604.54	27.93
	KVx780-4	1332.28	577.20	34.48
	IRON CLAY	1374.30	602.40	22.31
	IT93K-693-2	1260.13	908.21	19.75
	KVX442-3-25	1251.09	619.47	-55.49
	GOROM LOCAL	1158.14	770.60	10.25
	KVx780-1	1228.45	621.60	33.46
	KN1	1435.04	826.29	37.65
MODERATE	UC-524B	1171.15	479.41	49.60
	IT98K-1111-1	1111.61	940.96	37.36
	CB27	1180.75	903.05	41.05
	58-57	1214.22	757.10	50.49
	KVX771-10	1146.60	621.99	41.14
	IT84S-2246	1094.39	982.22	38.95
	ITOOK-901-6	946.06	735.03	58.65
	IT95M-190	1484.57	662.06	57.37
	KVx780-3	1192.63	999.64	45.75
	IT93K-503-1	1155.70	569.40	42.42
	KVx780-6	1138.28	694.94	44.87
	Donsin local	1104.97	692.15	46.02
	Djouroum local	988.56	847.35	55.40
	IT84S-2049	1051.43	843.74	42.37
	IT99K-573-2-1	1169.89	419.24	49.40
	IT97K-207-15	1073.28	781.84	41.18
	CB46	924.29	605.55	47.69
	Kaya local	872.05	467.92	46.34
	UCR-P-24	760.57	538.55	50.73
	F8/SR	961.14	379.12	50.60
	KVX421-2J	767.74	594.45	63.41
	Pobe local	768.09	797.47	56.17
LOW	KVX414-22-2	899.96	755.93	60.56
	MOUSSA LOCAL	896.49	528.44	59.07
	KVX525	696.56	498.58	64.16
	IT98K-205-8	806.60	711.61	61.46
	IT99K-499-39	796.12	429.74	56.68
	KVX745-11P	597.60	1046.59	58.80
	Bambey-21	381.04	592.46	65.37
MEAN		**1172.21**	**698.98**	
CV(%)		**28.84**	**32.06**	
Significance		***	ns	

***: significant at p<0.001 and ns: not significant

Table 4.5: Hundred seed weight of 49 cowpea genotypes under water-stressed and well-watered conditions at Saria research station

		100-seed weight		
Yielding ability	Genotypes	Non-stressed	Stressed	reduction (%)
HIGH	KVX396-4-5-2D	17.34	16.84	-28.81
	KVX775-33-2	23.30	23.39	6.47
	TN88-63	12.39	12.73	4.35
	IT96D-610	18.27	20.36	-9.11
	MELAKH	19.18	13.72	7.07
	KVX61-1	18.25	13.94	-7.42
	BulkF7/SR	20.49	22.80	7.09
	MOURIDE	18.52	17.21	25.03
	KVX30-309-6G	21.89	25.36	0.92
	KVx404-8-1	16.35	17.02	8.71
	IT95K-1479	19.19	18.59	11.96
	IT98K-317-2	12.28	17.64	15.27
	APAGBAALA	15.75	14.33	22.25
	KVx780-4	23.63	19.40	10.99
	IRON CLAY	15.81	17.97	-0.56
	IT93K-693-2	20.82	16.18	2.91
	KVX442-3-25	21.69	21.51	-4.87
	GOROM LOCAL	21.33	21.30	8.31
	KVx780-1	22.82	22.57	0.16
	KN1	14.51	15.82	5.02
MODERATE	UC-524B	28.66	23.66	-4.10
	IT98K-1111-1	17.74	17.58	-22.68
	CB27	19.80	14.85	14.24
	58-57	13.03	12.37	0.83
	KVX771-10	22.26	15.25	-15.87
	IT84S-2246	19.50	17.88	10.00
	ITOOK-901-6	18.13	18.23	23.61
	IT95M-190	22.07	23.02	7.07
	KVx780-3	23.34	20.55	31.47
	IT93K-503-1	15.29	17.43	-9.05
	KVx780-6	21.06	18.96	-11.42
	Donsin local	18.11	22.22	16.73
	Djouroum local	22.90	21.28	-4.29
	IT84S-2049	17.59	17.08	-2.74
	IT99K-573-2-1	23.57	24.48	1.08
	IT97K-207-15	22.22	20.29	-11.29
	CB46	21.97	19.55	-0.38
	Kaya local	16.15	18.25	-13.02
	UCR-P-24	22.41	18.99	-13.99
	F8/SR	24.45	20.83	3.12
	KVX421-2J	20.61	19.15	9.06
	Pobe local	16.41	17.91	-13.65
LOW	KVX414-22-2	20.73	19.83	14.82
	MOUSSA LOCAL	16.73	14.35	17.44
	KVX525	22.56	21.10	-3.89
	IT98K-205-8	16.34	17.56	2.90
	IT99K-499-39	20.70	17.24	17.88
	KVX745-11P	14.30	18.43	28.50
	Bambey-21	20.44	21.43	-43.56
MEAN		**19.44**	**18.69**	
CV(%)		**8.69**	**15.87**	
Significance		***	***	

***: significant at p<0.001 and ns: not significant

Correlation analysis indicated that grain yield was only significantly correlated with pod yield per plot in both stressed and non-stressed conditions (r=0.78, p<0.01; r=0.94, p<0.01), respectively (Table 4.6).

Table 4.6: Correlations among grain yield, pod yield, and 100-seed weight of 49 genotypes grown under stressed conditions

	100 Seed	Grain Yield	Pod Yield
100 Seed			
Grain Yield	-0.103		
	-0.112		
Pod Yield	-0.078	0.785**	
	-0.096	0.942**	

**: significant at p < 0.01

4.3.2. Grouping of 49 cowpea genotypes using quantitative stress indices

Forty nine (49) Genotypes that combined lower tolerance index and stress susceptibility index and higher mean productivity and stress tolerance index were drought tolerant (Table 4.7). Examples of these genotypes are KVx404-8-1, Gorom local, IT93K-693-2, CB27, IT98K-1111-1, and Djouroum local. In addition, genotypes KVx404-8-1 were high yielding and IT98K-1111-1, Gorom local, and Djouroum local were moderate yielding. In contrast, genotypes that combined higher tolerance index and stress susceptibility index and lower mean productivity and stress

tolerance index were drought susceptible. Examples of these genotypes are IT99K-573-2-1, Kaya local, Moussa Local, F8/SR, IT99K-499-39, and Bambey-21 (Table 4.7).

The stress intensity applied to this experiment was considered as moderate. The intensity of drought measured by the stress intensity (SI) was 0.38 (38%) and was then, between the intervals of 25% to 50%.

Table 4.7: Stress tolerance indices of the 49 cowpea genotypes, in 2012

Yielding ability	Genotypes	TOL[a]	MP[b]	GMP[c]	SSI[d]	STI[e]
HIGH	KVX396-4-5-2D	922.96	1081.52	978.12	0.03	1.26
	KVX775-33-2	636.40	1020.72	969.85	1.20	1.24
	TN88-63	551.42	952.59	911.82	1.19	1.09
	IT96D-610	538.72	948.61	909.57	1.17	1.09
	KVX61-1	452.77	943.25	915.68	1.11	1.10
	MOURIDE	472.83	907.70	876.37	1.09	1.01
	KVx404-8-1	369.06	906.75	887.77	0.54	1.04
MODERATE	BulkF7/SR	538.38	892.30	850.72	1.23	0.95
	KVX30-309-6G	418.78	891.10	866.15	1.58	0.99
	IT93K-693-2	230.96	843.84	835.90	0.64	0.92
	MELAKH	725.90	822.03	737.56	1.62	0.72
	IT95K-1479	557.39	795.92	745.53	1.37	0.73
	IT98K-1111-1	197.91	793.75	787.56	0.59	0.82
	GOROM LOCAL	282.38	789.96	777.24	0.80	0.80
	CB27	194.45	781.31	775.24	0.59	0.79
	KVX442-3-25	358.49	778.81	757.90	1.03	0.76
	APAGBAALA	538.22	750.79	700.91	1.40	0.65
	KVx780-3	191.56	740.89	734.67	1.62	0.71
	IT84S-2246	260.01	728.95	717.26	0.80	0.68
	Djouroum local	113.06	728.90	726.71	0.38	0.70
	58-57	294.90	727.23	712.12	0.89	0.67
	ITOOK-901-6	243.26	719.39	709.03	0.77	0.66
	KN1	381.66	713.53	687.53	1.01	0.62
	IRON CLAY	534.61	706.95	654.47	1.45	0.56
	KVx780-4	620.10	704.50	632.61	1.01	0.53
	KVx780-1	411.08	702.40	671.66	0.61	0.59
	KVX771-10	361.52	682.93	658.57	0.90	0.57
	IT98K-317-2	715.94	676.79	574.37	1.83	0.43
	IT84S-2049	230.46	669.02	659.03	0.78	0.57
	IT97K-207-15	167.39	657.15	651.80	0.60	0.56
	KVx780-6	309.24	656.27	637.79	1.28	0.54
	Donsin local	266.91	655.40	641.67	0.90	0.54
	IT95M-190	374.65	649.86	622.28	1.19	0.51
	UC-524B	511.28	642.25	589.18	1.51	0.46
	IT93K-503-1	430.71	616.74	577.92	1.37	0.44
	CB46	189.63	601.10	593.58	0.72	0.46
	KVX745-11P	-257.03	600.68	586.77	1.26	0.45
LOW	Pobe local	21.31	599.81	599.72	0.09	0.47
	KVX525	-33.64	595.29	595.05	-1.44	0.47
	KVX414-22-2	7.69	586.02	586.01	0.99	0.45
	UCR-P-24	146.40	562.41	557.62	0.61	0.41
	KVX421-2J	124.24	551.49	547.98	-0.15	0.40
	IT99K-573-2-1	442.97	550.22	503.68	1.52	0.33
	IT98K-205-8	113.16	511.55	508.41	0.53	0.34
	Kaya local	307.58	482.86	457.72	1.12	0.28
	MOUSSA LOCAL	209.97	479.39	467.75	0.95	0.29
	F8/SR	359.26	455.13	418.18	1.50	0.23
	IT99K-499-39	248.16	433.59	415.46	1.18	0.23
	Bambey-21	82.78	236.16	232.50	0.79	0.07

a : Tolerance index; b: Mean productivity; c: Geometric mean productivity; d : stress susceptibility index; e: Stress tolerance index

Correlation analysis between quantitative indices of stress tolerance and stressed and non-stressed mean yield are mentioned in Table 4.8. The results showed that the STI were positively and strongly correlated with MP, TOL, Ys, Yw, and GMP. The correlation between STI and GMP was almost equal to one. GMP was strongly correlated MP, Ys and Yw.

Table 4.8: Correlation among stress index scores and yield under stressed (Ys), and non-stressed (Yw) of 49 cowpea genotypes

	GMP[c]	SSI[d]	STI[e]	MP[b]	TOL[a]	Ys[f]	Yw[g]
GMP							
SSI	0.007						
STI	0.986**	0.009					
MP	0.989**	0.075	0.978**				
TOL	0.460	0.439	0.484	0.574**			
Ys	0.828**	-0.265	0.795**	0.749**	-0.113		
Yw	0.871**	0.247	0.875**	0.930**	0.835**	0.453	

a: Tolerance index; b: Mean productivity; c: Geometric mean productivity; d : stress susceptibility index; e : Stress tolerance index; f : yield under stressed condition; g : yield under non-stressed condition
**: significant at P< 0.01

The total variation explained by the first two axes was 91.49% (Figure 4.1). The PC1 explained 67.31% of the total variation of the data matrix and had high correlation among non-stressed yield (Yw), stress tolerance index (STI), geometric mean productivity (GMP) and mean productivity (MP). This dimension can be named as the yield potential-mean productivity component, which separates the high yielding from the low yielding genotypes. Because the angles and the directions between the attribute vectors indicate the strength and the direction of the correlation between two attributes, the biplot displayed indicates that there was significant and positive correlation between stress tolerance index and geometric mean productivity, stress tolerance index and mean

productivity, and stressed yield, and stress tolerance index and yield potential. The PC2 explained 24.17% of the total variation and had positive correlation with stressed yield (Ys), tolerance index (TOL), and stress susceptibility index (SSI). Thus, this dimension can be called stress tolerance dimension and it separates stress tolerant from stress susceptible genotypes. In relation to the two components of the biplot, the genotypes fell into distinct clusters that corresponded to their yield potentials and stress-tolerance. The stress tolerant attributes STI, GMP, MP and Yw were correlated with Djouroum local, KVx404-8-1, IT98K-1111-1, Gorom, Local, CB27, IT93K-693-2, Mouride, and KVx61-1 which represent the group of higher yielding and stress tolerant genotypes. The stress tolerant attributes SSI and TOL were correlated with high yielding and stress susceptible genotypes such as KVx396-4-5-2D, Melakh, Apagbala, KVx775-33-2, BulkF7/SR, and IT95K-1479. The genotypes were distributed over the biplot space according to their yielding ability and adaptation to stressed or non-stressed environments.

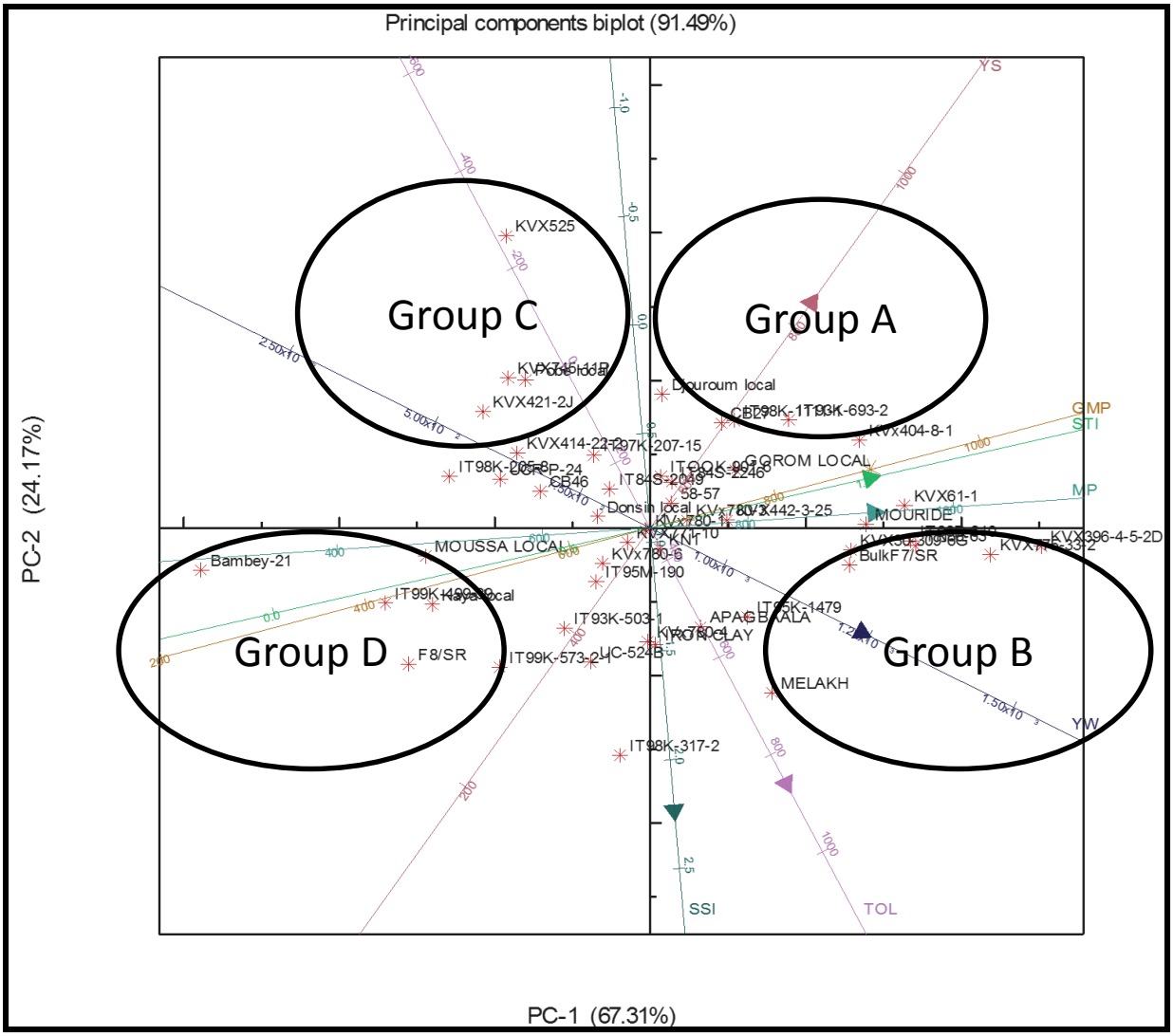

Figure 4.1: Biplot display of mean productivity (MP), geometric mean productivity (GMP), tolerance index (TOL), stress susceptibility index (SSI), stress tolerance index (STI), and yield of 49 cowpea genotypes under stressed (Ys) and non-stressed (Yw) conditions.

4.4. Discussion

Genetic variability is essential for the establishment of a breeding program in any crop. In this study, the genetic variation for yield, yield components, days to 50% flowering, and days to 95% maturity was detected under water-stressed and well-watered conditions indicating that improvement can be achieved using such germplasm. The strong and positive correlation between grain yield and pod yield suggested that yield improvement could be achieved by selecting genotypes based on the pod yield. These findings are in agreement with those of (Chiulele, 2010) who indicated that there were strong and positive correlations between grain yield and number of pods per plant and concluded that improvement could be achieved by using such germplasm. High yielding genotypes produced three times more than low yielding genotypes in normal conditions but under stressed conditions some low yielding remained stable across environment than some high yielding genotypes that yielded low. Because of that, (Richards, 2006) suggested that selection for yield is more efficient under stressed conditions than under non-stressed conditions. The stress intensity applied to this study was around 38%. This kind of stress is considered as moderate stress intensity.

The genotypes tested reacted differently to water stress indicating the existence of genetic variability for drought tolerance amongst the tested germplasm. For example, the yield of genotypes like KVx396-4-5-2D, Iron Clay, IT98K-317-2, UC-524B, F8/SR, IT99K-573-2-1, and IT93K-503-1 was severely reduced by the imposed stress while that of KVx745-11P, KVx525, KVx414-22-2, Pobe local, KVx421-2J, Djouroum local, CB27, UCR-P-24, IT97K-207-15, IT93K-693-2, IT98K-1111-1, and KVx780-3 was less affected. The reduction in grain yield is in general linked with reduction in pod yield. This is in agreement with the findings of Turk *et al.*

(1980) who reported that the reduction in grain yield of cowpea was a result of reduction in number of pods and seed weight due to detrimental effects of drought on pod set and grain filling. Likewise, Chiulele (2010) found that the reduction in yield was a result of reduction in number of pods per plant. As reported by Chiulele (2010), the difference in response of cowpea genotypes to drought is not surprising since the tested germplasm consisted of genotypes adapted to different growing conditions including the dry areas with high temperature of the Sahel and semi-arid Africa, and hot areas of California in the USA. Bahar and Yildirim (2010) reported that plants are most prone to damage due to limited water during flowering and pod setting stages. However, in this study hundred seed weight was less affected and most of the genotypes showed high hundred seed weight under stressed conditions and lower hundred seed weight under non-stressed conditions. Romanus *et al.* (2008) reported that additive gene action was more important than non-additive gene action for yield, number of seeds per pod, pod length, hundred seed weight and days to flowering. This implies that hundred seed weight passed from parents to offspring and the trait is less affected by environment. These results suggested that genetic improvement of cowpea yield using hundred seed weight as indirect selection criteria would be possible and it could be predicted based on performance of the parents. Nevertheless, results of phenotypic correlations between yield and yield components indicated that only the pods weight would be useful for improving yield since the correlation between yield and pods weight was high and positive.

The correlation between stressed and non-stressed yield was 0.45 for the tested genotypes. This correlation is not too large. These results suggested that selecting genotypes based on yield potential would improve yield only under non-stressed environments. These results are in agreement with that of Rosielle and Hamblin (1981) who reported that for most of the yield trials,

if the correlation between stressed and non-stressed yield is smaller it indicates that selection for yield potential would only increase yield under non-stressed environments while the selected genotypes would perform poorly under stressed conditions. The result is also consistent with the findings of Chiulele (2010) who reported that, it was better when looking for yield improvement for late maturing genotypes in cowpea to select under non-stressed conditions. Ishiyaku and Aliyu (2013), also reported that seed yield increases by 3.9 $kg.ha^{-1}$ with every mm increase in rainfall.

The correlation among quantitative indices of drought tolerance and stressed and non-stressed yield indicated that stress tolerance index (STI) was correlated with stressed (Ys) and non-stressed (Yw) yield, mean productivity (MP) and geometric mean productivity (GMP) suggesting that selection based on this index would improve both stressed and non-stressed yield. In addition, stress tolerance index (STI) enabled the identification of high yielding and stress tolerant genotypes, suggesting that this index was the best for selecting genotypes for drought tolerance. This statement is consistent with that of Fernandez (1992) showing that yield would be improved under both stressed and non-stressed environments when using stress tolerance index for selection. For Khayatnezhad and Gholamin (2010), the calculated gain from indirect selection from moisture stress environment would improve yield in moisture stress environment better than selection from non-stress environment. These author's conclusions about the positive correlations among Yw, STI, MP, and GMP are in concordance with the results of this study that showed that significant and positive correlation for Yp and MP, GMP and STI showed that these indices were more effective in identifying high yielding cultivars under different moisture conditions. In wheat selection, Akçura *et al.* (2011) found that SSI was a useful indicator when the stress is severe while MP, GMP, TOL and STI were useful indicators when the stress is less severe. Looking at the level of the stress intensity (SI= 38%) applied in this study which is moderate, the conclusions of Akçura

et al. (2011) can explain the fact that MP, GMP, TOL and STI were useful indicators for the grouping of the germplasm studied.

The principal component analysis showed that the PC1 explained most of the variation observed in yield. The PC1 was correlated with non-stressed yield (Yw) and mean productivity (MP) while PC2 was correlated with stress tolerance suggesting that PC1 was a yield potential dimension while the PC2 stress tolerance dimension. Plotting the genotypes over the PC1 and PC2 with quantitative indices of stress tolerance and stressed and non-stressed yield, genotypes were distributed over the coordinate space indicating different drought adaptation and yielding ability. Different clusters of genotypes were identified as described by Fernandez (1992). High yielding and drought tolerant genotypes (yield not significantly reduced by drought) (group A), high yielding and drought susceptible genotypes (reduced by drought) (Group B), low yielding and drought tolerant genotypes (group C) and low yielding and drought susceptible genotypes (group D). Genotypes like Djouroum local, KVx404-8-1, IT98K-1111-1, Gorom, Local, CB27, IT93K-693-2, Mouride, and KVx61-1 were clustered in group A. Genotypes like KVx396-4-5-2D, Melakh, Apagbala, KVx775-33-2, BulkF7/SR, and IT95K-1479 were found in group B. the varieties KVx745-11P, KVx525, KVx414-22-2, Pobe local, KVx421-2J, and IT98K-205-8 were clustered in group C while Bambey 21, Moussa Local, F8/SR, Kaya local, and IT99K-499-39 were in group D. The biplot displays showed a clear indication of a genetic variability for yield under drought conditions for the screened cowpea genotypes suggesting that improvement for yield under drought conditions could be achieved.

From the literature reviewed, some genotypes identified as drought-tolerant across countries seem to confirm their status in this study. This is the case of Gorom Local also known as Suvita2 (Muleba *et al.*, 1997; Hamidou *et al.*, 2007; Belko *et al.*, 2012), KVx61-1 (Hamidou *et al.*, 2007; Belko *et al.*, 2012), IT98K-1111-1 identified as type 2 drought-tolerant (Singh and Matsui, 2002). Other authors confirmed their susceptibility to drought. This is the case of Bambey 21 (Hamidou *et al.*, 2007; Sawadogo, 2009), Moussa Local (Sawadogo, 2009). Some genotypes identified as drought-tolerant in other parts of the world and in Burkina Faso were drought susceptible in this study. This is the case of UC-524B, IT99K-499-39, Apagbala (Sawadogo, 2009; Chiulele, 2010). The used of stress tolerance index in the identification of drought tolerant material is efficient. From the indices used the stress tolerance index (STI) is the best. It helps in cutting the population into groups.

4.5. Conclusion

The objective of this study was to identify cowpea genotypes tolerant to drought based on quantitative stress indices and yields under stressed and non-stressed conditions. Based on the results obtained the following conclusion could be drawn:

(i) Genotypic variability for drought tolerance existed amongst the tested genotypes

(ii) From the biplot displays of yields and quantitative indices for stress tolerance, four clusters of genotypes were identified based on yielding ability and drought tolerance; high yielding and drought tolerant genotypes were in group A, high yielding and drought susceptible genotypes in group B, low yielding and drought tolerant genotypes were in group C and low yielding and drought susceptible genotypes in group D.

(iii) Genotypes in group A were the best by combining high yield and tolerance to drought.

(iv) Amongst the quantitative indices of drought tolerance, stress tolerance index (STI) was the best because it enabled the identification of group A genotypes.

(v) The pod yield was strongly and positively correlated with yield. In general, drought tolerant genotypes did show high reduction in pod yield between normal and stress imposed conditions in the high yielding genotypes than the low yielding genotypes.

(vi) Some genotypes already identified as drought tolerant confirmed their tolerance status. Examples of such varieties are Gorom Local, KVx61-1, and IT98K-1111-1.

(vii) New drought tolerant genotypes were identified. Examples of these genotypes are Djouroum local, Pobe local, KVx404-8-1, KVx745-11P, KVx525, KVx414-22-2, and KVx421-2J. Of these, KVx745-11P, KVx525, KVx414-22-2, and KVx421-2J are low yielding genotypes but could be used in breeding program to improve high yielding drought-susceptible genotypes.

CHAPTER FIVE

5.0. SNP-BASED GENETIC DIVERSITY ASSESSMENT IN A SET OF COWPEA GERMPLASM

5.1. Introduction

Despite considerable phenotypic diversity that exists in cultivated cowpea germplasm, there is limited genetic variability in cowpea breeding programs (Pasquet, 1999, 2000). Breeding programs, which must focus most of their efforts on rapid delivery of varieties with a specific range of production and quality traits, tend to cross and re-cross cultivars containing these production and quality traits, and many of them are related to some degree. This leads to reduced genetic variability among cultivars that are released and among advanced breeding lines in the program, both of which are likely to be used as parents in new breeding cycles (Fang *et al.*, 2007). The lack diversity is a special concern because cowpea appears to have lower inherent genetic diversity than other cultivated crops as a result of a hypothesized single domestication event (Pasquet, 1999, 2000).

Markers based on single nucleotide polymorphisms (SNPs) have rapidly gained the center stage of molecular genetics during the recent years due to their abundance in the genomes and their amenability for high-throughput detection formats and platforms (Mammadov *et al.*, 2012). Of these available platforms, there is the LGC genomics' Kompetitive Allele Specific PCR (KASP) combined with the SNP line platforms in United Kingdom**.** SNP markers are increasingly being used for a large number of genetic studies including genetic diversities. Such studies have been

reported in pea (Deulvot *et al.*, 2010), cowpea (Huynh *et al.*, 2013; Egbadzor *et al.*, 2014), and cassava (Thompson, 2013). SNPs provide the simplest form of molecular markers as a single nucleotide base is the smallest unit of inheritance, and therefore, they can provide a large number of markers to be used in diversities or in marker assisted breeding. SNPs are co-dominant markers and they are most often linked to genes, and thus, they are the most attractive genetic markers in genetic studies (Jiang, 2013). The use of these markers could therefore help group germplasm for the use of breeding. SNP markers also help in decision making when knowing the variability within the germplasm.

Available breeding materials should be well known and described in any breeding programme for any crop for better exploitation of the potential variability. To do that, morphological, biochemical, and molecular characterization can be used. An important cowpea genetic materials exists in Burkina Faso cowpea breeding programme but, no in deep investigation has been made to establish the variability using molecular markers. Therefore, the objective of this study was to assess the genetic diversity in the set of cowpea germplasm using SNP markers.

5.2. Materials and Methods

5.2.1. Cowpea genotypes

The same 50 cowpea genotypes used in field screening of cowpea for drought tolerance (chapter four) were used for genetic diversity study using SNP markers. The origin and seed coat color of the material have been described in (Table 4.1).

5.2.2. SNP genotyping

Leaf samples of two weeks old plants were collected in a 96 wells plate and sent to LGC genomics in the United Kingdom for DNA extraction and SNP genotyping. The KASP technology as described by Thompson (2013) was used for the genotyping at LGC genomics. The DNA was extracted using LGC genomics internal protocol described in Appendix 4. One hundred and eighty one (181) SNP markers selected from the Generation Challenge Programme (GCP) platform were used (Appendix 1). After excluding the SNPs that were not informative enough (more than 10% missing data) a total of 170 markers and 47 cowpea lines were used for further analysis.

5.2.3. Analysis of genetic diversity

Pair-wise genetic distances between genotypes were measured with the software GGT 2.0 (Van Berloo, 2008) based on the allele-sharing method (Bowcock *et al.*, 1994). The simple matching algorithm considers both presence and absence of markers in calculating degrees of similarity. Phylogenetic relationships dendrogram were generated based on the genetic-distance matrix using the unweighted pair group method (UPGMA) with the software MEGA 6.0 (Tamura *et al.*, 2013).

Descriptive statistics like polymorphism information content (PIC) value, major allele frequency (MAF), and expected heterozygosity (He) were calculated for all the SNPs using PowerMarker 3.25 software (Lui and Muse, 2005). A core collection of genotypes was generated from GGT2.0 software based on the maximum diversity sum.

5.3. Results

5.3.1. Descriptive statistics

The summary statistics for major allele frequencies (MAF), expected heterozygosity (H_e), and polymorphic information content (PIC) is presented in Table 5.1. A low expected heterozygosity (0.08) was observed with the SNP marker (1_0992) that has the high major allele frequency (0.96). The mean of the expected heterozygosity was 0.41 and that of the major allele frequency was 0.68. The allele frequencies of all the SNP markers were greater than their corresponding expected heterozygosity values. The allele frequencies of all the markers were below 0.95 except 1_0992 (0.96), indicating the polymorphic nature of the SNP markers used. The PIC values ranged from 0.08 (1_0992) to 0.38 with an average of 0.32. Out of the 181 SNPs used 4 did not amplify. 177 SNPs were successful and out of that, 170 were useful representing 96.04% of the total. 103 SNPs were the most informative markers with a PIC value greater than the mean which represents 60.59% of the useful SNPs. Out of the 103 SNPs seven have a PIC of 0.38, 40 a PIC of 0.37, 26 a PIC of 0.36, 13 a PIC of 0.35, nine a PIC 0.34, and eight a PIC of 0.33. The seven most informative markers are 1_0126, 1_0351, 1_0362, 1_0594, 1_1130, 1_1367, and 1_1393.

Table 5.1: Summary statistics of genetic variation using 170 SNP markers among 47 cowpea lines

Marker	MAF	Avail	He	PIC	Marker	MAF	Avail	He	PIC
1_0126	0.50	0.94	0.50	0.38	**1_1021**	0.57	0.94	0.49	0.37
1_0351	0.50	0.98	0.50	0.38	**1_1371**	0.57	1.00	0.49	0.37
1_0362	0.50	0.98	0.50	0.38	**1_0136**	0.58	0.96	0.49	0.37
1_0594	0.50	0.94	0.50	0.38	**1_0923**	0.58	0.96	0.49	0.37
1_1130	0.50	0.94	0.50	0.38	**1_0993**	0.58	0.96	0.49	0.37
1_1367	0.50	0.98	0.50	0.38	**1_1038**	0.58	0.96	0.49	0.37
1_1393	0.50	0.94	0.50	0.38	**1_0259**	0.59	0.98	0.48	0.37
1_0531	0.51	1.00	0.50	0.37	**1_1117**	0.59	0.98	0.48	0.37
1_0605	0.51	1.00	0.50	0.37	**1_1189**	0.59	0.98	0.48	0.37
1_0123	0.51	0.96	0.50	0.37	**1_0987**	0.59	0.94	0.48	0.37
1_0771	0.51	0.96	0.50	0.37	**1_0127**	0.60	1.00	0.48	0.37
1_1467	0.51	0.96	0.50	0.37	**1_0388**	0.60	1.00	0.48	0.37
1_0183	0.52	0.98	0.50	0.37	**1_0449**	0.60	1.00	0.48	0.37
1_1007	0.52	0.98	0.50	0.37	**1_0401**	0.60	0.96	0.48	0.36
1_0001	0.52	0.94	0.50	0.37	**1_0752**	0.60	0.96	0.48	0.36
1_0982	0.52	0.94	0.50	0.37	**1_0806**	0.60	0.96	0.48	0.36
1_1141	0.52	0.94	0.50	0.37	**1_1135**	0.60	0.96	0.48	0.36
1_0905	0.53	1.00	0.50	0.37	**1_0052**	0.61	0.98	0.48	0.36
1_0604	0.53	0.96	0.50	0.37	**1_0377**	0.61	0.98	0.48	0.36
1_0425	0.54	0.98	0.50	0.37	**1_0397**	0.61	0.98	0.48	0.36
1_0565	0.54	0.98	0.50	0.37	**1_0657**	0.61	0.98	0.48	0.36
1_1072	0.54	0.98	0.50	0.37	**1_0670**	0.61	0.98	0.48	0.36
1_0081	0.55	0.94	0.50	0.37	**1_0437**	0.61	0.94	0.47	0.36
1_0146	0.55	0.94	0.50	0.37	**1_1360**	0.61	0.94	0.47	0.36
1_0153	0.55	0.94	0.50	0.37	**1_0025**	0.62	1.00	0.47	0.36
1_0056	0.55	1.00	0.49	0.37	**1_0945**	0.62	1.00	0.47	0.36
1_1103	0.56	0.96	0.49	0.37	**1_1512**	0.62	1.00	0.47	0.36
1_0058	0.57	0.98	0.49	0.37	**1_0917**	0.62	0.96	0.47	0.36
1_0062	0.57	0.98	0.49	0.37	**1_0567**	0.63	0.98	0.47	0.36
1_0525	0.57	0.98	0.49	0.37	**1_0652**	0.63	0.98	0.47	0.36
1_0690	0.57	0.98	0.49	0.37	**1_0706**	0.63	0.98	0.47	0.36

MAF: major allele frequency; Avail: allele availability; He: Expected Heterozogosity; PIC: polymorphic information content

Table 5.1: Summary statistics of genetic variation using 170 SNP markers among 47 cowpea lines (continued)

Marker	MAF	Avail	He	PIC	Marker	MAF	Avail	He	PIC
1_1214	0.57	0.98	0.49	0.37	**1_0937**	0.63	0.98	0.47	0.36
1_1246	0.57	0.98	0.49	0.37	**1_0977**	0.63	0.98	0.47	0.36
1_1431	0.57	0.98	0.49	0.37	**1_1096**	0.63	0.98	0.47	0.36
1_1129	0.63	0.98	0.47	0.36	**1_0022**	0.70	0.91	0.42	0.33
1_1370	0.63	0.98	0.47	0.36	**1_0746**	0.70	0.91	0.42	0.33
1_0256	0.64	0.94	0.46	0.36	**1_0807**	0.70	1.00	0.42	0.33
1_0319	0.64	0.94	0.46	0.36	**1_0647**	0.71	0.96	0.41	0.33
1_1151	0.64	0.94	0.46	0.36	**1_0709**	0.71	0.96	0.41	0.33
1_0699	0.64	0.96	0.46	0.35	**1_0392**	0.72	0.98	0.41	0.32
1_0290	0.65	0.98	0.45	0.35	**1_0755**	0.72	0.98	0.41	0.32
1_0823	0.65	0.98	0.45	0.35	**1_0853**	0.72	0.98	0.41	0.32
1_0246	0.66	0.94	0.45	0.35	**1_0242**	0.72	1.00	0.40	0.32
1_0317	0.66	0.94	0.45	0.35	**1_0957**	0.72	1.00	0.40	0.32
1_0757	0.66	0.94	0.45	0.35	**1_0142**	0.73	0.96	0.39	0.31
1_0482	0.66	1.00	0.45	0.35	**1_0775**	0.73	0.96	0.39	0.31
1_0730	0.66	1.00	0.45	0.35	**1_0983**	0.73	0.96	0.39	0.31
1_1271	0.66	1.00	0.45	0.35	**1_0107**	0.74	0.98	0.39	0.31
1_0033	0.67	0.96	0.44	0.35	**1_0330**	0.74	0.98	0.39	0.31
1_0065	0.67	0.96	0.44	0.35	**1_0529**	0.74	0.98	0.39	0.31
1_0306	0.67	0.96	0.44	0.35	**1_0679**	0.74	0.98	0.39	0.31
1_0649	0.67	0.96	0.44	0.35	**1_1281**	0.74	0.98	0.39	0.31
1_0438	0.67	0.98	0.44	0.34	**1_0060**	0.74	1.00	0.38	0.31
1_0473	0.67	0.98	0.44	0.34	**1_0238**	0.76	0.96	0.37	0.30
1_0834	0.67	0.98	0.44	0.34	**1_0451**	0.76	0.96	0.37	0.30
1_1037	0.67	0.98	0.44	0.34	**1_0583**	0.76	0.96	0.37	0.30
1_1042	0.67	0.98	0.44	0.34	**1_0053**	0.76	0.98	0.36	0.30
1_1062	0.67	0.98	0.44	0.34	**1_0323**	0.76	0.98	0.36	0.30
1_1520	0.68	1.00	0.43	0.34	**1_0740**	0.76	0.98	0.36	0.30
1_0322	0.68	0.94	0.43	0.34	**1_0876**	0.76	0.98	0.36	0.30
1_0911	0.69	0.96	0.43	0.34	**1_1087**	0.76	0.98	0.36	0.30
1_0111	0.70	0.98	0.42	0.33	**1_1170**	0.76	0.98	0.36	0.30
1_0157	0.70	0.98	0.42	0.33	**1_0128**	0.77	1.00	0.36	0.29
1_0370	0.70	0.98	0.42	0.33	**1_0663**	0.77	1.00	0.36	0.29

MAF: major allele frequency; Avail: allele availability; He: Expected Heterozogosity; PIC: polymorphic information content.

Table 5.1: Summary statistics of genetic variation using 170 SNP markers among 47 cowpea lines (continued)

Marker	MAF	Avail	He	PIC	Marker	MAF	Avail	He	PIC
1_0082	0.77	0.91	0.36	0.29	**1_0866**	0.83	0.98	0.29	0.25
1_0105	0.77	0.94	0.35	0.29	**1_1092**	0.83	0.98	0.29	0.25
1_1333	0.77	0.94	0.35	0.29	**1_0074**	0.83	1.00	0.28	0.24
1_0171	0.78	0.96	0.35	0.29	**1_0262**	0.83	1.00	0.28	0.24
1_1073	0.78	0.96	0.35	0.29	**1_1039**	0.84	0.91	0.27	0.24
1_1157	0.78	0.96	0.35	0.29	**1_0067**	0.85	0.98	0.26	0.22
1_0139	0.78	0.98	0.34	0.28	**1_0703**	0.85	0.98	0.26	0.22
1_0510	0.78	0.98	0.34	0.28	**1_0878**	0.85	0.98	0.26	0.22
1_0718	0.78	0.98	0.34	0.28	**1_0432**	0.87	0.96	0.23	0.20
1_0889	0.78	0.98	0.34	0.28	**1_0420**	0.87	0.98	0.23	0.20
1_1255	0.78	0.98	0.34	0.28	**1_0588**	0.87	0.98	0.23	0.20
1_0514	0.79	1.00	0.33	0.28	**1_0754**	0.87	1.00	0.22	0.20
1_1517	0.80	0.96	0.32	0.27	**1_1492**	0.87	1.00	0.22	0.20
1_0773	0.80	0.98	0.31	0.27	**1_0732**	0.88	0.91	0.21	0.18
1_0801	0.80	0.98	0.31	0.27	**1_0678**	0.89	0.98	0.19	0.17
1_1121	0.80	0.98	0.31	0.27	**1_1249**	0.91	0.96	0.16	0.15
1_0280	0.81	1.00	0.31	0.26	**1_0421**	0.91	0.98	0.16	0.15
1_0691	0.81	0.91	0.30	0.26	**1_0539**	0.91	0.98	0.16	0.15
1_0014	0.83	0.98	0.29	0.25	**1_1217**	0.93	0.98	0.12	0.11
1_0436	0.83	0.98	0.29	0.25	**1_0992**	0.96	0.98	0.08	0.08
1_0519	0.83	0.98	0.29	0.25					
1_0625	0.83	0.98	0.29	0.25	**Mean**	**0.68**	**0.97**	**0.41**	**0.32**

MAF: major allele frequency; Avail: allele availability; He: Expected Heterozogosity; PIC: polymorphic information content

5.3.2. Core collection of cowpea germplasm

Twenty cowpea genotypes forming a core collection is presented in Table 5.2. This collection comprises mainly improved varieties from Burkina Faso (15) followed by improved varieties from International Institute of Tropical Agriculture (IITA) in Nigeria (IITA/Nigeria) (three), one line from Niger and one from Senegal.

Table 5.2: Core collection of cowpea germplasm

Genotypes	Origin
MOURIDE	Senegal
KVX525	Burkina Faso
KVX396-4-5-2D	Burkina Faso
KVX780-3	Burkina Faso
KVX780-4	Burkina Faso
IRON CLAY	IITA/Nigeria
KVX30-309-6G	Burkina Faso
KVX61-1	Burkina Faso
TN88-63	Niger
KVX404-8-1	Burkina Faso
KVX780-6	Burkina Faso
IT98K-317-2	IITA/Nigeria
F8_SR	Burkina Faso
BULKF7_SR	Burkina Faso
KVX771-10	Burkina Faso
KVX775-33-2	Burkina Faso
KVX421-2J	Burkina Faso
KOMSARE	Burkina Faso
IT99K-499-39	IITA/Nigeria
KVX414-22-2	Burkina Faso

5.3.3. Phylogenetic relationships between cowpea lines

The cowpea lines were grouped into seven clusters based on genetic distance based on the allele sharing similarity. The cluster analysis showed that lines generally grouped together according to their geographical origin and traditional genetic background (Figure 5.1). Cluster VII and IV, can be considered as outliers as they contained only one line (Mouride, IT86D-610). Cluster I consisted of 16 genotypes, cluster II had six lines, Cluster III had 14 lines, Cluster V contains seven lines, and cluster VI has two lines. United State and Burkina Faso landraces respectively fell into clusters II (US) and V (BF_2Loc) while the improved varieties were all in cluster III (BF_1). The genetic

material from IITA fell into two main clusters I ($IITA_1$) and VI ($IITA_3$) with slight mixture of some improved varieties from Burkina, Senegal, and Ghana.

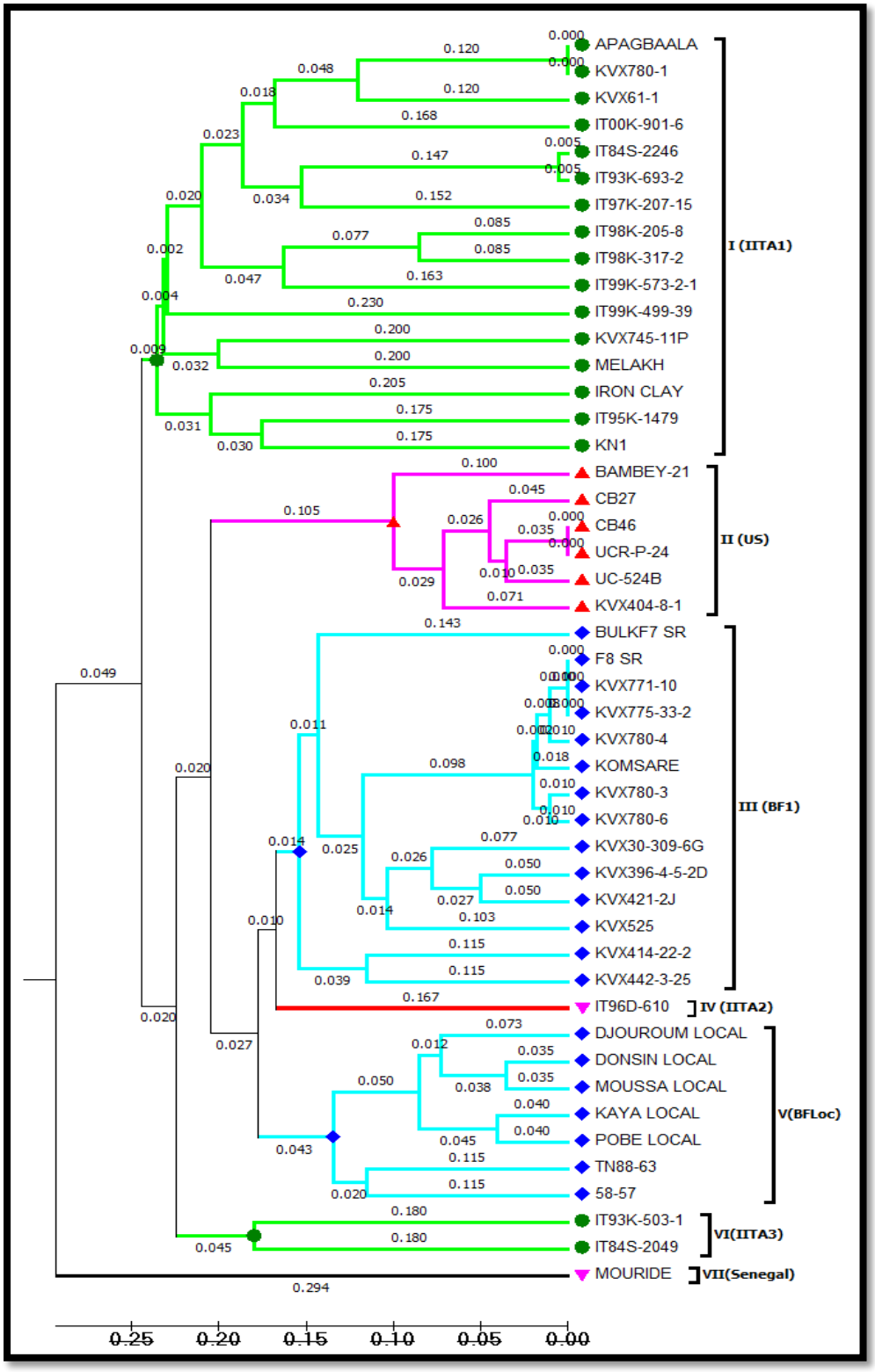

Figure 5.1: UPGMA dendrogram of 47 cowpea genotypes constructed using 170 SNP markers

5.4. Discussion

In the present study one hundred and seventy (170) SNP markers were used to genotype forty seven (47) cowpea lines. The results showed a good level of polymorphism but a moderate level of diversity based on the average polymorphic information content values (0.32). Almost all of the 47 lines shared a very narrow genetic distance (≤0.29), which is consistent with the results reported by Li *et al.* (2001). Moreover, the markers enabled the grouping of lines based on their similarity. Likewise, the SNP markers were able to associate more or less the cluster to the geographical origin of the line. Breeding programs generally work within restricted pools of genetic variation (Huynh *et al.*, 2013) and might be the cause this narrow genetic diversity observe in this study. A number of authors have come to the conclusion that cowpea lacks significant variability (Pasquet, 1999, 2000; Fang *et al.*, 2007). Narrow genetic base has been also observed within different lines from breeding programs (Li *et al.*, 2001). The materials from IITA collection have been widely used by different breeding programs in different countries. This can explain the relatedness between some cowpea improved varieties from Burkina Faso (KVx745-11P, KN1, KVx780-6, and KVx61-1). Looking at also the pedigree of Melakh (IS86-292xIT83S-742-13) (Diouf and Hilu, 2005), it becomes easy to confirm that this line fell into the IITA varieties cluster because of its relatedness with line from the IITA breeding program. Huynh *et al.* (2013) provided some useful assumptions that tend to explain the reduction of the genetic distance between cowpea wild types, landraces, and improved germplasm within African germplasm accessions and among African and Non-African germplasm accessions. These authors concluded that the small genetic differentiation observed between the African and non-African collections indicated that the entire genetic diversity in the African germplasm might already have spread over cowpea-growing regions in the world as a whole although not completely within any single region. Nevertheless,

the clustering of these forty seven lines into seven distinct groups gives important insights that can improve the efficiency of germplasm used in cowpea for breeding purposes. Except the material from Senegal (Bambey in cluster II, Mouride in cluster VII, 58-57 in cluster III, and Melakh in cluster I), from Niger (TN88-63 in cluster III), and from Ghana (Apaagbala in cluster I) that were not grouped according to their geographical origin, the rest were clustered in a country basis. That could be helpful for new ways of genetic improvement of cowpea by exchanging material from different countries to broaden the genetic base of the crop. In contrast with these findings, a numbers of genetic diversity studies conducted on cowpea have reported absence of correlation between geographical origin of the accessions and their clustering pattern (Asare *et al.*, 2010; Egbadzor *et al.*, 2014). This was also observed in a genetic diversity study in maize using SSR markers (Oppong, 2013). In this study, the genotypes were clustering following a regional basis of maize cultivation in Ghana. The differences shown between Burkina Faso landraces and the improved varieties may also be useful as a little diversity still exists among the local germplasm for new variety development. SNP markers have demonstrated their capacity in assessing genetic diversity in cowpea (Huynh *et al.*, 2013; Egbadzor *et al.*, 2014). Varshney *et al.* (2007) reported on the robustness of SNP markers. As compared to SSR markers, SNPs are more robust as they are able to detect slight changes in the genome and discriminate genotypes. This assumption is confirmed by the findings from a genetic diversity study on sweet cherry (*Prunus avium*, L.) (Fernandez I Marti *et al.*, 2012). In this study SNP markers were able to discriminate mutants from their original parents than SSR markers. In addition, SNP markers confirmed parentage and also determined relationships of the accessions in a manner consistent with their pedigree relationships. The latter statement confirmed our findings. Lines like Melakh from Senegal, KVx745-11P from

Burkina Faso grouped with the IITA accessions because of the large contribution in their genome of materials from IITA.

Extension of gene pool is important for crop improvement (Varshney *et al.*, 2007). As such a core collection of 20 lines was proposed from this study based on the maximum diversity between them. Several genetic diversity studies have been conducted in cowpea (Pamella and Gepts, 1992; Vaillancourt and Weeden, 1992; Fotso *et al.*, 1994; Coulibaly *et al.*, 2002; Ba *et al.*, 2004). Despite of the presence of little diversity within the collection used for this study and the core collection, the separation of the broader germplasm of cowpea landraces into gene pools as done by Huynh *et al.* (2013) could be useful for expanding the genetic diversity within breeding materials and could lead to development of more efficient strategies and genetic gain within future breeding programs.

5.5. Conclusion

The present study was undertaken to determine the genetic variability in a set of germplasm used by INERA cowpea breeding program in Burkina Faso using SNP markers. The germplasm used has some moderate variability with narrow genetic base. These results were comparable to previous studies that have also reported the narrow genetic base of cowpea.

The phylogenetic patterns and clustering of relatively similar individuals into groups providing important information on the germplasm used for cowpea improvement. The materials grouped based on the geographic origin and the genotypic background. Materials from United

State/University of California Riverside clustered together. Likewise, materials from IITA/Nigeria, Burkina Faso clustered in country base.

SNP markers were able to group the genotypes in a way that they could be used to link the genotype clusters and their pedigree. A panel of 20 genotypes representing the maximum variability of the germplasm used in the study was generated based on the maximum diversity sum. This panel constituted a core collection could be together with the information on the clustering of great importance for further plant breeding to develop superior varieties of cowpea.

CHAPTER SIX

6.0. QTL INTROGRESSION FOR DROUGHT TOLERANCE IN COWPEA

6.1. Introduction

Backcrossing is a traditional breeding method commonly employed to transfer alleles at one or more loci from a donor to an elite variety (Allard, 1960). This method can take a lot of time before recovering the background of the elite cultivar. According to Semagn *et al.*(2006), the recovery of 99.2% of the elite cultivar could take at least six generations when there is no deviation. It is time and labor consuming. The molecular breeding technology allows transferring target genomic regions resulting in extensive genetic mapping experiments aiming at the development of molecular markers for marker assisted backcrossing (MABC) and marker-assisted selection (MAS) (Semagn *et al.*, 2006). This technology may be defined in a broad-sense as the use of genetic manipulation performed on DNA at molecular levels to improve characters of interest in plants and animals, including genetic engineering or gene manipulation, molecular marker-assisted selection, genomic selection (Jiang, 2013).

During the past two decades, the technology development has opened a way for gene transfer using molecular markers. As a result of that many studies were carried out in order to exploit the technology in crop improvement. The use of MAS for introgression of major quantitatively inherited trait loci for stress tolerance is increasingly being applied in crop improvement. MAS has been used in selection for drought tolerance in maize (Ribaut and Ragot, 2006), pearl millet

(Howarth and Yadav, 2002), and rice (Courtois and Lafitte, 2003). Howard and Yadav (2002) have also reported a successful introgression of disease resistance using MAS.

SNP markers derived from specific DNA sequences quantitative trait loci (QTLs) are cheaper and more useful for marker-assisted selection (MAS) and have become the marker of choice when high-throughput genotyping assays have been developed. It is an approach that has been developed to avoid problems resulting from conventional plant breeding by changing the selection criteria from selection of phenotypes towards selection of genes that control traits of interest, either directly or indirectly. However, the technology is sometimes not accessible.

Until recently, cowpea was an orphan in the molecular domain. Recent development in the area moved cowpea from orphan to one the most important in terms of markers availability and uses. In terms of availability there is a physical map (Http://Phymap.Ucdavis.Edu/Cowpea/, 2013) with a density in average of 0.6 cM and no gap > 4 cM marker density. In many cases it will be possible to identify flanking markers that to be used in drought tolerance QTLs introgression with minimum linkage drag. These advances in plant molecular genetics have provided plant breeders with powerful tools to identify and select Mendelian components underlying both simple and complex agronomic traits (Ribaut and Hoisington, 1998). In terms of uses, the SNP markers available have extensively been used in diversity studies (Huynh *et al*., 2013) and to identify drought tolerant QTLs (Muchero *et al*., 2008 and Muchero *et al*., 2009b). This study was therefore undertaken to make use of the MABC method to introgress drought-tolerant (yield under drought and stay green) QTLs from two IITA cowpea varieties into Moussa local, a farmer-preferred variety using SNP markers.

6.2. Materials and methods

6.2.1. Leaf sampling procedure

The kit for leaf sampling was provided by LGC Genomics Ltd. (Hoddesdon, UK), including:

1. 1× 96-well tube storage rack (the tubes can be removed from the rack if needed) with lid.
2. 1× perforated, gas permeable, heat-sealable film seal.
3. 1× 50g desiccant pack in a small sealed bag that should be kept in the sealed bag until use .as they will rapidly absorb moisture when exposed to the air.
4. 1× heavy-duty, sealable bag into which to return the samples.
5. Harris Uni-Core leaf cutting tool for cutting 6mm leaf discs.
6. Harris self-healing cutting mat for use with Harris Uni-Core cutting tool.

The sampling kit is designed to facilitate both the cutting of leaf discs and their transport and concomitant desiccation, for eventual DNA isolation. For each sample, four leaf discs of 6mm diameter were cut using the Harris Uni-Core leaf cutting tool supported by the Harris self-healing cutting mat and placed into a well of a storage plate. The plate was sealed with a perforated (gas-permeable) heat seal by applying a medium hot household iron to the top of the seal for about 2 seconds. The sealed plate was then, placed in a heavy-duty, sealed bag in the presence of a desiccant to dehydrate and hence preserve the leaf tissue during transit at ambient temperature. Decontamination of the leaf cutting tool should be carried out between sampling different plants to eliminate the possibility of cross-contamination. This is achieved using 70% ethanol or 2% NaClO (sodium hypochlorite).

For this study, leave from two weeks old plants sown in pot under greenhouse were collected using the kit and sent to LGC Genomics. The samples were collected from the fully expand trifoliate leaves of the two weeks old plants. The samples were then put in a 96 well plate and map of the plate was made to help identified each sample.

6.2.2. DNA extraction

The leave samples were sent to LGC Genomics for DNA extraction and SNP genotyping using an internal protocol described in Appendix 4. The SNP genotyping followed the KASP technology system as described by Thompson (2013).

6.2.3. Selection of markers

A set of 184 SNP markers spanning every 2-cM internals was selected for this study using the BreedIt© SNP Selector tool (Http://Breedit.Org/, 2014) developed by the cowpea team at University of California Riverside (UCR). The SNP_Selector provides an interface to generate customized lists of SNPs based on cM distance between markers genome-wide, and between markers at known trait positions. The installer generates a folder C:\BreedIt\SNP_Selector\ and place three Excel spreadsheets inside this folder: (1) the master SNP spreadsheet including 1536 cowpea SNPs from the Illumina Golden Gate assay and the genotypes of some cowpea parental lines, (2) the SNPs that work well with the KASP platform (LGC Genomics), and (3) the markers linked to traits of interest.

All SNP markers were developed from EST of drought-stressed tissues, so there is a chance the markers are associated with drought tolerance candidate genes (Muchero *et al.*, 2009b). The interface of the SNP_selector is shown in Figure 6.1.

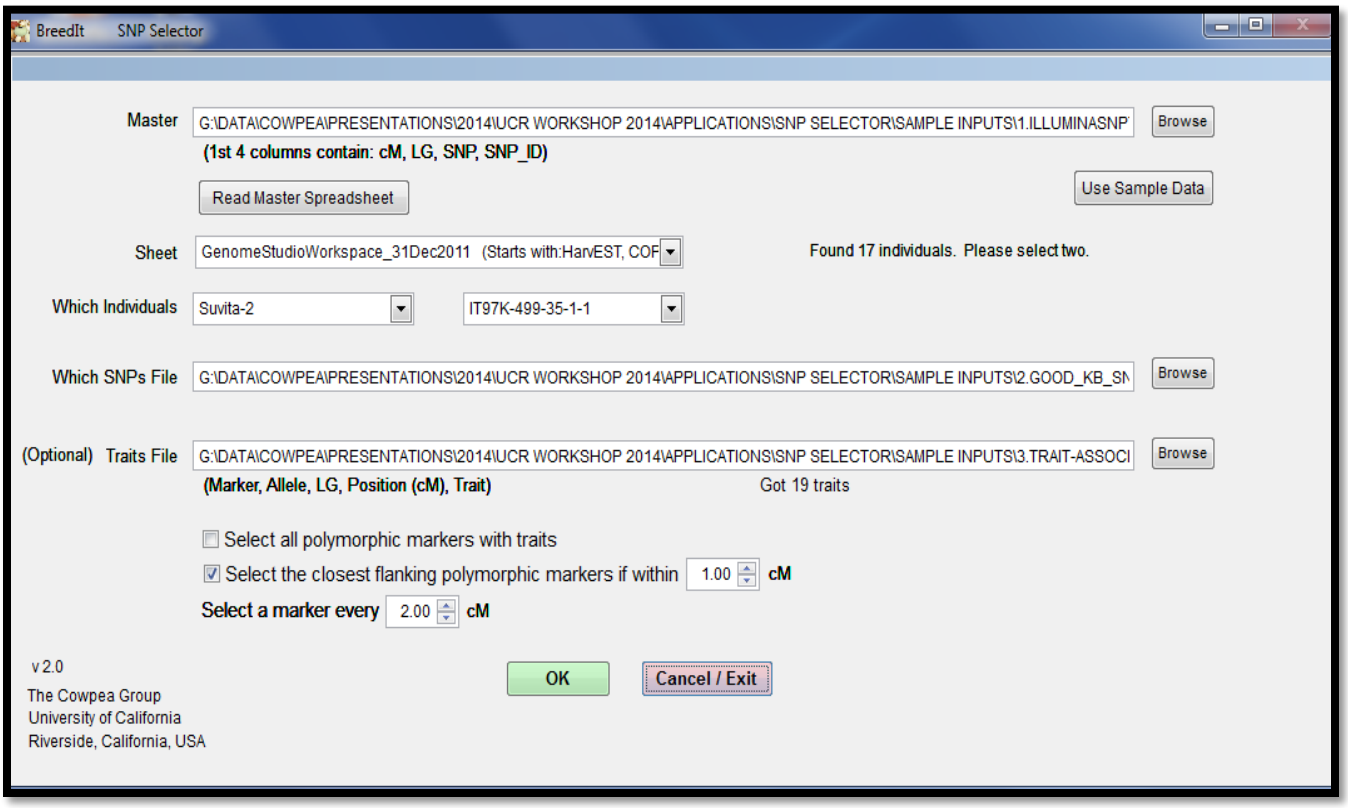

Figure 6.1: The interface of the SNP_selector tool

6.2.4. Plant materials and QTL introgression procedures

Two drought-tolerant lines from IITA (IT93K-503-1 and IT97K-499-35) that were found to be drought tolerant in Burkina Faso (Sawadogo, 2009) and in which drought-tolerant QTL have been discovered and mapped (Muchero *et al.*, 2008; Muchero *et al.*, 2009a; Muchero *et al.*, 2009b; Muchero *et al.*, 2010; Muchero *et al.*, 2011) were used as donors of positive drought QTLs, striga and nematode resistance genes. Moussa local that is a locally farmer preferred variety from

Burkina Faso was used as a recurrent parent. The donor alleles for yield, and nematode resistance were selected based on results from the UCR/INERA on-going and collaborative projects. The donor alleles for *Striga* were selected based on synteny with the *Striga* locus reported in Ouedraogo *et al.* (2002). Table 6.1 shows the position of trait-linked markers on cowpea consensus genetic map related to the QTLs introgressed in this study.

Table 6.1: Position of trait-linked markers on cowpea consensus genetic map

Trait	Marker	LG	cM	Donor
Nematode	1_1170	3	28.568	IT93K-503-1
Yield, Stay green	1_0678	4	25.390	IT93K-503-1
Yield, Stay green	1_0128	4	27.408	IT93K-503-1
Yield, Stay green	1_0157	4	30.339	IT93K-503-1
Yield, Stay green	1_0992	4	33.146	IT93K-503-1
Yield	1_0022	8	7.935	IT97K-499-35
Yield	1_1370	8	9.173	IT97K-499-35
Yield	1_0567	8	19.501	IT97K-499-35
Striga	1_0583	10	50.534	IT97K-499-35

For the MABC scheme, IT93K-503-1 and IT97K-499-35 were crossed to Moussa local to obtain F_1 progenies. The F_1s were backcrossed to Moussa local to obtain 95 BC_1F_1 seeds for each recurrent-donor combination. The BC_1F_1 seeds were planted in boxes in the greenhouse. Two weeks after planting, leaf samples were collected from each plant and sent to LCG Genomics for genotyping with the 184 SNPs. This allowed the selection of BC_1F_1 individual plants that were heterozygous for SNPs associated with drought tolerance, *Striga* and/or nematode resistance (foreground selection SNPs) and carried as many recurrent-parent alleles as possible at other SNP loci (background selection SNPs). The selected BC_1F_1 individual plants were backcrossed with Moussa local to obtain 95 BC_2F_1 individuals for another round of genotyping. In the BC_2F_1

generation, the individual plants that were heterozygous for foreground SNPs and carried as many recurrent-parent alleles as possible at background SNPs were identified.

In the next cycle, each of the selected BC_2F_1 individual plants was backcrossed to Moussa local to create BC_3F_1 lines. Four BC_3F_1 individuals from the cross Moussa local/IT97K-499-35 and three BC_3F_1 individuals from the cross Moussa local/IT93K-503-1 were selfed to obtain about forty BC_3F_2 seeds per line. Seed from BC_3F_2 were used for morphological characterization of the families and yield performance estimation. Ten entries (six MABC lines and four checks) were planted using a randomized complete block design with two replications and two water regimes (water-stressed WS, and well-watered WW). Two rows of 4 m with row spacing of 0.2 m between plants and 0.8 m between rows constituted the plot size per line. The checks comprised the three lines involved in the introgression process (IT97K-499-35, IT93K-503-1, and Moussa local) and one drought tolerant variety (Gorom local). The trial was conducted during the 2014 off-season from April to June under a drip-water irrigation system at the Kamboinse research station. Watering was withheld in the stressed block at 50% flower bud initiation till harvest.

6.3. Results

6.3.1. QTLs introgression

Genotyping of the BC_1F_1 identified the plant named M503_BC1F1_31 carrying the donor IT93K-503-1 alleles for yield and stay green under drought, *Striga*, and nematode resistance, and about 67% of variety Moussa local alleles at background markers. In the other cross, the plant named M499_BC1F1_04 carried the donor IT97K-499-35 alleles for yield under drought and *Striga* resistance, and about 70% of Moussa local alleles at background markers. In addition, some other

BC_1F_1 plants (M499_BC1F1_49, M499_BC1F1_48, M499_BC1F1_44, M503_BC1F1_54 and M503_BC1F1_92) carrying donor alleles but less of Moussa local background than M503_BC1F1_31 and M499_BC1F1_04 were also selected for backcrossing to Moussa local. In total, 190 individuals were obtained from the BC_2 backcrosses. The genotyping of these BC_2F_1 plants identified 10 individuals carrying different combinations of donor IT93K-503-1 alleles and 80–97% of Moussa local background (Table 6.2). Three selected plants with highest Moussa local background (M503_BC2F1_54P15, M503_BC2F1_54P8, and M503_BC1F2_92P27) were backcrossed to Moussa local to generate the M503_BC3F1 families. Likewise, 21 plants families were selected in the BC_2F_1 population; they carried different combinations of donor alleles for yield and *Striga* resistance and 69 – 93% of Moussa local background (Table 6.3). Five selected plants with highest Moussa local background (M499_BC2F1_48P90, M499_BC2F1_44P19, M499_BC2F1_48P93, M499_BC2F1_48P85, and M499_BC2F1_4P67) backcrossed to Moussa local generated the BC_3F_1 families. Of the total of six families derived from two donors were retained and selfed (five M499_BC3F2s and one M503_BC3F2s) enabled seed increase seed for further studies.

Table 6.2: Percentage of Moussa background and genotypes of BC_2F_1 plants carrying donor alleles (for yield, stay green, and nematode resistance) from the cross Moussa local /IT93K-503-1//Moussa local. Alleles A and B are designated for Moussa local and IT93K-503-1, respectively.

	Nematode	Yield, stay-green				Moussa background	
Plant	1_1170	1_0678	1_0128	1_0157	1_0992	(%)	Note
M503_BC2F1_54P15	AA	AB	AB	AB	AB	92	BC3 parent
M503_BC2F1_54P8	AA	--	AB	AB	AB	89	BC3 parent
M503_BC2F1_54P14	AA	AB	AB	AB	AB	88	
M503_BC2F1_54P11	AA	AB	AB	AB	AB	87	
M503_BC2F1_82P19	AA	AB	AB	--	AB	86	
M503_BC1F2_92P27	AB	AA	AA	AA	AA	86	BC3 parent
M503_BC1F2_83P34	AB	AA	AA	AA	--	86	
M503_BC2F1_54P16	AA	AB	AB	AB	AB	81	
M503_BC1F2_92P24	AB	AA	AA	AA	AA	80	
M503_BC1F2_77P55	AB	AA	AA	AA	AA	80	

Table 6.3: Percentage of Moussa background and genotypes of BC_2F_1 plants carrying donor alleles (for yield and Striga resistance) from the cross Moussa local /IT97K-499-35//Moussa local. Alleles A and B are designated for Moussa local and IT97K-499-35, respectively.

	Yield			Striga	Moussa background	
Plant	1_0022	1_1370	1_0567	1_0583	(%)	Note
M499_BC2F1_4P67	AA	AA	AA	AB	93	BC3 parent
M499_BC2F1_49P28	AB	AB	AB	AA	93	
M499_BC2F1_48P90	AB	AB	AB	AA	93	BC3 parent
M499_BC2F1_44P17	AB	AB	AB	AA	92	
M499_BC2F1_49P32	AB	AB	AB	AA	92	
M499_BC2F1_44P19	--	AB	AB	AA	90	BC3 parent
M499_BC2F1_4P72	AA	AA	AA	AB	89	
M499_BC2F1_49P31	AB	AB	AB	AB	88	
M499_BC2F1_49P25	AA	AA	AA	AB	88	
M499_BC2F1_48P85	AB	AB	AB	AA	88	BC3 parent
M499_BC2F1_48P93	AB	AB	AB	AA	84	BC3 parent
M499_BC2F1_10P39	AB	--	AB	AB	83	
M499_BC2F1_49P26	AB	AB	AB	--	83	
M499_BC2F1_31P47	AB	AB	AB	AA	83	
M499_BC1F2_67P86	AB	AB	AB	AB	79	
M499_BC2F1_49P23	AB	AB	AB	AB	79	
M499_BC2F1_31P51	AB	AB	AB	AB	77	
M499_BC2F1_70P38	AB	AB	AB	AA	76	
M499_BC2F1_66P77	AB	AB	AB	AA	72	
M499_BC2F1_66P75	AB	AB	AB	AA	69	
M499_BC2F1_29P63	AB	AB	AB	AB	69	

6.3.2. Morphological characterization of the MABC selected lines

Seed from BC_3F_2 were not enough to undertake a multi-location trial, so they were used for morphological characterization of the families and a single site yield trial. The morphological characteristics of the selected families and the recurrent parent are shown in Table 6.4. The plant type or growth habit of the lines and their dry pods form and colour in comparison with Moussa local are shown in Figure 6.2.

Table 6.4: Morphological characteristics of MABC selected lines and their recurrent parent

Genotype	Flower colour	Green Pod colour	Dry pod colour	Plant growth habit	*Striga* presence
Moussa local (RP)	**White**	**Purple**	**Purple**	**Spreading**	**1**
M499_BC3F3_44P19	White	Purple	Purple	Spreading	0
M499_BC3F3_4P67	White	Purple	Purple	Spreading	0
M499_BC3F3_48P90	White	Purple	Purple	Spreading	0
M499_BC3F3_48P85	White	Purple	Purple	Spreading	0
M503_BC3F3_92P27	White	Purple	Purple	Semi-erect	0
M499_BC3F3_48P93	White	Purple	Purple	Semi-erect	1

RP: recurrent Parent; 1: Presence of *Striga*; 0: absence of *Striga*

Figure 6.2: Plant growth habit of selected MABC lines (A); selected lines dry pod curvature and colour compared to Moussa local (B)

6.3.3. Grain yield performance of the MABC selected lines

The grain yields of the ten lines per water regime are represented in Figure 6.4. The grain yields ranged between 287.16 and 1187.70 kg.ha^{-1} in the water-stressed environment, while in the well-watered environment the yields were higher, ranging from 272.29 to 1771.00 kg.ha^{-1}. Three BC_3F_2 families (M499_BC3F3_48P85, M499_BC3F3_4P67, and M499_BC3F3_48P90) yielded better than all parents and the local check. All BC_3F_2 families appeared to perform better than the recurrent parent Moussa Local under water limited condition.

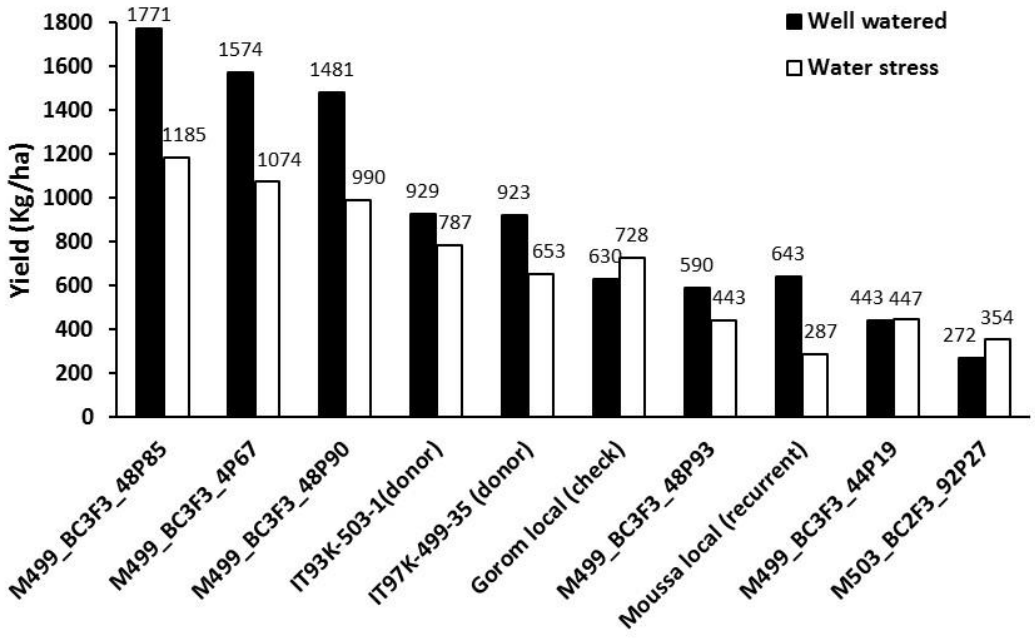

Figure 6.4: Yield performance of selected BC_3F_2 families, their parents and a local check under well water and water limited conditions. Values are the yield mean (kg.ha^{-1}) of two replications.

6.4. Discussion

This study involving MABC methodology using SNP markers seems to be the first report in cowpea breeding, particularly in the selection for drought tolerance. The methodology has allowed a quick recovery of the recurrent-parent (Moussa Local) background (up to 97%) with only two backcross cycles (BC_2) by using ninety-five individuals for each set of backcross in BC_1. The quick recovery of the recurrent parent background allows early selection and helps to reduce the population to be carried to next generation and therefore reduce time and work load. The levels of recovery of the recurrent parent background confirmed the findings reported by Jiang (2013) that revealed a percentage of recovery of 98% at BC_3 with a number of 100 individuals selected at BC_2. In the present study, several donor loci (yield under drought, stay-green, *Striga* resistance, and nematodes resistance) were introgressed at the same time. This decreases the chance to identify a line carrying all donor alleles and high Moussa background and, therefore, limits the number of offspring to be selected in the subsequent generations. Sebolt *et al*. (2000) also reported that the rate of success decreases when large numbers of QTLs are targeted for introgression; by using MABC for two QTLs for seed protein content in soybean introgression, they eventually found that only one QTL was confirmed in $BC_3F_{4:5}$. Compared to MABC, conventional backcross breeding, however, needs a much larger backcross population, as such 500 plants or more must be produced to ensure that there are sufficient plants for background selection after the foreground and recombinant selection have been performed. During the process, unless breeders screen the material to identify those that are carrying the gene of interest, they may end up doing blind crossing to the recurrent parent. In such condition conventional backcrossing is time consuming. By using flanking markers and better distributed markers across the recurrent parent genome, effective introgression can be done to avoid linkage drag and the use a large number of individuals.

In Burkina Faso, most of cowpea landraces have a prostrate growth habit and are susceptible to *Striga* and are often grown with cereal crops like sorghum and millet. The prostrate growth habit allows soil conservation and soil humidity maintaining by the cover of the vines. These morphological characteristics are in accordance with the expectations (Figure 6.2). Moussa local, a farmer preferred landrace has some purple pods which remain purple even for dry pods. The six lines bore the purple green and dry pod colour character confirming that they recovered this character from the recurrent parent. The prostrate (spreading) growth habit of Moussa local is also found in the selected MABC lines. These results, therefore, confirmed the molecular results that showed a high level of recovery of the recurrent parent which is the same Moussa local. The same trend was observed for *Striga* resistance. Only one line (M499_BC3F3_48P93) that had no *Striga* donor allele had *Striga* emergence in the well-watered environment. The Striga-resistant checks did not emerge *Striga* confirming their resistance while Moussa local had emerged *Striga* confirming its susceptibility to *Striga*. This result also confirms that the lines selected based on the presence of the *Striga* gene through the MABC introgression are effective in controlling *Striga*.

Yield is by far the first criteria for varietal selection by African farmers (Tignegre, 2010; Some, 2012; Traore, 2013). It was a promising achievement from the preliminary yield performance trial where three lines yielded better than the parents and the drought-tolerant check (Gorom local, Figure 6.5). In addition, the general performance of these lines reached the potential yield of the released variety in Burkina Faso which is around 1.5 tha^{-1}(Ouedraogo *et al.*, 2012b). The low yields of certain lines could be attributed to the fact that there was drought spells due to water shortages during the growing period at Kamboinse. However, other authors have reported in maize, three

QTLs for two traits (earliness and yield) were introgressed between maize elite lines with MABC but the results were function of numbers of other genes controlling the traits (Bouchez *et al.*, 2002).

In the domain of molecular breeding, a lot of conventional breeding methods have been associated with markers to design a large number a marker-aided selection methods. Some examples are marker-assisted selection (MAS), marker-assisted recurrent selection (MARS), marker-assisted backcrossing (MABC), and the new method which involves genotyping by sequencing (GBS).

Among the molecular breeding methods, MABC has been the most widely and successfully used in plant breeding up to date. Marker-assisted backcrossing (MABC) is an effective method for developing improved versions of widely cultivated varieties, also referred to as Mega varieties (Neeraja *et al.*, 2007). It has been applied to different types of traits (e.g. disease/pest resistance, drought tolerance and quality) in many species, e.g. rice, wheat, maize, barley, pear millet, soybean, tomato, etc. (Collard *et al.*, 2005; Dwivedi *et al.*, 2007; Xu, 2010). In maize, for example, *Bacillus thuringiens*is is bacterium that produces insecticidal toxins, which can kill corn borer larvae when they ingest the toxins in corn cells was used (Ragot *et al.*, 1995). The integration of the *Bt* transgene into various corn genetic backgrounds has been achieved by using MABC. Genotyping by sequencing (GBS) was also used by Asante (2012) to introgress effectively rice fragrance from jasmine rice into a local Ghanaian variety Digang.

6.5. Conclusion

In this study, the use of the technology has allowed rapid recovery of the background of the farmer preferred landrace. In two backcrosses the recurrent parent background recovered at 97% in some lines. The recovery captured all the characteristics of Moussa local, the farmer preferred that was used as recurrent parent. Pod colour and curvature, plant growth habit, flower colour were successfully transferred to the new lines together with QTLs for yield and stay-green under drought stress. This demonstrates that MABC can be used in introgressing important traits in cowpea.

The morphological observation demonstrated that these lines could be good candidates for intercropping in farmers' field. Until now, no *Striga* resistant variety combining farmers' preferences has been proposed by INERA for intercropping. Since Moussa local was used in cowpea intercrops, these lines are promising. From this study three lines out of the six are more promising based on the good yield and their probable resistance to *Striga*. These three promising lines M499_BC3F3_48P85, M499_BC3F3_4P67, and M499_BC3F3_48P90 need to be advanced for multi-location trials to measure their performance and ability to withstand drought and *Striga* attacks. The lines could be phenotyped and genotyped to confirm lastly the presence of the introgressed donor alleles affecting yield under drought, stay-green, and *Striga*.

The next decade will possibly see the marker assisted breeding technology spreading to benefit cowpea farmers in Africa. It is now practical to use marker-assisted methods for step-wise maintenance and enhancement in cowpea breeding program to more efficiently target step-wise improvement that best meets farmers' needs.

CHAPTER SEVEN

7.0. GENERAL DISCUSSION AND CONCLUSION

7.1. Introduction

Drought, manifested in the form of high variability in amount and distribution of rainfall over seasons and agro-ecologies, is a major constraint threatening cowpea production in Burkina Faso. This study is a step towards the development of farmers' preferred and drought tolerant cowpea cultivars in the country. This chapter aims at providing an overview of the research findings, the breeding implications of such findings, and the way forward. The objectives of this research were to:

- Determine farmer perceptions on the impact of drought on cowpea
- Identify sources of tolerance to drought stress in the cowpea germplasm
- Assess the SNP-based genetic diversity of a set of cowpea germplasm
- Introgress drought tolerant QTLs into Moussa local, a farmer preferred cowpea landrace using marker-assisted backcrossing.

7.2. Main research findings and breeding implications

Research investigations were conducted in Burkina Faso from 2012 to 2014 to achieve these objectives. The overall research was supported by a literature review that reveals the following:

- Cowpea (*Vigna unguiculata* (L.) Walp.) is an important staple in semi-arid areas of West Africa including Burkina Faso, where large quantities of cowpea is being produced. The

production at farmers' level is 300 kg ha^{-1} which is below the potential of 7000 kg ha^{-1} achieved in USA (Ehlers and Hall, 1997).

- Drought due to erratic pattern of rainfall is one of the main constraints to cowpea production. Drought is a quantitative trait and as such breeding for drought tolerance is more complex than breeding for other simply inherited traits (Krishnamurthy *et al.*, 1996).

- Molecular markers are increasingly being discovered and used in cowpea for many kinds of studies (gene mapping, genetic diversity, molecular breeding, fingerprinting etc.). QTLs have been mapped for drought-tolerance, stay-green, nematode resistance, *Striga* resistance in cowpea (Ouedraogo, 2001; Muchero *et al.*, 2009b).

The breeding priorities for cowpea drought-prone areas of Burkina Faso were investigated using participatory research methods. Grain yield reduction, fodder yield reduction, total loss of crop and landraces, difficulties in planning cropping season, loss of arable land were most of the time during this study the main impact of drought on cowpea production in the areas where the study was conducted.

- Farmers demonstrated that they have a deep knowledge about cowpea production constraints and difficulties to access improved variety seeds was the first constraint in all the areas where the study has been conducted. Other important constraints include: drought tolerance, lack of equipments, soil fertility, limited access to fertilizer and pesticicdes.
- The preferred grain traits for all regions were white, large seeded, with a rough texture for food and market purposes, except for the northern region where brown grain was preferred for food.

Therefore, the development of new drought-tolerant cultivars for Burkina Faso will need a simultaneous selection for genotypes with resistance to the major abiotic and biotic constraints for farmers as well as for market preferred grain traits. These grain characteristics should be included in cowpea breeding programs to ease the adoption of improved varieties by Burkina Faso farmers. The participatory variety selection (PVS) during this participatory rural appraisal (PRA) paved the way for need-based selection by the farmers, and thereby could help promote quicker adoption of useful varieties in the farming community. In this way, improved varieties seeds could be availed to framers. That could help fill the gap of the unavailability of improved variety seeds at farmers' level.

Field screening was conducted in 2012 to identify cowpea genotypes tolerant to drought based on quantitative stress indices and yields under stressed and non-stressed condition.

- Genotypic variability for drought exists among the tested genotypes.
- Different cowpea varieties were found to be highly or moderately drought tolerant based on the biplot analysis.
- Gorom local, KVx61-1, and IT98K-1111-1 confirmed their tolerance to drought.
- Djouroum local, Pobe local, KVx404-8-1, KVx745-11P, KVx525, KVx414-22-2, and KVx421-2J were new drought tolerant genotypes identified in this study.
- Of these, KVx745-11P, KVx525, KVx414-22-2, and KVx421-2J are low yielding genotypes.

These genotypes low or high yielding could be used in breeding program to improve high yielding drought susceptible genotypes. The existence of genetic variability for drought tolerance implied that genetic improvement of cowpea for drought tolerance could be conducted using this germplasm.

Diversity or genetic variation is a pre-requisite for new variety development. Genetic variation can be found in traditional varieties, landraces, commercial cultivars, and other plant materials developed through breeding. A study was conducted to determine the genetic diversity in a set of germplasm used in INERA cowpea breeding program exploiting SNP markers technology. This study showed that:

- The germplasm used showed some moderate diversity
- Genotypes grouped into clusters based on their genetic background or their geographic origin
- The SNP markers were able to group the genotypes in a way that they could be used to link the genotypes and their original parents

A core collection of 20 genotypes was generated from the germplasm based on the maximum diversity between accessions. These findings implied that measures should be taken for introduction of new cowpea accessions from other breeding programs to broaden the genetic base of cowpea in Burkina Faso.

Marker-assisted backcrossing (MABC) methodology was used to transfer drought tolerance QTL and *Striga* resistance into a farmer preferred landrace. The use of the technology in this study has allowed rapid recovery of the background of the farmer preferred landrace. Some promising lines

were selected from $BC_1F_1$and BC_2F_1 populations using SNP markers for background and foreground selection.

- Three lines out of the six are more promising based on the good yield and their probable resistance to *Striga*. These three promising lines M499_BC3F3_48P85, M499_BC3F3_4P67, and M499_BC3F3_48P90 need to be advanced for multi-location trials to measure their performance and ability to withstand drought and *Striga* attacks.

- The morphological observation demonstrated that these lines could be good candidates for intercropping in farmer field.

Until now, no *Striga* resistant variety combining farmers' preferences has been proposed by INERA for intercropping. Since Moussa local was used in intercropping these lines are promising. It will also be practical to use marker-assisted methods for step-wise maintenance and enhancement cowpea breeding program in Burkina Faso to more efficiently target step-wise improvement that best meets farmers' needs.

7.3. General conclusion and way forward

In this study, cowpea production constraints, farmers' preferences, and perceptions on drought impact on cowpea cultivars and traits in three major districts of cowpea production were identified and ranked. Genotypes with high variability for grain yield and drought tolerance were identified. A core collection of diverse cowpea lines based on the maximum diversity using SNP markers was built representing the variability within the germplasm used for in this study. Six promising cowpea lines were selected using MABC. Three of them showed good preliminary yield that need to be confirmed in additional trials.

Efforts should be made to address the major production constraints through breeding to increase cowpea production in Burkina Faso. During the breeding process farmers' preferences should be considered and farmers themselves should be involved to ensure varietal acceptance and adoption. Market aspects need to be considered to develop suitable varieties that meet the needs of farmers. A crossing program among diverse parents for the traits (drought and yield) and selection may generate a pool of individuals for the improvement of the crop. In the selection process farmers should be involved. Phenotyping and genotyping of the advanced MABC lines should be conducted to confirm the presence of the introgressed donor alleles affecting yield under drought, stay-green, and *Striga*. Further studies need to be conducted using more sites and seasons for firm recommendations.

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Appendices

Appendix 1: SNP ID and sequences used for MABC selection

SNPID	SNPNum	AlleleY	AlleleX	Sequence
1_0105	12650001	G	A	AAGTATGGCCAGACTTC[G/A]AATCTTGAGATCC
1_0709	12650016	G	A	AAGCCTGTCCGCAA[G/A]TTGTCTCTAGTCCAC
1_0917	12650017	G	A	ATAGCAAAGAAATG[G/A]TAAAAAGAAAGAAGG
1_0866	12650029	C	A	AACGCAAACTGTCGC[A/C]GGTTATATTTTCCT
1_1217	12650034	G	A	AAGCAGAGCCTGGA[G/A]TCGGACTCCGCCGGA
1_0594	12650038	C	A	ATTCTGTGCTGCCAC[A/C]TTAAGCAGGCTGTC
1_1370	12650039	G	A	TTCAATGCATTTCAC[A/G]TCTTCTGGCGGAAT
1_0706	12650043	G	A	TTTGTTGATGATTGT[A/G]TTCAAAGTGACATA
1_0754	12650049	G	A	GGACAGCACAAGTCT[A/G]ACTTCAGAAAAGCT
1_1413	12650048	C	A	ACTCCTCCTATGGC[C/A]GCAAAGGTCAAACCA
1_0256	12650056	G	A	GGCTCTTGGTAAGC[G/A]TATGCATAACGTTGT
1_0649	9030007	G	A	GTGAAAGTTGAAAAA[A/G]GTGAAACTGTCAAG
1_0262	12650063	T	A	AATCCCCGCCGCGTT[A/T]GCTCCACAGGGTCA
1_1103	12650070	G	C	AGCTTGCAGGATCAA[C/G]CCACCCTCCAGATT
1_1249	12650073	G	A	AAGTCATTGACGAT[G/A]TGAGGAATTTCATCG
1_0755	12650079	G	C	TGCTGCGGGGCATGT[C/G]AGAGAAGAATGTGA
1_0992	12650082	C	G	AGGGCAGAGATAAT[C/G]AATGAGGTAAAAAAT
1_0775	12650084	C	G	AGAAGAGTTCGAAA[C/G]AGATAAAATTATTTA
1_0392	12650095	G	A	CTGTTTCTTTGAGC[G/A]TCAAGTTGGGGTGGT
1_0370	12650098	G	A	TCGATGGACGATCC[G/A]GGAAGATTGGGCAGT
1_0126	12650104	G	A	ATTCGCATTTGGCG[G/A]GACTGAGGACCATCA
1_0757	12650116	C	A	TTATGAAGCTCTTGG[A/C]CTCACTTCCAAGCA
1_0081	12650121	G	A	AGAGCAAATATTTA[G/A]AACAAAATATCCCTC
1_0401	12650131	C	A	ATGCAAACTGAGAG[C/A]ATGCAAATACAAAAG
1_0432	12650136	C	A	CTTCGATTAAGTGCA[A/C]ACTCCTACTCTACC
1_0022	12650139	C	A	CCTCGTCTTCAAGTC[A/C]GGCATGGCCAAGTC
1_0307	12650150	G	A	ACACGTTTGTACATA[A/G]GAGTGTGTAAAGTT
1_0053	12650157	G	A	TTGCAGCAAGTACTC[A/G]TTTGACATGAGCTA
1_1360	12650159	T	A	TGGGTATGTAACTAA[A/T]GCCCTTAACCTTCA
1_0982	12650162	G	A	AAATTATTTTTGGTG[A/G]GCCTGAGGTTACAA
1_0993	12650174	G	A	TTGGGAAACACAAA[G/A]ATGTCACCTTTGTTA
1_0652	12650181	G	A	ACCTTAATTGGGGAC[A/G]TTGATCCAGTTCAA
1_0183	12650189	G	A	TCCGGAGAAACAGC[G/A]ACAGTGTTCACATAC
1_0052	12650197	C	A	TAGTTCTGGTGTGG[C/A]YTTGCAGGTACAGAA
1_1039	12650199	T	A	GATGAAACAGACTTA[A/T]GGGCTTATGATGTA
1_0033	12650200	G	A	CAAAAARATGTCCA[G/A]GCTAAAAAACAAAAG
1_0678	12650222	G	A	TGCTTCTTTTGATG[G/A]AAAATTTAGTTGTAC

Appendix 1: SNP ID and sequences used for MABC selection. Continued

1_0983	12650225	G	A	CAGAGTTCCTCCTC[G/A]ACGTCCCCGAACCTT
1_0670	12650228	G	A	AGCTCAACCATTCA[G/A]GCCTCAAAATTCAAA
1_0142	12650229	A	T	TTTGCAGTTCCACA[A/T]CCTATAGACAGCAAC
1_0139	12650234	C	A	GGCTACCATGAATC[C/A]GGAAAATTGATCGTG
1_0547	12650239	G	A	CATAAAACACTGTCG[A/G]AAACAAAAAAATGT
1_0703	12650262	G	A	AAGCATTCTATTGG[G/A]AAGTTCTCCAGGTTA
1_0082	12650269	G	A	TCTAAGGAAAGATGG[A/G]AAGAAGCCCAGTGC
1_0290	12650276	G	A	TCAAAAGGTAGTGGT[A/G]GTGCGGTGCGAAGA
1_0987	12650281	G	A	CAGAGGAACTGTGT[G/A]GTGGAAGTCCATCTG
1_1517	12650284	G	A	CTACTGATTGGATA[G/A]CAGGCCCAATATTGG
1_0565	12650286	C	G	CTAAAGCACCARTA[C/G]ACACTGCCAACAACA
1_1151	12650294	G	A	AGTGTATCTGTTAC[G/A]TGGGCAAAATAAAAG
1_0153	12650304	G	A	TATTATAAGAATGTG[A/G]GAATATGCAATGGC
1_1042	12650308	G	A	GATAGATGAGTCATC[A/G]CCTGCTAAATACCG
1_0732	12650314	G	A	TGAACTCCGTGGCC[G/A]AACGTGTAAACCTCC
1_0519	12650322	G	C	TCTCATCCATGCTTT[C/G]TGCTCCTTTGGATC
1_0679	12650323	G	A	GCTCCAACAATTTC[G/A]GTGGGTTCCTCTGCA
1_0127	12650329	G	A	AACCCAGAGAAAAC[G/A]AACTTACAAGACCTA
1_0823	12650331	C	A	TCCCACCTCGAAAA[C/A]GACGTTTGGGTTGGA
1_0322	12650336	C	A	ATCAAATGTTACGGT[A/C]AATTTGGAAGGACA
1_1189	12650339	G	A	CAGTCTCACTGCCA[G/A]CAACTACATCACGGG
1_0280	12650342	G	A	ATGACGCGATCTGC[G/A]ACCTCGGACTTGTCG
1_0567	12650348	C	G	GTCGCCGGTTCGGA[C/G]TGCGAGTCGGACAGC
1_0539	12650356	G	A	ACACAAAAATATTG[G/A]CATYAATCTCAAGTG
1_0242	12650357	C	A	ACAGGGGATTCACC[C/A]TGCGAACCCGTTGCA
1_0598	12650360	G	A	GTAGGGAAGAAARAG[A/G]GAGAGATAAAATAC
1_0171	12650366	G	A	AACTGTGAAAGATGG[A/G]AAACTATACATCTG
1_1072	9030019	G	A	CCTAGACAACCAGCA[A/G]AGTATGTTCAGATT
1_1021	12650373	G	C	ATGTCTAACCCTCCT[C/G]GGTCGTAGATTTCA
1_0136	12650380	G	A	CTCGCTGAATACCA[G/A]AGGGGGCTGGTGCTT
1_0377	12650390	G	A	GGGTCATCTCGACCC[A/G]GGGGCCATTAGTTT
1_1467	12650393	G	A	CAACATATGCAGTG[G/A]TAAATCCCTGAGGTT
1_0317	12650396	G	A	CAACAACATTTACAA[A/G]CGCAAGTATGAGGA
1_0531	12650417	C	G	CAGTGCCTATCCTC[C/G]GCAAGCTCAACAATA
1_0067	12650411	G	A	TGAATGGCGCAGAG[G/A]TTAGTGTCTTCAAAG
1_1333	12650420	C	A	ATTTTTTTTTTACTT[A/C]CAAAAAAAAATGTT
1_0436	12650421	C	G	CGCAGAAGAGATTT[C/G]GAAGCCAACCCATCT
1_0111	12650431	G	A	TTGGCTTCTTGCCAG[A/G]ATGGTGTTGCAAAT
1_0420	12650436	G	A	AGCTGAAGGWCTTGA[A/G]AATGGTCCCTCAGC

Appendix 1: SNP ID and sequences used for MABC selection. Continued

1_1214	12650443	C	G	AAGGCAAGCCAGAC[C/G]GCGGTGTTGCACTTG
1_0748	12650447	G	A	TCATTTTCATTCTGG[A/G]ACATGGGAAGATCG
1_0801	12650461	G	A	GGCCCTGAAAGTAGG[A/G]TTGTCCAGTCTGTT
1_1135	9030013	G	A	CCTCGCTTTAATCGT[A/G]CGCCACTGGGTTGA
1_1170	12650475	G	A	CAATGCGGCGACTA[G/A]CGTGAACACAACGGT
1_1431	12650476	G	A	TTCGAGCTCCAATA[G/A]ATTAGGTTGTTGCAA
1_0351	9030014	C	A	TTGCCTTAGTCTCAT[A/C]TCTCTGTTTTACGT
1_0752	12650483	G	C	GTTTCATGTGTATTT[C/G]ATGATTGCTATTGC
1_0937	12650516	G	C	GCCATACGACGTCGT[C/G]GCTGCGCTGCTCTG
1_1371	12650518	G	A	TCTGAACATATCTT[G/A]GCTTTCATTTCTTTA
1_0806	12650520	G	A	ATGCAGGAGTTACAT[A/G]TTAGAGGATGAGAA
1_1073	12650521	G	A	AGAGGAAAAGAAGGT[A/G]GAAGAGAAGAAGGA
1_0306	9030025	G	A	GCCACAGGAACCGGC[A/G]CCTGCTCCTTCAAC
1_0691	12650551	G	A	AACTCTTGAATTGGT[A/G]GCTATTGATGAGCC
1_1520	12650555	C	G	GAAACGACCCGATC[C/G]GTGATAACATCAATC
1_0157	12650562	G	A	GAAACCCTAGGTAAG[A/G]AAAAATGCCGGCTG
1_0807	12650566	C	G	CTAATCTGCGCTAC[C/G]GCAGAATTTAAAATC
1_1246	12650568	T	A	TCCGTCCGCTTCCTC[A/T]CCCGTCGGCGTTTC
1_0084	12650577	C	A	CGTTTTTCGTGATCG[A/C]ATGCCACGTTTGCA
1_0583	12650579	G	A	CTAGATCCCAAGACC[A/G]CCATAGATATCAAG
1_0794	12650583	G	A	TAGTCAATTTTAAC[G/A]GATCTTCAAAACTTG
1_1281	12650587	G	A	TGGTTTTGGCTCAAC[A/G]GAGTCTAAACAGGA
1_1157	12650589	G	A	ATTGAACAAGTGAA[G/A]AGAAAAATAGAAGGA
1_0060	12650602	C	A	TTATTTGTTGGTGGT[A/C]CCATTCATTCTGAT
1_0025	12650606	G	A	AATTTTCTTCCTTTC[A/G]GTTTCGTTAGCCAG
1_0123	12650616	G	A	AAAGGGAATTGGTAA[A/G]AGTGGAAAGCCTCT
1_0473	12650618	G	A	GCTCACGGATCTGGA[A/G]GAGGTTGAGGAGGT
1_0771	12650624	C	A	AACAGAAAATAATG[C/A]AACAGAGGAGGATCC
1_0388	12650635	T	A	GGCTACTTCCCACTT[A/T]CGCTTCACTTTAGT
1_0525	12650642	G	A	TGATGCTTTGATACA[A/G]AAAGTAAATGCTGA
1_0690	12650651	G	A	GGGCACCAGAGTCAG[A/G]GCACAAACCATGAA
1_1271	12650657	G	A	AATTACAAAATTCT[G/A]CGCATTACATCATCT
1_0330	12650662	A	T	TGGAGGCCAGGGTT[A/T]GCACTGCTGAAGATA
1_0438	12650667	G	A	CGTGAGTACCTCATC[A/G]CCAATTTTTAGCAG
1_1393	12650668	G	A	AAGAAAAAGAATGAA[A/G]TTAAAGAAGATTTT

Appendix 1: SNP ID and sequences used for MABC selection. Continued

1_0065	12650669	C	A	GTGGCAGTGGCATCA[A/C]CTACAATCCTAGGA
1_1087	12650674	G	A	GTTCATGTTCCATA[G/A]CTAACTTTTCTTCAG
1_0625	12650675	G	A	CAAGTATCATATGTA[A/G]AAGACTGCAGACAT
1_1007	12650677	G	A	GATATATATTCAGT[G/A]CCAATTATATGGCCA
1_1141	12650698	G	A	TTATATTAATGTTGC[A/G]AATCATTGCAACAA
1_0853	12650709	G	A	CGGCGGAGGACGCC[G/A]GAGATAATGCGGCTG
1_0056	12650712	G	A	TCCATGAGGAAAACA[A/G]CCTCTAAGTCTGTT
1_1129	12650713	G	A	ATGTTCATGGTATT[G/A]TAGTCATTTATCAAC
1_1096	12650718	G	A	TCACTTAATCACTCA[A/G]TCACTTTCATCTTC
1_0730	12650735	G	A	ATGGTTTTGGTTTC[G/A]GTCTGAAGAAGCTCG
1_1117	12650741	C	A	GTTTGTGTGCATTG[C/A]AGTCTGGGAGTTCTG
1_0514	12650751	G	A	GGAATCCTCTATCA[G/A]AGGCACCCAGTAAGA
1_0923	12650752	G	A	GCAAGCATTAACAGT[A/G]GCGGCTGCAGTTGG
1_0397	12650767	C	A	TGGTTCTCTTTGTGG[A/C]CCTGTTGTTGATCA
1_0222	12650773	G	A	AACCTTTGACTCCR[G/A]AGATTCTTGGTGAGT
1_1038	12650777	G	A	TGAGGAAGAGCGTA[G/A]CCCTCATAAATGGGG
1_0014	12650685	C	A	CCCTTTGCAGGTTT[C/A]GTCTGCACCAAAACA
1_1492	12650785	C	G	ACAATCTACCGTTT[C/G]TGAAACGCGTTACCT
1_1092	12650786	G	A	TGATACTACTGTCAA[A/G]ATTTACAATGGGAA
1_0449	12650788	G	A	TGAACATTAAAATG[G/A]GAAACATCTTATTAT
1_0058	12650793	G	A	GGAAACTGAGGAAAA[A/G]AAGGGGTTTCTTGA
1_0421	12650794	G	A	ACAGCACGCAATAT[G/A]TTTGCACCAGCGCCT
1_0529	12650804	G	A	TCATCCTGCTGTCAA[A/G]GGCCTTCTCCCAGA
1_0482	12650805	C	G	AAGAATTTGCACTT[C/G]AAGGATATCTTCCAA
1_0905	12650809	G	A	AGATCCAAGGACAGG[A/G]GAAGTGATTACGAA
1_0232	12650812	G	A	GAGGAATCGTGGTC[G/A]TGGATCTTCCCGGAA
1_0957	12650816	G	C	TAAAACTGCAAATGT[C/G]GGAACGAAGATATG
1_0510	12650817	G	A	GAGATCTGGAAGTTA[A/G]TTGTCATTTTGAAC
1_0657	12650821	G	A	CACTGACTTGGCCA[G/A]CACGGTGTAGTCCTC
1_0773	12650823	G	A	ACTGATGGAAGGAAC[A/G]CTGAAGAGAAGGGA
1_0451	12650833	C	G	CTGCCTCTTCTGGA[C/G]GATCACTCTGTGGAG
1_0062	12650864	G	C	AAGGAGGTAGGGCTA[C/G]CCAATGGGYTTTTA
1_0437	9030018	G	A	TAGTACCCCTCTTCT[A/G]ATATCTTTTATTTG
1_0605	12650911	C	A	GGATAACCGGACCGT[A/C]CTGGACGGGACCTT

Appendix 1: SNP ID and sequences used for MABC selection. Continued

1_1130	12650915	G	A	ATGATGTTGGCTTT[G/A]TGGACGGCGGTGACT
1_0319	12650924	G	A	GGAACCTGCTCAGC[G/A]CATGTAAGTAATTCA
1_0740	12650940	G	A	ATGAAGCTGCTTCT[G/A]TGTGGCTTCCTCTGG
1_0001	9030026	G	A	TTTAGAGATCTAAGG[A/G]ATGTGGTTTTTAAT
1_0107	12650955	G	C	CCGCCACAACCCCAA[C/G]CTCTCTTTCCTTCA
1_0178	12650964	C	A	TYTGGTTGGTGCACC[A/C]GGTGGCCTAAAAGC
1_0362	12650966	G	A	TGGGGTTCGATTCGC[A/G]GTTGAACCCGAACA
1_0718	12650968	G	A	GAGAAAAAATCGTTC[A/G]TTGTAACGTTTTCG
1_0425	12650971	G	A	AGATGCAAGTCCTTC[A/G]GGAAACGCTGCCGG
1_0246	12650978	G	C	ATTGGGCTCTYCTCT[C/G]CGCTATTAGTTTTC
1_1512	12651001	A	T	GCAATGATGAGCAT[A/T]CAGAGACCATTATTC
1_0834	12651004	G	A	AGTGCCGGCAGGGT[G/A]TTGCACAACTCCGGA
1_0699	12651008	C	G	CATGCAAGATACTT[C/G]GTAAACTGATCAATT
1_0663	12651013	G	A	GGATTCTGCTTCAA[G/A]TCGCCAAAAGACGGG
1_0878	12651014	G	A	TCCATTGAACCACA[G/A]GCAAGTCGTTTCCCA
1_0911	12651029	C	A	ACGGCTGAAACTGAG[A/C]AGAGGAGGATAGTC
1_0746	12651032	C	A	ATCATTTTCCTCAT[C/A]AATGTCGTCGTCGTC
1_0442	12651034	C	G	CGATTGATCGGCAT[C/G]GACGAGATGAAGAAC
1_0146	12651037	G	A	TTGACGACGAGGTT[G/A]GTGACGGAGTAGAGG
1_0876	12651065	T	A	TAGGATATTTTGACA[A/T]GTTATGTATCCGAT
1_0945	12651066	G	A	TTTCTCCTCACAGAA[A/G]CAGAGAATGCAGCG
1_1062	12651070	C	A	TTTAGTTAACAAAGC[A/C]TTGGTTCTCATAAC
1_0604	12651075	A	T	CAACCATCTATGAA[A/T]TGCCCTTTTGATGGA
1_0977	9030009	C	A	TGTAGTGGTCAATGG[A/C]TGTGCTCACATATA
1_0323	12651082	C	A	GAAACCAACTCTTA[C/A]CAAAAGGCGCAACAA
1_0128	12651083	G	C	GACCCTTCACCTTGT[C/G]CTCAGGCTTCGCGG
1_0588	12651090	G	A	TTTCGAGACTGTGTT[A/G]ATGGTTTAATGTAT
1_1367	12651092	G	A	TCAAAGATTAAACAT[A/G]CCTCTCATGTATCA
1_1121	12651101	G	A	CTGTGGGAGCTATGG[A/G]GATTATCCTGTGGA
1_0259	12651106	G	A	CTGCTGCACCGTTT[G/A]GAGTTATCCATTGCA
1_0889	12651110	C	G	TTTCAATACTGTTT[C/G]TTGTTAGTACTATCT
1_1255	12651114	T	A	ATCGATACAGTGTTG[A/T]GGAAGTGAAGAAAG
1_0238	12651129	G	A	CATCACCGATCTTAA[A/G]GGTGGCAAAGTCGG
1_0074	9030020	C	A	CTGGACACTTATGTG[A/C]GAGGAAATCTTGTG
1_0647	12651138	G	A	GAAAGAAGCTCAGG[G/A]AACTCTGTCTTCAAT
1_1037	12651147	G	A	ACAGACGAGATCAT[G/A]CATGACGATTTATAA

Appendix 2: Full genotyping results for selecting BC_2F_1 line carrying yield, stay green, and nematodes QTL in the cross Moussa local /IT93K-503-1//Moussa local

	Nematode resistance	Yield, stay-green				
Plant	1_1170	1_0678	1_0128	1_0157	1_0992	Moussa background (%)
M503_BC2F1_54P10	AA	AA	AB	AB	AB	97
M503_BC2F1_54P9	AA	--	AB	AB	AB	95
M503_BC2F1_82P21	AA	AA	AA	AA	AA	94
M503_BC2F1_54P15	AA	AB	AB	AB	AB	92
M503_BC2F1_54P7	AA	AB	AB	AB	AA	92
M503_BC2F1_54P17	AA	AA	AA	AA	AA	92
M503_BC2F1_54P8	AA	--	AB	AB	AB	89
M503_BC2F1_54P13	AA	AA	AA	AA	AA	89
M503_BC2F1_54P14	AA	AB	AB	AB	AB	88
M503_BC1F2_77P61	AA	AA	AA	AA	AA	88
M503_BC2F1_82P20	AA	AA	AA	AA	AA	87
M503_BC2F1_54P11	AA	AB	AB	AB	AB	87
M503_BC2F1_82P18	AA	AA	AA	AA	AA	87
M503_BC2F1_82P19	AA	AB	AB	--	AB	86
M503_BC1F2_92P27	AB	AA	AA	AA	AA	86
M503_BC1F2_83P34	AB	AA	AA	AA	--	86
M503_BC1F2_83P31	AA	AA	AA	AA	AB	86
M503_BC2F1_54P12	AA	AA	AA	AA	AA	86
M503_BC1F2_83P45	AA	AA	AB	AA	AA	82
M503_BC1F2_83P49	AA	AA	AA	AA	AA	82
M503_BC1F2_83P32	AA	AA	AA	AA	--	82
M503_BC1F2_83P39	AA	AA	AB	AA	AA	81
M503_BC1F2_77P64	AA	AA	AA	AA	AA	81
M503_BC2F1_54P16	AA	AB	AB	AB	AB	81
M503_BC1F2_92P24	AB	AA	AA	AA	AA	80
M503_BC1F2_77P55	AB	AA	AA	AA	AA	80

Appendix 3: Full genotyping results for selecting BC_2F_1 line carrying yield and striga QTL in the cross Moussa local /IT97K-499-35//Moussa local

Plant	Yield			Striga	Moussa background (%)
	1_0022	1_1370	1_0567	1_0583	
M499_BC2F1_47P2	AA	AA	AA	AA	97
M499_BC2F1_47P15	AA	AA	AA	AA	95
M499_BC2F1_47P13	AA	AA	AA	AA	95
M499_BC2F1_47P6	AA	AA	AA	AA	95
M499_BC2F1_47P11	AA	AA	AA	AA	95
M499_BC2F1_47P1	AA	AA	AA	AA	94
M499_BC2F1_48P94	AA	AA	AA	AA	94
M499_BC2F1_47P14	AA	AA	AA	AA	94
M499_BC2F1_47P9	AA	AA	AA	AA	93
M499_BC2F1_4P67	AA	AA	AA	AB	93
M499_BC2F1_29P64	AA	AA	AA	AA	93
M499_BC2F1_47P3	AA	AA	AA	AA	93
M499_BC2F1_49P28	AB	AB	AB	AA	93
M499_BC2F1_48P84	AA	AA	AA	AA	93
M499_BC2F1_48P90	AB	AB	AB	AA	93
M499_BC2F1_47P12	AA	AA	AA	AA	92
M499_BC2F1_49P33	AB	AA	AB	AA	92
M499_BC2F1_47P7	AA	AA	AA	AA	92
M499_BC2F1_44P17	AB	AB	AB	AA	92
M499_BC2F1_47P16	AA	AA	AA	AA	92
M499_BC2F1_47P5	AB	AA	AA	AA	92
M499_BC2F1_47P8	AA	AA	AA	AA	92
M499_BC2F1_48P81	AA	AB	AA	AA	92
M499_BC2F1_49P32	AB	AB	AB	AA	92
M499_BC2F1_27P4	AB	AA	AB	AA	91
M499_BC2F1_44P20	AA	AA	AA	AA	91
M499_BC2F1_44P21	AB	AA	AB	AA	91
M499_BC2F1_47P4	AA	AA	AA	AA	90
M499_BC2F1_44P19	--	AB	AB	AA	90
M499_BC2F1_31P55	AA	AA	AA	AA	90
M499_BC2F1_29P61	AA	AA	AA	AA	90
M499_BC2F1_47P10	AA	AA	AA	AA	90
M499_BC2F1_38P79	AB	AA	AB	AA	89
M499_BC2F1_48P83	AA	AA	AA	AA	89

Appendix 3: Full genotyping results for selecting BC_2F_1 line carrying yield and striga QTL in the cross Moussa local /IT97K-499-35//Moussa local. Continued

M499_BC2F1_48P86	--	AA	AA	AA	89
M499_BC2F1_4P72	AA	AA	AA	AB	89
M499_BC2F1_49P31	AB	AB	AB	AB	88
M499_BC2F1_49P25	AA	AA	AA	AB	88
M499_BC2F1_27P2	AA	AA	AA	AB	88
M499_BC2F1_48P92	AA	AA	AA	AA	88
M499_BC2F1_31P48	--	AA	AA	AA	88
M499_BC2F1_48P85	AB	AB	AB	AA	88
M499_BC2F1_39P66	AA	AA	AA	AA	88
M499_BC2F1_31P44	AB	AA	AB	AA	87
M499_BC2F1_10P41	AA	AA	AA	AB	86
M499_BC2F1_27P1	AB	AA	AB	AA	86
M499_BC2F1_31P45	AA	AA	AA	AA	86
M499_BC2F1_31P49	AA	AA	AA	AB	86
M499_BC2F1_31P50	AA	AA	AA	AA	86
M499_BC2F1_39P65	AA	AA	AA	AA	86
M499_BC2F1_48P82	AB	AB	AB	AA	86
M499_BC2F1_10P40	AA	AA	AA	--	86
M499_BC2F1_48P88	AB	AB	AB	AA	86
M499_BC2F1_48P91	AB	AA	AB	AA	85
M499_BC2F1_29P58	AA	AA	AA	AB	85
M499_BC2F1_49P36	AB	AB	AB	AA	85
M499_BC2F1_49P22	AB	--	AB	AA	85
M499_BC2F1_27P3	AA	AA	AA	AB	85
M499_BC2F1_31P56	AB	AA	AB	AA	84
M499_BC2F1_48P93	AB	AB	AB	AA	84
M499_BC2F1_31P53	AA	AB	AA	AB	84
M499_BC2F1_48P87	AA	AA	AA	AA	84
M499_BC2F1_38P80	AA	AA	AA	AA	84
M499_BC2F1_44P18	AB	--	BB	AA	84
M499_BC2F1_29P59	AB	AA	AA	AB	84
M499_BC2F1_31P46	AB	AA	AB	AB	84
M499_BC2F1_10P39	AB	--	AB	AB	83
M499_BC2F1_49P26	AB	AB	AB	--	83
M499_BC2F1_49P35	AA	AA	AB	AB	83
M499_BC2F1_48P89	AA	AA	AB	AA	83
M499_BC2F1_31P47	AB	AB	AB	AA	83
M499_BC2F1_4P71	AA	AA	AA	AB	83

Appendix 3: Full genotyping results for selecting BC_2F_1 line carrying yield and striga QTL in the cross Moussa local /IT97K-499-35//Moussa local. Continued

M499_BC2F1_31P52	AA	AA	AA	AA	81
M499_BC2F1_4P69	AA	--	AA	AB	81
M499_BC1F2_27P51	AA	AA	AA	AA	81
M499_BC1F2_67P80	AA	AA	AA	AA	80
M499_BC2F1_49P27	AA	AA	AA	AB	80
M499_BC2F1_49P37	AA	AA	AA	AB	80
M499_BC2F1_49P34	AA	AA	AA	AB	80
M499_BC2F1_31P54	AA	AA	AB	AA	79
M499_BC2F1_29P57	AB	AB	AA	AB	79
M499_BC2F1_27P5	AB	AA	AB	AA	79
M499_BC1F2_67P86	AB	AB	AB	AB	79
M499_BC2F1_4P68	AA	AA	AA	AB	79
M499_BC2F1_4P70	AA	AA	AA	AB	79
M499_BC2F1_49P23	AB	AB	AB	AB	79
M499_BC2F1_49P24	AA	AB	AA	AB	79
M499_BC1F2_67P85	AA	BB	AA	AB	78
M499_BC1F2_27P50	BB	AA	BB	AA	77
M499_BC2F1_31P51	AB	AB	AB	AB	77
M499_BC2F1_20P42	AB	AB	BB	AB	77
M499_BC2F1_20P43	AB	--	AB	AB	77
M499_BC2F1_38P78	AA	AA	AA	AB	77
M499_BC2F1_66P73	AB	AA	AB	AA	77
M499_BC1F2_27P73	AA	AA	AA	AB	76
M499_BC1F2_67P87	AB	AB	BB	BB	76
M499_BC2F1_70P38	AB	AB	AB	AA	76
M499_BC1F2_67P95	AA	AA	AA	AB	75
M499_BC2F1_66P74	AB	AA	AB	AA	73
M499_BC2F1_66P77	AB	AB	AB	AA	72
M499_BC1F2_67P91	AA	BB	AA	AB	71
M499_BC2F1_27P6	AB	AA	AB	AB	71
M499_BC1F2_27P77	AA	BB	AA	BB	71
M499_BC1F2_67P94	AA	AA	AA	AB	70
M499_BC1F2_27P68	AA	AA	AA	AA	70
M499_BC2F1_49P30	AB	AB	AB	BB	70
M499_BC1F2_67P89	AA	AA	AA	BB	70
M499_BC2F1_66P75	AB	AB	AB	AA	69
M499_BC2F1_29P63	AB	AB	AB	AB	69

Appendix 4: LGC Genomics DNA extraction Protocol (Plant tissue, sbeade)

1. Samples are received in 96-well sample tube storage racks or 96-well plates. These racks / plates are centrifuged briefly to ensure that samples are at the bottom of the tubes / wells.

2. The seals are removed from the racks/plates, and a single ball bearing is added to each sample using a plate ball dispenser (Size of ball bearing used is adjusted based on sample type, standard size is 4 mm).

3. This step varies based on the type of sample being processed:

a. *Dessicated / frozen samples* - Plates are resealed using a heat sealer, and transferred to the Genogrinder.

b. *Fresh samples* – 75 µl lysis solution is added to each well. Plates are resealed using a heat sealer and transferred to the Genogrinder.

4. Samples are disrupted in the Genogrinder; the standard protocol is 1 min at 1500 rpm although this will be adjusted based on sample properties to ensure that samples are fully disrupted. Samples are then centrifuged briefly to ensure that all material is at the bottom of the tube / well.

5. The plate seal is removed, an appropriate volume of lysis solution is added to each well using a multidrop, and the plate resealed. The plate is vortexed thoroughly and centrifuged briefly.

6. Lysis plates are incubated at 65ºC for 1 hour.

7. An appropriate volume of binding solution is prepared and dispensed across the wells of a 1.2 mL process plate.

8. The lysis plates are centrifuged to ensure all liquid is at the bottom of the wells. Lysate is then transferred to the corresponding wells of the process plate containing binding solution. Plates are heat sealed, briefly vortexed to mix, and incubated at room temperature for 30 min.

9. After incubation, plates are centrifuged and placed on a magnetic rack for 1 min.

10. The plate seal is removed and supernatant removed by inverting the plate and magnetic rack.

11. An appropriate volume of wash buffer PN1 is added to each well, the plate is sealed, vortexed briefly and incubated at room temperature for 5 min.

12. After incubation, plates are centrifuged and placed on a magnetic rack for 1 mm