GENETIC ANALYSIS AND MARKER ASSISTED BREEDING FOR DROUGHT TOLERANCE AND YIELD IN CHICKPEA (*Cicer arietinum* L.)

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN PLANT BREEDING

WEST AFRICA CENTRE FOR CROP IMPROVEMENT

COLLEGE OF BASIC AND APPLIED SCIENCES

UNIVERSITY OF GHANA

LEGON

DECEMBER, 2014

DECLARATION

I hereby declare that this thesis is my original work, except for reference to work of other authors which have been duly cited, it has not been presented to any other University for any degree.

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ABSTRACT

Chickpea is an important legume crop in the arid and semi-arid lands (ASALs). It is commonly grown during the post-rainy season under receding soil moisture conditions. This exposes the crop to drought especially terminal drought which occurs towards the end of the cropping period, causing high yield losses. Developing drought tolerant and high yielding chickpea genotypes, incorporating farmer prefer traits, is an important goal for plant breeders in the ASALs for increased productivity. The objectives of this study were to a) identify production constraints and farmer preferred traits b) determine the inheritance of root traits and yield components c) introgress drought tolerance root traits through marker assisted backcrossing, and d) evaluate genotypes and identify quantitative trait loci (QTL) for yield components in chickpea. The study was conducted in Kenya during a three year period (2012 to 2014).

Participatory rural appraisal (PRA) through focus group discussions established that the major chickpea production constraints were drought, pest infestation, late maturing varieties, diseases and lack of markets. Farmers preferred chickpea that were high yielding, drought tolerant, early maturing and resistant to pests and diseases. Farmers especially in the dry highlands needed varieties that could fit in the short rainfall duration period as they planted chickpea as a relay crop. Desi (brown seeded) varieties were preferred over Kabuli (white seeded) by farmers.

Genetic analysis through generation mean analysis revealed that total root length, root length density, root dry weight, shoot dry weight and 100-seed weight were governed by additive genes. However, root dry weight was also controlled by non - additive genes; dominance, additive x additive and dominance x dominance interactions. Similarly, shoot dry weight was also governed by additive x dominance while 100-seed weight was also

controlled by dominance and additive x additive genes. Five genes controlled 100-seed weight.

Introgression of drought tolerance root traits using marker assisted backcrossing (MABC) from donor parent ICC 4958 into two varieties (*Chania Desi II* and *LDT 068*) was achieved. However, low polymorphism from both simple sequence repeats (SSR) and single nucleotide polymorphisms (SNPs) markers was detected among the parents. Two families (EUC-03-BC_2F_2-P22-1-2-1 and EUC-03- BC_2F_2-P22-1-2-3) were significantly different for seed weight per plant (g) and one family (EUC-03- BC_2F_2-P22-1-2-1) for 100-seed weight from the recurrent parent (*Chania Desi II*). The BC_2F_3 families were significantly different for root dry weight (RDW), shoot dry weight (SDW), total plant dry weight (PDW) and root to shoot dry weight ratio (R/S) for *Chania Desi II* x ICC 4958 and R/S for *LDT 068* x ICC 4958. However, 20 families had better root traits: root length density (RLD), rooting depth (RDp), RDW, and total root length (TRL), than their recurrent parents in both crosses. SDW was positively correlated with TRL, RLD and RDW which could be used as indirect selection criteria for root traits.

Evaluation of 188 $F_{3:5-6}$ genotypes from ICCV 94954 x ICCV 05107 under irrigated and rainfed conditions indicated that six lines namely: ICCX-060045-F3-P188-BP, ICCX-060045-F3-P4-BP, ICCX-060045-F3-P159-BP, ICCX-060045-F3-P76-BP, ICCX-060045-F3-P179-BP and ICCX-060045-F3-P91-BP had 38% higher yields than the better parent, ICCV 05017 (689 kg/ha), across the environments. These lines were also among the 20 best performers under rainfed conditions. Similarly under irrigated conditions, four lines had over 100% yield increment than better parent. These were: ICCX-060045-F3-P174-BP, ICCX-060045-F3-P146-BP, ICCX-060045-F3-P23-BP and ICCX-060045-F3-P62-BP. Three lines, ICCX-060045-F3-P188-BP, ICCX-060045-F3-P111-BP and ICCX-060045-F3-P4-BP had over 50% yield increase compared to the better parent, ICCV 05017, under rainfed

conditions. Positive significant correlations were obtained between yield and biomass, harvest index, seed weight and days to maturity. Quantitative trait loci (QTL) using IciMapping for yield traits generated a linkage map spanning a total length of 335.04 cM with 49 simple sequence repeat (SSR) markers and identification of eight QTL. Three QTL for above ground biomass were mapped, one on LG 3 and two on LG 4 (8.67-32.4% phenotypic variation expressed, PVE). Two QTL for yield were mapped on LGs 4 and 6 (8.24-11.08% PVE). One QTL each was mapped for 100-seed weight on LG 1 (12.19% PVE), HI on LG 8 (9.9% PVE) and days to maturity on LG 4 (13.31% PVE).

Breeding for multiple traits such as high yield, drought tolerance and pest resistance chickpea, as per farmers' preferences will enhance chickpea adoption. Given that most of these traits are controlled by many genes, selection should be done in the later stages of a breeding programme, from F_4 generations onwards, when plants are approaching homozygozity. Families developed through MABC that had higher mean root traits and yield compared to recurrent parents and lines selected across environments and those under irrigated and rainfed conditions should be extensively tested for possible release as commercial varieties. This will enhance chickpea production especially in ASALs. Further, validation of identified QTL and its application through introgression will be useful in accelerating conventional methods through marker assisted breeding.

DEDICATION

To my father Daniel K. Komen and my mother Mary Kipkosgei

To my sons James Kigen and Moses Kibet

To my sisters and brothers

ACKNOWLEDGEMENTS

I am thankful to Almighty God for his love, grace and mercy. I am grateful to the Alliance for a Green Revolution in Africa (AGRA) for provision of scholarship throughout the entire study period. Thanks to management and staff of West Africa Centre for Crop Improvement (WACCI), University of Ghana, for being prompt in dealing with administrative and financial issues of my fellowship. I also thank the Ministry of Agriculture, Livestock Development and Fisheries for granting me a study leave to undertake this programme.

I am indeed grateful to my supervisory committee at WACCI, Prof Eric Danquah, Prof. Samuel Offei and Dr. Martin Yeboah for their immense contributions to the completion of this study. I sincerely appreciate my in - country supervisors Prof. Paul Kimurto of Egerton University, Kenya and Dr. Pooran Gaur of ICRISAT, India for their consistent support and contribution during the research period in the two institutions and for provision of genotypes.

I am indebted to Generation Challenge Programme (GCP) for financial support for molecular work and six weeks of training. I also thank ICRISAT, India for training on how to do crosses in chickpea and financial support in genotyping. Special thanks to Dr. Rajeev Varshney and Dr. Pooran Gaur for facilitating. To Drs. Trusha Shah and Abhishek Rathore, for their guidance during selection of heterozygous plants and quantitative trait loci (QTL) analysis. I am also grateful to ICRISAT, Nairobi, Dr. Emmanuel Monyo and Dr. Damaris Odeny for financial support in genotyping services.

I am also grateful to Dr. Pascal Okwiri of Egerton University for his guidance during field research in Kenya and my appreciations to Prof. Stephen Mwangi of Jomo Kenyatta

University of Agriculture and Technology for his great inputs in the write up. I am also thankful to Drs. Beatrice Ifie (WACCI) and John Elelbu for their contributions.

I also extend my thanks to the staff of Genomics laboratory ICRISAT, India, Drs. Thudi Mahendar and Yakahia, Deepa, Swathi, and all those who assisted in one way or another. To Samuel Manthi, Saumu and of ICRISAT, Nairobi and Jantor of ICRAF Nairobi, thank you for sparing your time to assist.

I appreciate the assistance I received from the administration and staff of Egerton University, Prof. John Gowland Mwangi (DVC, Research), Prof. Alfred Kibor (Research extension) Prof. Joshua Ogendo (Chairman, CHS department), and Mr. Joseph Macharia (Lecturer, CHS department). I extend my thanks to Kenya Agricultural Livestock and Research Institute – Perkerra, director Mr. Timon Moi and Principal Koibatek Agricultural Training Centre, Mrs. Rachel Kipruto, for facilitating and providing field for research. Also, to the farmers of Bomet, Chepalungu and Mbeere South districts, this work would not have been achieved without your participation. My appreciation also goes to Egerton University department technicians, Mr. Caleb Otieno and Mr. Felix Kipchirchir for assisting during laboratory work. Also thanks to Mr. Nyakundi (Egerton University, CHS department), Dennis, Alice, Leah, Liz, Wesley and Philemon, for assistance in conducting crosses and management of the crops in the field.

Lastly I deeply appreciate the strong moral support from my colleagues at WACCI (Liliane, T., Ernest, B., Nasser, L., Lawan, M., Joseph B, Joseph D, Oumarou, S., and Hortense, M), friends especially Ruth Muriuki, Nancy Njoku and Mr. Charles Hamza Onyari. To my sisters and brothers thank you for your consistent prayers and support. To my parents thanks you for being there for me and for your support in prayers and above all you took care of my sons while I was in Ghana for two years. To my kids you deserve special thanks for your understanding during the entire study period, you are my inspiration.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Chickpea (*Cicer arietinum* L.) (Fabales: Fabaceae) is a self-pollinated, diploid ($2n$ = $2x$ =16) cool season grain legume. It has a genome size of 740 Mbp (Gaur *et al.*, 2011b) with an outcrossing rate of less than 1% (Singh *et al.*, 2008). Chickpea is the third most important food pulse crop after beans (*Phaseolus vulgaris* L.) and common peas (*Pisum sativum* L.), fifth among grain legumes, and 15th among grain crops of the world (Varshney *et al.*, 2011). It is cultivated in over 50 countries worldwide (Upadhyaya *et al.*, 2008) and is grown on about 12.3 million hectare (Mha) globally, with 95% cultivation in the developing countries (Kumhar *et al.*, 2013). Global chickpea annual production is 11.62 million tons (Mt) average yield production of 1.4 tons/ha (FAOSTAT, 2012; ICRISAT, 2013). Approximately 88% of the world production is from Asia, 5% in Oceania, 5.6% in Africa, 1.2% in Americas and 0.5% in Europe with India leading in production with 7.7 m tons (68%) (FAOSTAT, 2012). Also, India is the largest importer of chickpea (~1 million tons) followed by Pakistan (~ 0.1 million tons) (Mantri, 2007). Africa contributes 5.6% (0.64 m tons) of the total world production, Ethiopia leading in production with 400,000 tons (3.5%) from 231,000 ha followed by Tanzania with a production of 67,000 tons from 115,000 ha (FAOSTAT, 2012). The current chickpea area in Kenya is about 55,000 ha and production is approximately 15,000 tons to 18,500 tons (KARI, 2012).

In Kenya, chickpea is a relatively new crop that is grown by farmers in the Eastern and Rift Valley Provinces mainly in Mwea, Mbeere, Machakos, Kitui, Bomet, Nakuru, Koibatek and Timau districts. It was introduced into Kenya in the 1980s mainly in the

Eastern provinces; Machakos, Kitui and Mwea and it spread to Njoro district, in the Rift Valley province, in early 1990 (ICRISAT, 1989). However, production and land acreage of chickpea has been declining over the last 10 years, but recent efforts to introduce the crop in dry highlands as a relay crop (grown in rotation after harvesting the main crop) has shown significant increase and adoption of new varieties (Kimurto *et al.*, 2009; Kimurto *et al.*, 2013a; Kimurto *et al.*, 2013b). Several varieties have since been evaluated and released for commercial production in dry lowlands (Baringo, Mbeere, Kerio valley), medium altitude (Koibatek, Karaba, Naivasha) and dry highlands (Njoro, Bomet, Narok, Timau) (KARI, 2010; KHEPHIS, 2010). These varieties are Desi types; *Chania Desi I* (ICCV 97105), *Chania Desi II* (ICCV 92944) and *Chania Desi III* (ICCV 97126) and Kabuli types (e.g. *Saina K1* (ICCV 95423) with yield ranging between 1.0 - 3.2 tons ha^{-1} during both the short and long rainy seasons (Thagana *et al.*, 2009; Kimurto *et al.*, 2009; Onyari *et al.*, 2010). More recently, a drought and heat tolerant genotype, ICCV 92318, was reported from on-station evaluation of 123 chickpea genotypes in Kabete and Kiboko (Kaloki, 2010). This indicates that Kenya has a high potential for chickpea production and can be a leading exporter to deficit countries especially if production is enhanced in the dry highlands and in the arid regions.

Chickpea provides quality source of protein through its seeds and it is also a source of carbohydrates, minerals and vitamins (Upadhayaya *et al.*, 2008). Chickpea unlike soybean does not contain high amounts of isoflavones (USDA-ARS, 2004), but provide more beneficial carotenoids such as *β*-carotene, cryptoxanthin, lutein and zeaxanthin than genetically engineered "Golden Rice" (Abbo *et al.*, 2005; Millan *et al.*, 2006). The green leaves/twigs are also used in preparing nutritious vegetable soup. In India, it serves as a major food for the vegetarian population and is considered a healthy food in western countries (Abbo *et al.*, 2005). Chickpea fodder provides rich nutrients for feeding livestock.

In one growing season, chickpea can fix up to 140 kg N ha^{-1} (Saraf *et al.*, 1998) but the range that has been more commonly reported is between 20 to 60 kg N ha^{-} (Kumar and Abbo, 2001). It has become an important crop for the resource poor farmers because chickpea has the tendency to tolerate drought by utilizing low amounts of water to complete its life cycle. It is mostly grown as single crop or intercropped with maize (KARI, 2009), barley, linseed, mustard, pea, sweet potato, wheat, or sorghum (Ahmad *et al.*, 2010).

Abiotic stresses constrain chickpea yields worldwide. These stresses include drought, low temperature (cold) and salinity. Global chickpea production losses due to abiotic stresses have been estimated to be approximately 3.7 million metric tons, amounting to average losses of 40 – 60% (Varshney *et al.*, 2009). Among these environmental stresses, drought is the most important constraint accounting for 40 - 50% yield reduction globally (FAOSTAT, 2003; Varshney *et al.*, 2009). In addition to its direct effect on yield, it can also reduce the potential beneficial effects of improved crop management practices such as fertilizer application and intercropping (Serraj *et al.*, 2003). There are various ways of reducing the effect of drought or addressing the problem of drought stress i.e. irrigation and breeding. However, irrigation requires large capital outlay and availability of water throughout the growing season, especially at flowering and pod filling stages. This makes it less feasible especially for small scale farmers in Africa.

Developing drought tolerant varieties is a sustainable option of managing drought since there would be no additional cost to the farmer once drought tolerant seeds are available. Conventional genetic mapping approaches to traits like drought tolerance and grain yield are governed by multiple genes. These genes interact with the environment and due to large genotype x environment (G x E) interactions, they lack precision and accuracy. In chickpea research, efforts have led to discovery of root traits such as root biomass, root length density and rooting depth as some of the main drought avoidance traits contributing to

seed yield under terminal drought (end of season) environments (Kashiwagi *et al.*, 2005). Hence, indirect selection using physiological traits like high root mass, smaller leaf area, osmotic adjustments and early vigor growth and maturity, short-duration, may be easier to use (Saxena, 2003). High root mass has been of interest because of the potential for greater water absorption efficiency. This gives a plant more advantage under less soil moisture conditions.

Similarly, breeding for early phenology (flowering, podding and maturity) is important in chickpea adaptation in water-limited areas since early maturing crops escape terminal drought stresses. Such cultivars establish and mature before the end of rains and make use of residual moisture before the soil dries completely. ICRISAT has made several advances resulting in development of extra early maturing varieties (ICCV 2 and ICCV 96029) that mature in 75 - 85 days which has increased cultivation of chickpea in tropical environments (ICRISAT, 1990; Kumar and Rao, 2001). In Kenya several elite lines, both Desi such as *Chania Desi I* (ICCV 97105), *Chania Desi II* (ICCV 92944), *LTD 068* (ICCV 00108), *Chania Desi III* (ICCV 97126), ICCV 05107 and ICC 4958) and Kabuli types such as *Saina K1* (ICCV 95423), *LDT 065* (ICCV 00305), ICCV 97306, ICCV 95306 and ICCV 92311), have also been evaluated and some have been shown to have high to moderate yields, drought tolerance, early maturity and wider adaptability. Some of these lines have recently been released due to their adaptability and high yields. These lines could be used as parents in breeding efforts to accumulate good alleles for yield and drought tolerance in known local genetic backgrounds. However, further improvement of these varieties is crucial to meet specific farmers' needs based on challenges and preferred traits which is predicted to differ according to Kenya's wide and varied agro-ecological zones. Their involement in breeding progresss is crucial for adoption of developed varieities. Farmers were reported to prefer certain traits over others (Thagana *et al.*, 2009; Kaloki, 2010) and choices across localities

differed in other crops (Ojwang, 2010; Were, 2011; Kiiza *et al.*, 2012). These traits and challenges need to be identified through involvement of farmers in order to breed for lines that target specific areas to further enhance adoption.

Successful breeding programs for yield improvement in chickpea require information on: (a) nature of gene action and interactions involved in the inheritance of grain yield and its components and (b) the efficiency of such genetic patterns in the selection process (Deb and Khaleque, 2009). In any improvement program, genetic information regarding the inheritance of quantitative characters, especially the nature and magnitude of gene action governing the inheritance of the trait should be determined (Hinkossa *et al.*, 2013). Polygenically controlled traits such as yield and drought are affected by both genetic and environmental factors. These genes have small effect contributing to phenotype and cannot be easily identified (Babu *et al.*, 2004). Selecting traits controlled by many genes has not been easy. In addition, they require repeated field tests /sites to accurately characterize the effects of quantitative traits loci (QTL). Molecular technologies in which selection is based on molecular marker(s) tightly linked to the trait of interest, unlike direct selection of the trait, helps accelerate the generation of new varieties, especially for traits that are difficult to score (Bharadwaj *et al.*, 2011). Quantitative trait loci (QTL) identification or analysis is based on the principle of detecting an association between phenotype and the genotype or the marker (Collard *et al.*, 2005). Identification of QTL for important quantitative traits such as drought tolerant traits and yield is expected to effectively improve breeding of difficult characters. An important root QTL trait referred to as '*QTL – hotspot region'* that harbors yield and drought related yield has been identified (Varshney *et al.*, 2013a). These markers are used for foreground selection to track the introgressed region in recurrent parent. Application of marker assisted selection (MAS) for drought tolerance is still low with few successes reported (Oyier, 2012; Varshney *et al.*, 2013a). The application of these markers in chickpea is expected to

accelerate breeding cycle and more drought tolerant varieties developed for ASALs of Kenya leading to improved productivity.

1.2 Objectives

The objectives of this study were to:

a) identify constraints to chickpea production and farmer preferred traits,

b) determine inheritance of root traits and yield components,

c) introgress drought tolerance into adapted Kenyan chickpea varieties through marker assisted backcrossing (MABC), and

d) determine performance of genotypes for high yield and identify quantitative trait loci (QTL) associated with yield under drought stressed conditions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Chickpea types and centers of origin

There are mainly two types of cultivated chickpeas. Desi types are characterized by small seeds (100 to 200 mg), angular shape with rough surface, coloured seeds of various shades combinations of brown, yellow, green and black with a high percentage of fibre. The flowers are generally pink and the plants show various degrees of anthocyanin pigmentation, although some Desi have white flowers and no anthocyanin pigmentation on the stem. Kabuli types are large seeded (200 to 680 mg), ram-head shape, beige coloured seeds, thin seed coat, smooth seed surface with a low percentage of fibre but high sucrose, white flowers and lack of anthocyanin pigmentation on the stem. A third type, is also reported that has pea-shaped or intermediate, medium to small seed size, and cream coloured seeds (Kumar and Abbo, 2001; Upadhyaya *et al.*, 2007; Upadhyaya *et al.*, 2008; Gaur *et al.*, 2010). The Desi are primarily cultivated in South Asia, mainly the Indian sub-continent and East Africa while Kabulis are mainly cultivated in the Mediterranean region and Near East (Upadhyaya *et al.*, 2008). Vavilov (1950), cited by Kumar and Abbo, (2001) suggested two primary centers of diversity, Southwest Asia and the Mediterranean center, and designated Ethiopia as a secondary center. He observed that large-seeded varieties were cultivated in the Mediterranean basin and progressively small-seeded varieties abounded Eastward. It is believed that Kabuli chickpea was introduced into India through Kabul, Afghanistan (therefore named Kabuli) in the mid-to late 17^{th} century. Allelic variations of chickpea loci were described to be important in adaptive success from its origin e.g. genes for time to flowering (Kumar and Abbo, 2001).

2.2 Genetic diversity in chickpea

Genetic diversity is defined as the probability that two randomly chosen alleles from the population are different and that it provides an assurance to future genetic resource and insurance to unforeseen threat to agricultural production (Upadhyaya *et al.*, 2008). Greater genetic options and genetic diversity are necessary for breeding success (Krishnamurthy *et al.*, 2010) and in management and conservation of genetic resource and is particularly useful as a guide in choice of parents for generating hybrids (Talebi *et al.*, 2008). Its knowledge and management are critical for any crop improvement program (Upadhyaya *et al.*, 2008), tagging of germplasm, identification and or elimination of duplicates in accessions (Dwevedi and Lal, 2009) and establishment of core collections (Nisar *et al.*, 2007). It has been used as a powerful tool in the classification of cultivars and also to study taxonomic status (Kuruma *et al.*, 2010). When diverse lines are involved in breeding programs, recombination occurs sometimes resulting in transgressive segregants with beneficial traits that can be selected to extract high yielding lines with desirable trait combinations (Upadhyaya *et al.*, 2007).

Chickpea has a narrow genetic base (Singh *et al.*, 2008; Upadhyaya *et al.*, 2008), despite a large collection of germplasm and active genetic researches (Upadhyaya *et al.*, 2008), probably as a consequence of its monophyletic descendence from its wild progenitor *C. reticulatum* in the Fertile Crescent (Abbo *et al.*, 2003). Similarly in cowpea, low genetic variability was reported (Kuruma *et al.*, 2010). The narrow genetic variation in cultivated chickpea limits molecular markers development and identification of QTL for certain stresses (Coram *et al.*, 2007). Loss of genetic diversity has been attributed to farmers adopting new high yielding varieties and abandoning landraces and this has increased crops' vulnerability to biotic and abiotic stresses (Kuruma *et al.*, 2010). However, it has also led to increased crop yield. Thus the use of domesticated and wild relatives is useful in widening genetic

diversity. The use of powerful tools such as microsatellites (SSRs) and single nucleotide polymorphisms (SNPs) markers will help in distinguishing polymorphism. Diverse lines are necessary for success in breeding to develop varieties that contain traits that are preferred by farmers and fit in their varied agro-ecological conditions. These farmer preference and challenges in production of chickpeas need to be identified to enable breeders to develop varieties that are easily adopted by farmers.

2.3 Participatory rural appraisal (PRA)

Farmers will choose varieties that overcome their challenges and meet their needs. Engaging farmers in order to understand their needs and preferences would help in developing varieties that are adaptable to farmers' diverse conditions and hence ease their adoption. By involving farmers in breeding, scientists can develop varieties that have multiple traits. The involvement of farmers should be from beginning of a breeding programme and one way is by conducting participatory rural appraisal (PRA). Farmers are also involved in breeding through practices like participatory plant breeding (PPB) and participatory variety selection (PVS). Employing farmers in participatory plant breeding (PPB) and participatory variety selection (PVS) helps to reduce the possibility of farmers being given unacceptable varieties (Kiiza *et al.*, 2012).

PRA started in late 1980s with its methodologies adopted from agro-ecosystems analysis and anthropology in combination with participatory research and elements of practice of Rapid Rural Appraisal (Cornwall and Pratt, 2011). Since its introduction in Kenya, several PRA studies have been conducted both in livestock and crop production sectors (Bebe *et al.*, 2003; Kamau, 2006; Leley, 2007; Ojwang, 2010) aimed at involving farmers in breeding to improve the adoption of developed breeds and varieties. The aim of involving farmers in a breeding activity is to empower farmers in skills and knowledge for

utilizing genetic diversity, and on processes of maintaining and exchanging seed of preferred varieties (Witcombe *et al.*, 2006).

2.3.1 Chickpea farmer-preferred traits

Farmers in Kenya grow chickpea towards end of the rainy season (Muthisiya *et al.*, 1990) to maximize land utilization after the main crop, mainly maize, and increase yield per unit land. Thus, chickpea in Kenya is prone to drought, especially terminal drought (towards end of reproductive period). Although moisture stress in ASALs may be minimized using minimum tillage and herbicides in large scale farms (Oplinger *et al.*, 1997), herbicide application under small scale Kenyan farming systems is not feasible. Farmers will therefore choose varieties that are able to tolerate drought and get reasonable yields. In on-farm trials conducted in Naivasha and Bomet districts in Kenya, farmers participated in selecting varieties based on their preferred traits, which included disease resistance, earliness, plant vigour, taste and seed yield at flowering and harvest (Thagana *et al.*, 2009). A PRA report showed that farmers were also interested in chickpea that are bold seeded, easy to thresh, drought and heat tolerant, easy to cook and better taste (Kaloki, 2010).

Kenyan chickpea is largely marketed locally where the market demand factors determine the chickpea prices. Yield is a major concern for farmers and Kabuli (large seeded) was shown to fetch a higher price although this was dependent on production costs involved such as pest and disease management (Shiferaw *et al.*, 2007). Kabuli chickpea with a size of 6 mm was reported to sell for $ 260 while 10 mm size sold for $ 650 per ton in Ethiopia (ICRISAT, 2009). This indicates that the size of chickpea drives the price for both local and export markets. However, seeding rates for the Kabuli types were reported to be higher than the Desi types if a higher maximum population density is to be achieved (Thagana *et al.*, 2009). In the Mediterranean region, large seeds with a smooth texture and a thin seed coat are

preferred when the crop is consumed as whole grains (Cobos *et al.*, 2009). This large seeded trait of Kabuli can be utilized in improving seed size of Desi types which have small seed size, hence improving its market price. Water shortage was shown to decrease seed size and consequently yield. Despite this, small seed size under normal conditions was reported to contain enough food supply for germination and plant establishment in chickpea (Varshney, 2003). Other than seed size, other seed quality traits include uniformity, color and shape, freedom from external damage and foreign material, and ease of processing (Siddique, 1993) especially in South Asia where Desi types are split and used as dahl or flour (Turner *et al.*, 2005). Seed size and uniformity were noted as important in determining market price especially for the Kabuli type (Davies *et al.*, 1999). Under environments prone to terminal drought, a reduction in seed size is common especially those that are formed late. Seed weight and carotenoids content were reported to be negatively correlated but researchers recommended that this could be overcome by use of markers to improve both traits (Abbo *et al.*, 2005). Quality of chickpea is important in improving human nutrition, especially in most African countries where large populations live below poverty line. Therefore effective breeding requires identification of farmers' perceived constraints and their preferences for cultivars. These traits of farmers' interest are controlled by genes at different levels. Some are controlled by major gene such as days to flowering (Kumar and Abbo, 2001) and others have complex genetic basis such as drought tolerance (Turner *et al.*, 2001) and yield components. The understanding of gene action controlling these traits is important in developing successful breeding programs.

2.4 Genetics of drought tolerance in chickpea breeding

2.4.1 Drought resistance mechanisms

Drought is the major constraint which reduces the productivity of crops (Parameshwarappa and Salimath, 2007) preventing them from expressing their full genetic potential. In agriculture drought resistance refers to ability of crops' to achieve economic production with minimum loss in water-deficit environment comparative to the water-constraint free management (Mitra, 2001). A drought situation can be classified as either terminal or intermittent. Terminal drought results due to progressive decrease in available soil water resulting in severe drought stress at the later period of crop growth while intermittent droughts occurs at one or more intervals due to limited periods of inadequate rain or irrigation during the crops' growing period (Rehman, 2009). Terminal drought stress is more important as most chickpea globally is sown as post-rainy season crop under rainfed conditions (Serraj *et al.*, 2004; Gaur *et al.*, 2008).

Drought is an interaction between precipitation, evapotranspiration, irradiation, soil physical properties and nutrient availability, and biological interactions, making it complex to define a 'typical drought' (Price *et al.*, 2002). An understanding of drought resistant trait will facilitate development of drought resistant crops and efficient management practices for drought-prone areas. Plants have evolved a number of morphological, physiological, biochemical, and metabolic responses to survive against drought with a number of genes identified that are responsible for drought-induced gene expression (Gao *et al.*, 2008). The expression of drought depends on action and interaction of different morphological (earliness, reduced leaf area, leaf rolling, wax content, efficient rooting system, awn, stability in yield and reduced tillering), physiological (reduced transpiration, high water use efficiency, stomatal conductance and osmotic adjustment) and biochemical (accumulation of proline,

polyamine, trehalose, etc, increased nitrate reductase activity and increased storage of carbohydrates) characters (Mitra, 2001). Drought responses such as stomatal closure, leaf rolling, enhanced root growth and enhanced abscisic acid (ABA) production, act to minimize water deficits (Price *et al.*, 2002). The mechanisms of drought resistance have been classified as drought escape, drought avoidance and drought tolerance (Mitra, 2001; Blum, 2005; Gaur *et al.*, 2008; Rehman, 2009; Bhatnagar-Mathur *et al.*, 2010).

Drought escape was defined as plants' ability to complete its life cycle before a serious plant water deficit develops (Mitra, 2001; Rehman, 2009). This mechanism involves rapid phenological development (early flowering, early podding and early maturity) (Gaur *et al.*, 2008), developmental plasticity (variation in duration of growth period depending on the extent of water deficit) and remobilization of pre-anthesis assimilates to grain (Turner *et al.*, 2005). Selection for rapid phenological development is a common approach in breeding for drought resistance in crops. Early maturing varieties were shown to be better adapted under stress conditions compared to late maturing (Grzesiak *et al.*, 1996). However, studies confirm that there is a positive association between long duration growth and yield potential (Caliskan *et al.*, 2008). This indicates that any reduction of crop duration below the optimum would have a yield penalty, which is more problematic in environments where moisture content is unpredictable. If the environment is more predictable, crop duration can be optimized (Rehman, 2009).

Dehydration avoidance is defined as plant capacity to sustain high plant water status or cellular hydration under the effects of drought (Blum, 2005). In most cases, a plant's first response to water stress is to avoid low tissue water potential, which is achieved by increasing water uptake or limiting water loss mainly by stomatal closure (Rehman, 2009). Other mechanism for control of water loss include reduction of quantity of radiation that they intercept when suffering from drought stress either by leaf folding and paraheliotropism or by

leaf rolling (Asim and Rabiye, 2007) and reduced leaf area (Mitra, 2001). These are important traits in breeding for drought tolerance.

When water stress becomes more severe and plant tissue is not protected from dehydration avoidance, cells lose turgor pressure. Mechanisms related to dehydration tolerance are more or less related to survival mechanisms (Rehman, 2009; Bhatnagar-Mathur *et al.*, 2010). Dehydration tolerance was defined as a plant's ability to resist water-deficit with low tissue water potential (Mitra, 2001). Its mechanism involves changes in biochemical composition in order to protect macromolecules and membranes or maintenance of turgor pressure through osmotic adjustment (solute accumulation in cell) (Bhatnagar-Mathur *et al.*, 2010). Most of the dehydration tolerance traits are primarily involved with protection of cellular structure from the effect of dehydration. However, decrease in water content and turgor to some extent is important in triggering abscisic acid (ABA) accumulation which causes stomatal closure to prevent further decrease in water content (Rehman, 2009; Jain and Chattopadhyay, 2010). Chickpea being a drought tolerant crop can further be improved for the above mechanisms thus making it more adapted to drought stressed environments hence meeting farmers' needs.

2.4.1.1 Drought adaptation mechanisms in chickpea

Drought is the most common abiotic stress limiting chickpea production in different parts of the world. Chickpea frequently suffers from drought stress towards the end of the growing season in rain-fed conditions (Serraj *et al.*, 2003). It is generally grown without irrigation, planted in the post-rainy season, surviving during the growing period on progressively declining residual soil moisture (Gaur et al. 2008). Thus, chickpea grows during the time of the year when many other legumes are rarely cropped, displaying considerable drought avoidance and/or tolerance (Jayashree et al. 2005). Ninety percent of the world's chickpea is produced in areas relying upon conserved, receding soil moisture (Kumar and Abbo, 2001). Chickpea requires only 152.4 – 254 mm of rainfall and/or irrigation water during the growing season and thus is well suited to dryland or limited-irrigation production, however, its exposure to terminal drought is one of the major constraints to increasing productivity (Kanouni *et al.*, 2012). Terminal drought was reported to cause seed yield reduction between 58 - 95% in comparison to yield under irrigation (Leport *et al.*, 2006). Water stress during the reproductive period has been attributed to impairment in pollen viability and stigma functioning, reduced flowers and pods and their abortions, especially on secondary branches, with Kabuli being more sensitive than Desi (Fang *et al.*, 2010). These factors have a negative impact on seed yield under terminal drought. Earlier reports indicated that chickpea pod set was sensitive to temperatures (Turner et al., 2005). Seed size especially those formed late during the cropping season were noted to be affected by terminal drought which further decreased yield. Reports indicated that the date of sowing and tillage method affected water use (evapotranspiration), leaf area index (LAI) and dry matter production (Kibe and Onyari, 2007). Further, delayed sowing led to significant decrease in shoot biomass and number of pods and yield (Onyari *et al.*, 2010).

Root development is fundamentally involved in the response to many plant stresses, mainly drought and mineral deficiency (Price *et al.*, 2002). Further, nutrient uptake in plants under drought may have an important role in drought tolerance (Samarah *et al.*, 2004). Decreasing water availability under drought generally results in reduced total nutrient uptake and frequently causes reduced concentration of nutrients in plants (Gunes *et al.*, 2006). The most important effect of water deficit is observed on transport (uptake) of nutrients by roots and on root growth and extension (Gunes *et al.*, 2006). Further, roots have a major role in dehydration avoidance as deep and prolific root systems are able to obtain moisture from deeper layers even when the upper layer becomes dry (Serraj *et al.*, 2004, Kashiwagi *et al.*, 2005, Rehman 2009). Research in Ethiopia showed that reduced water loss from the plant and extensive extraction of soil moisture are factors involved in the adaptation of chickpeas to drought conditions (Anbessa and Bejiga, 2002). In another report, chickpea root systems responded to water deficit stress by increasing roots numbers deeper in the soil profile (Benjamin and Nielsen, 2006). Chickpea genotypes differing in root traits were reported to be consistent over contrasting environments especially in relation to soil bulk density (Ali *et al.*, 2005). Most tolerant chickpea varieties have a higher increase in root to shoot ratio than non-tolerant varieties (Labidi *et al.*, 2009). Thus the information on the genetic variability of chickpea root traits provides baseline knowledge for further progress on the selection and breeding for drought avoidance root traits in chickpea.

In addition to root trait response to drought, Anbessa and Bejiga (2002) reported that drought tolerance was found to be associated with high leaf water potential (LWP) at dawn and that the Indian cultivar 'Annigeri' showed maximum LWP with highest yield. Under water stress, LWP was lower in water stressed plants and a decrease in concentration of total chlorophylls in leaf tissues under water stress conditions (Labidi *et al.*, 2009). Water stress lowered the leaf number, but this effect was only significant in one of the varieties studied.

Early shoot growth vigor was shown to contribute to terminal drought stress (Turner *et al.*, 2001). Drought tolerance index (DTI) (calculated as differences between actual and estimated yield under stress) was shown to potentially offer a selection criteria, devoid of yield potential and phenology effects, for drought tolerance across wider agro-ecological zones (Krishnamurthy *et al.*, 2010). Drought is therefore, a complex trait and further, drought tolerance traits and related traits are all controlled by quantitative genes. Understanding the nature of these genes is necessary for developing a breeding programme.

2.5 Gene action/gene effects in chickpea drought tolerance

In order to develop an effective breeding program or strategies, an understanding of the nature of genes operating in the trait(s) of interest and its associated traits is crucial. This is more so especially under stress-prone environment where genotype x environment (G x E) plays a role in expression of a trait. Gene action is about how genes express themselves, and these are studied by use of Mendelian and biometrical genetic approaches. In any improvement program, genetic information regarding the inheritance of quantitative characters, especially the nature and magnitude of gene action governing the inheritance of the trait should be determined (Hinkossa *et al.*, 2013). Most of the traits of interest such as yield, drought and traits associated with these are usually controlled by many genes. In order to formulate an efficient breeding program for developing drought tolerant varieties, it is essential to understand the mode of inheritance, the magnitude of gene effects and their mode of action (Farshadfar *et al.*, 2008). Traits like drought and yield are affected by environmental factors. These factors are not inherited and therefore there is need to determine the genotypic factors affecting these traits.

There are three types of gene effects i.e. additive, dominance and epistatic where these parameters were represented differently by different authors (Gamble, 1961). The

dominance and epistatic constitute the non-additive part (Kearsey and Pooni, 1998). The additive gene effects reflect the degree to which progenies are likely to resemble their parents, as reflected in narrow-sense heritability (Derera, 2005). The dominance can either be ambidirectional, a situation of positive and negative dominance at different genes or unidirectional, dominance in one direction (Kearsey and Pooni, 1998). Epistasis refers to interaction of alleles at different loci. Epistatic gene action occurs when additive-dominance model cannot explain variation alone (Derera, 2005). Non-additive gene action is observed when the additive model cannot adequately explain the variation (Falconer and Mackay, 1996).

Different genetic models have been used in estimating genetic effects (Kearsey and Pooni, 2004). Majority of these models are mainly additive-dominance models or just additive without considering the epistatic or non-allelic interactions which are of frequent occurrence in controlling trait-expression of continuous variation (Farshadfar *et al.*, 2008). The non-allelic gene actions could inflate the measures of additive and dominance components (Kumhar *et al.*, 2013). Gene interaction is considered to be complementary when the dominance [d] and dominance x dominance [dd] estimates have the same signs and to be duplicating when the signs differ (Mather and Jinks, 1982). Several procedures have been used to test the deviation from additivity or apistasis. These include Wr-Vr tests of additivity, tripple test cross, computation of interaction *per se* and scaling test (Viana, 2005). Phenotypic means are consummated by additive, dominance and interaction effects (complementary, additive x additive [aa] or duplicate, additive x dominance and dominance x dominance [ad and dd]), where analysis of generation means detects presence or absence of interaction by scaling test and where present it measures appropriately (Farshadfar *et al.*, 2008). Generation means analysis provides basic information to determine the inheritance and pattern of quantitative traits in F_1 and later generations among parents of divergent nature. In addition to

determining the gene action, determining how these genes are inherited is important. Heritability represented by a symbol h^2 is a measure of the amount of genetic variation and its estimation in relation to genetic interpretation is important in determining the response to selection for the traits under observation. It also indicates genetic gain resulting from selection and both depend on repeatability in addition to method of estimation, type of cross, generation, sample size and environmental influence (Pandey and Tiwari, 1983). Heritability is determined from variance components; additive (V_A), dominance (V_D) and environmental (V_E) and there are two ways of estimating heritability namely; broad-sense and narrow-sense (Kearsey and Pooni, 1998).

Several researches have been done to demonstrate gene effects and their implication in crop improvement. In mungbean, non-additive effects and directional dominance were observed in pods per plant, seeds per pod and grain yield per plant (Ajaml *et al.*, 2007). Reports in lentils indicated that additive [a], dominance [d] and at least one of the epistatic effect (additive × additive [aa], additive × dominance [ad] and dominance × dominance [dd]) were involved in the inheritance of the studied traits (Khodambashi *et al.*, 2012). Sucrose content was reported to be controlled by additive and non-additive genes and at least five epistatic genes affected accumulation of sucrose content in cowpea seed (Tchiagam *et al.*, 2011). Researches in chickpea showed that additive gene effect were reported for pod and seed traits (length and width) while both additive and dominance gene effects were reported for pod thickness and width (Bicer and Sakar, 2010). The number of primary branches at first flower, plant height at maximum flower, plant weight just after harvest, pod weight per plant, number of pods and seeds per plant and seed weight per plant showed an additive-dominance relationship which would help in planning a successful breeding programme for development of potential lines (Deb and Khaleque, 2009). Both high heritability and genetic advance for plant height and seed yield per plant revealed that additive gene effects were important for

these traits (Ali *et al.*, 2008). Seed yield, pods per plant, seeds per plant, and stem chlorine concentration were controlled by additive effects under saline conditions (Samineni *et al.*, 2011b). Additive and additive x additive gene effects were reported to affect root biomass and root length density (RLD) where additive gene effects were shown to increase root growth (Kashiwagi *et al.*, 2008a). In two crosses of chickpea (ICC 283 × ICC 8261 and ICC 4958 × ICC 1882), the additive and additive × additive interaction effects played an important role in governing the root length density and root dry weight which have been shown to provide adaptation to drought (Kashiwagi *et al.*, 2008a). Significant genetic variation was observed amongst the RIL population, developed from a cross between a large root system (ICC 4958) and an agronomically preferred variety (Annigeri), for root length density, root dry weight and shoot dry weight at 35 days after sowing and for shoot biomass and seed yield at maturity (Serraj *et al.*, 2004). The authors reported a major putative quantitative trait locus (QTL) for RLD involving a profuse rooted variety ICC 4958 and the contrasting Annigeri. These QTL will provide a faster and easier technique to select for bigger roots than the time-consuming phenotyping of roots (Vadez *et al.*, 2008). The understanding of gene action of important traits is useful in determining a successful breeding programme.

2.6 Breeding for drought tolerance in chickpea

2.6.1 Conventional breeding

Selections in chickpea were mainly from native lines and introductions from other regions. Development of new varieties has been largely through hybridization. Plant breeding involves two main activities, i.e., pre-breeding/germplasm enhancement and cultivar development *per se* and that these are determined by factors such as breeding goals, genetics and agronomy of the crop, breeder's long term objectives, availability of

testing facilities and national cultivar registration requirements (Shimellis and Laing, 2012). According to these researchers conventional breeding involves four steps; parental selections, making crosses among selected parents, and selection from recombined parents followed by extensive field evaluations of the selected cultivars. Depending on breeders' objectives, the breeding programme can be single, three way or multiple crosses (Gaur, *et al.*, 2012). The major methods that have been used in chickpea breeding are backcrossing, pedigree and bulk. A combination of bulk and pedigree has been utilized in chickpea with early segregating generations at F_2 - F_3 where selection was suggested for simple traits such as disease and seed traits (Gaur *et al.*, 2012). Several chickpea lines have been developed using different breeding methods for important traits such as drought tolerance in an effort to improve yield.

Breeding strategies such as selection for early maturity to escape terminal drought have been exploited for many years. Development of early maturing chickpea varieties, such as Kabuli ICCV 2 and ICCV 96029, escaped terminal drought in India (ICRISAT, 1990; Kumar and Rao, 2001 and Upadhyaya et al., 2007). An extra early maturing variety was developed from a cross between ICCV 2 and JG 62 by single-seed-descent (Kumar and Van Rheenen, 2000). In Canada, a significant advance in maturity date of chickpea was achieved by incorporating early flowering, double podding and other favorable alleles into the desirable genetic backgrounds (Anbessa *et al.*, 2007). Days to flowering have been reported as an important trait for crops' adaptation and productivity under late season drought environments and high temperatures (Cho *et al.*, 2002). Days to 50% flowering and days to maturity, when plotted against seed yield under drought stress, showed a linear negative relationship (Krishnamurthy *et al.*, 2010), indicating that late flowering and maturing in chickpea result in a reduced yield. Further, according to the authors, heritability estimates for these traits (50% flowering and maturity) under stressed environments were highest

compared to irrigated conditions, which were also shown to prolong flowering and maturity period. Improved grain yields were also associated with high harvest index (HI), early flowering and maturity, where those with high HI had high ability to partition photosynthates into grain (Rehman, 2009).

Since a plant obtains its water and mineral requirements through its roots and the availability of these resources often imposes a limit to plant productivity, it is imperaive to emphasize the importance of roots to plant productivity. Several root traits have been reported to play important role(s) in plant survival under stress conditions. Root length density (RLD), expressed as root proliferation, and maximum root depth (RDp) were found to positively influence the seed yield under terminal drought environments (Gaur *et al.,* 2008, Ali *et al.,* 2005). However, differences in root proliferation were clearly observed compared to rooting depth (Ali *et al.,* 2005). Higher RLD was observed in the 0 - 30 cm soil layer, which also had higher genetic variability, indicating more branching of roots at this depth (Kashiwagi *et al.*, 2005). Under irrigated conditions, Ali *et al.,* (2005) reported that a greater root proliferation of roots was observed in surface layers (0 - 30 cm), except for Annigeri, unlike non irrigated conditions where they observed higher RLD below 30 cm at 36 days after sowing (DAS). However, this was not the case at podding stage as more roots were found at the top 15 cm and root numbers decreased between 15-30 cm followed by slight increases at 30-90 cm then a decrease (Ali *et al.*, 2005). The increased root numbers was to increase water uptake at deeper soil surface necessary for pod filling. According to Rehman (2009), water deficit affected the distribution of root weight density (RWD) and root length density (RLD) at various depths, providing increased water absorption capacity in deeper soil layer to cope with drought. Total RLD and total RWD had significant positive correlations with days to flowering and days to maturity, indicating that higher root densities might lead to continued water uptake for longer periods and delay the maturation process. Greater root

density deeper in the soil profile and larger proportion of fine roots was reported in chickpea compared to field pea or soybean, which could lead to better exploitation of water stored at lower soil depths (Benjamin and Nielsen, 2006). On the other hand, genotypes with relatively smaller root densities might get drought stress signals earlier than large rooted genotypes and thus start the maturation process earlier. Combining early maturity and drought avoidance through deep rooting and high transpiration efficiency resulted in high yield under terminal drought (Soltani *et al.*, 2000). Hence there is need to breed for early maturing varieties combined with traits such as large root systems to improve chickpea yield performance under drought.

Other important traits in breeding for drought include regulation of stomatal conductance (gs) by plants and canopy temperature. Stomata provide a means of controlling water loss from plants while allowing photosynthesis. When stomatal conductance is limited, it can result in high water-use efficiency (WUE), which is a trait that postpones stress (Bhatnagar-Mathur *et al.*, 2010). The high positive correlation of gs with grain yield and HI and the negative correlation with drought susceptibility index (DSI) in chickpea indicates the importance of considering gs for the improvement of grain yield under drought conditions (Rehman, 2009). Differences in transpiration efficiency (TE) are brought about by changes in stomatal conductance, where improvement in TE means maximization of crop production per unit of water use which is an important component of improving drought tolerance (Kashiwagi *et al.*, 2006). Thus, selection of genotypes for low stomatal conductance under drought stress conditions could help improve yields.

Using canopy temperature in plant breeding, with interest on genotypes that maintain lower canopy temperature is a potential trait useful in screening chickpea for drought tolerance. In wheatgrass it was observed that canopy temperature is one criterion for selecting plants with greater water use efficiency (WUE) (Frank *et al.*, 1997). Low canopy

temperature was indicated as a useful marker for breeding for drought tolerance in wheat and could be used as an index to evaluate physiological capacities under drought conditions (Feng *et al.*, 2009). Under stress treatments, chickpea showed significant negative correlations between canopy temperature and grain yield and harvest index (HI) and a positive correlation with drought susceptibility index (DSI) where ICCV 2 had lower canopy temperature than air temperature compared to ILC 3182, CDC Chico, Amit and ILC 588 (Rehman, 2009). This indicated that cooler canopies were associated with higher grain yield, HI and lower DSI. In other chickpea accessions, the canopy temperature of those tolerant to drought were found to maintain cooler temperatures (Kashiwagi *et al.*, 2008b) while highly sensitive accessions maintained warmer temperatures. This was believed to result from differences in extraction of water (Krishnamurthy *et al.*, 2010). Therefore, modifications to the root system, controlling stomata, leaf area and canopy temperature and matching of the crops' phenology to environment will improve production under drought conditions. However, most of the traits involved in drought tolerance are controlled by many genes or recessive genes that are difficult to detect and/or select, especially phenotypically and this has led to use of DNA markers to enhance breeding.

2.6.2 Molecular breeding in chickpea

Important agronomic traits such as yield and drought tolerance are complex and often highly regulated by many genes. The difficulty in manipulating such traits is related to their genetic complexity in terms of the number of genes involved, interactions among genes (epistasis) and environmental influence. These genes, in general, have smaller individual effects on the phenotype, and individual gene effects are not easily identifiable (Babu *et al.*, 2004). This requires repetitions of field evaluations to accurately determine the effects of quantitative traits loci (QTL) and to evaluate their stability across these environments.

Molecular technologies help in improving the efficiency of breeding several folds since selection is based on molecular marker(s) tightly linked to the trait of interest, unlike direct selection of the trait, accelerating generation of new varieties, especially for traits that are difficult to score (Bharadwaj *et al.*, 2011). Molecular markers associated with genes conditioning desirable traits have been used for efficient pyramiding of these traits (Coram *et al.*, 2007). However, use of molecular markers will depend on ease, cost and stage of traditional selection for that character as compared to the cost, time saving and enhanced precision of indirect selection based on molecular markers (Crouch and Ortiz, 2004). Molecular marker systems allow high-density DNA marker maps to be constructed that are useful in detecting putative genes affecting traits of interest. Genetic studies and molecular breeding approaches require basic genomic resources, such as molecular markers, genetic maps and sequence information (Varshney *et al.*, 2010).

Molecular breeding (genomics-assisted breeding) uses several modern breeding strategies such as marker assisted selection (MAS) and genomics selection (GS) (Varshney *et al.*, 2005), Marker assisted selection includes marker assisted backcrossing (MABC), marker assisted recurrent selection (MARS) and most currently genome-wide selection (GWS) (Ribaut *et al.*, 2010). GS uses all available marker data for a population as predictors of breeding value i.e integrating marker data from a training population with phenotypic and, when available, pedigree data collected on the same population to generate a prediction model (Varshney *et al.*, 2014a). MAS involves selection of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers. With the development and availability of an array of molecular markers and dense molecular genetic maps in crop plants, it has become possible for traits both governed by major genes as well as QTL to be selected and used in breeding (Babu *et al.*, 2004) and used in breeding. Molecular markers serve as efficient and powerful tool for MAS of agronomically important traits

(Bharadwaj *et al.*, 2011) and also in biotic and abiotic tolerance traits. Several successes in of MAS have been reported. In Soyabean, QTL for resistance to soyabean cyst nematode (SCN), rgh1 and Rhg4 have been introduced into several soyabean lines (Cahill and Schmidt, 2004). Four QTL for resistance to *Phytophtora capsici* were transferred through MABC from perennial pepper into yellow wonder pepper double haploids using markers (Thabius *et al.*, 2004). The application of RAPD markers were successfully used in selection for resistance to common bean blight in common beans, and this selection was also economical compared to conventional greenhouse screening (Yu *et al.*, 2000).

Marker assisted backcrossing was also indicated as the most acceptable and efficient method of gene pyramiding. It involves transfer of a target allele from a donor variety to a popular cultivar by a repetitive process called backcrossing with the help of markers (Nayak *et al.*, 2010). The ultimate goal is to have the highest possible percentage of the recurrent parent genome with the trait of interest present. The gene of interest from the donor is transferred into the background of a preferred variety. The result is a line containing only the major gene from the donor parent, with the recurrent parent genotype present everywhere else in the genome. According to Nayak *et al.* (2010), the use of MABC is effective in selection in that i) it is possible to select on target allele whose effects are difficult to select phenotypically, ii) rare progeny can be selected in which recombination near the target gene have produced chromosomes that contain the target allele and as little as possible surrounding DNA from the donor parent and iii) rare progeny that are the result of recombination near the target gene, hence minimizing the effects of linkage drag is achievable (Nayak *et al.*, 2010). Drought tolerance QTL introgression lines developed through MABC were significant for yield under terminal stress in pearl millet (Serraj *et al.*, 2005). Three stay green QTL for drought tolerance in sorghum were transferred into a Kenyan famer-preferred variety by MABC (Ngugi *et al.*, 2010). In chickpea, molecular

markers associated with QTL for resistance to biotic and abiotic stresses and some morphological traits have been located on chickpea linkage maps and the genotypes have been identified (Flandez-Galvez *et al.*, 2003; Lichtenzveig *et al.*, 2006; Millan *et al.*, 2006; Kottapalli *et al.*, 2009; Rehman, 2009). However, despite the several candidates QTL identified, few of these have been utilized for marker assisted selection. This has been attributed to (a) QTL for drought tolerance explaining only a small proportion of the phenotypic variation, (b) QTL identified for drought tolerance themselves explaining only a portion of the yield variation (Ravi *et al.*, 2012). Reported examples include: introgression of genomic regions flanked by SSR markers TAA 170 and ICCM 0249 into three chickpea cultivars (JG 11, KAK 2 and Chefe) using MABC (Gaur *et al.*, 2011a), introgression of root QTL named *QTL- hotspot* into adapted chickpea variety, ICCV 97105 released as *Chania Desi I* through MABC (Oyier, 2012) and JG 11 (Varshney *et al.*, 2014b) from the same donor parent ICC 4958. More adapted varieties to Kenya need to be improved for drought tolerance given the current effect of climate change and increased population.

2.7 Chickpea genome mapping

2.7.1 DNA marker systems for chickpea genetic enhancement

New biotechnology techniques have increased interest in drought tolerance breeding using new genomic tools in an effort to enhance water use in crop production. DNA markers have various applications ranging from improved access and germplasm resource utilization, selection of parents and progeny prediction performance, fingerprinting, marker assisted selection, enhanced marker assisted backcrossing, gene pyramiding, gene isolation, function and manipulation. Molecular marker technology has made it possible to generate genetic maps of chickpea (Winter *et al.*, 2000; Cobos *et al.*, 2005; Radhika *et al.*, 2007; Tar'an *et al.*, 2007; Nayak *et al.*, 2010; Hiremath *et al.*, 2012) that holds promise for use in marker-assisted

selection and positional cloning of agronomically important genes. However, chickpea genome is considered homogeneous based on the minimal polymorphism for molecular markers prior to the use of simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) markers (Muehlbauer and Rajesh, 2008). Using different markers, identification and detection of several quantitative trait loci controlling useful traits in chickpea have been reported (Radhika *et al.*, 2007; Tar'an *et al.*, 2007; Kottapalli *et al.*, 2009; Hossain *et al.*, 2010; Imtiaz, 2010; Gaur *et al.*, 2011a; Gowda *et al.*, 2011). Characterization of chickpea genotypes for resistance to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* was done using RAPD markers (Soregaon and Ravikumar, 2010). SSRs have been shown to detect very high levels of polymorphism among chickpea (Ahmad *et al.*, 2010). Inter simple sequence repeat (ISSR) markers were used in genetic analysis of chickpea germplasm (Bhagyawant and Srivastava, 2008). These genetic studies are useful in germplasm bank management, conservation programs, and breeding purposes (Ahmad *et al.*, 2010). The ISSR markers linked to the traits of agronomic importance have been sequenced and used as sequence-tagged site (STS) markers in marker aided selection (Reddy *et al.*, 2002).

Microsatellites or simple sequence repeat (SSR) markers are DNA-based molecular markers for indirect selection due to their robust, repeatable, co-dominant and high polymorphic nature. The variability of microsatellites is exploited by a polymerase chain reaction (PCR) - based technique that uses microsatellite flanking sequences as primers to amplify the microsatellite (Rehman, 2009). SSR markers in chickpea were identified as being highly polymorphic compared to other types of markers (Muehlbauer and Rajesh, 2008). Detection and identification of single nucleotide polymorphism (SNP) markers will enhance polymorphism in various chickpea accessions and improve map saturation, which

are useful in detecting QTL controlling useful traits in drought and yield (Muehlbauer and Rajesh, 2008; Nayak *et al.*, 2010). However, progress in genomic research in chickpea, including bacterial artificial chromosome (BAC) end sequencing and expressed sequence tags (EST) will provide additional opportunities for developing sequence based markers that target specific genomes (Jayashree *et al.*, 2005; Gao *et al.*, 2008; Muehlbauer and Rajesh, 2008). Drought and salinity responsive genes have been identified with 10,996 high quality drought-responsive expressed sequence tags (ESTs) (Varshney *et al.*, 2009). Drought responsive sequences were identified with varying degrees of responses including response to abscisic acid (ABA) using differential display reverse transcription – PCR (DDRT-PCR) (Medini *et al.*, 2009). The completion of genome sequence of chickpea highlighting position of candidate genes for disease resistance and agronomic traits will allow the tracking of these genes controlling traits of interest rather than using markers linked to a QTL (Varshney *et al.*, 2013c). The use of markers, for example, has helped in identifying reliable QTL for root traits and osmotic adjustment (Courtois *et al.*, 2003)**.** This will enhance faster, efficient and effective methods of breeding chickpea for drought tolerance and improved chickpea yields in addition to genome diversity and domestication information.

2.7.2 Quantitative trait loci (QTL) mapping in chickpea

The process of constructing linkage maps and conducting QTL analysis to identify genomic regions associated with traits is known as QTL mapping, also referred as ‘genetic,’ ‘gene’ or ‘genome’ mapping (Mohan *et al.*, 1997). By producing genetic mapping populations based on crosses of parental varieties contrasting for the trait of interest, it is possible to identify which parts of the genome improve the trait (Price *et al.*, 2002). It is also possible to identify genomic regions that influence the component trait that is linked to the

main trait and approximately quantify the contribution of these component traits (Price *et al.*, 2002). With the use of molecular markers, chickpea genetic maps have been developed. This has been useful in identifying QTL linked to important traits. Three steps are needed for construction of a linkage map: development of mapping population, identification of polymorphism and linkage analysis (Collard and Mackill, 2008). Several methods/software have been used in construction of linkage maps and QTL detection. Commonly used ones are mapmaker (Lincoln *et al.*, 1993), MapManager QTX (Manly *et al.*, 2001) and JoinMap (Stam, 1993).

Based on these steps and methods, several chickpea maps have been generated. A chickpea linkage map was established that comprised of nine linkage groups containing 116 markers covering a map distance of 981.6 centiMorgans (cM) with an average distance of 8.4 cM between markers (Santra *et al.*, 2000). The genetic map was generated from 53 Sequence Tagged Microsetallite Markers (STMS) primer pairs based on the population of Recombinant Inbred Lines (RILs) from an intraspecific cross between PI359075(1) and FLIP84-92C(2) (Cho *et al.*, 2004). Flandez-Galvez, *et al.*, (2003) reported 51 chickpea-STMS markers (94.4%), three ISSR markers (100%) and 12 resistant gene analog (RGA) markers (57.1%) mapped into eight linkage groups. They further reported that chickpea-derived STMS markers were distributed throughout the genome, while the RGA markers clustered with the ISSR markers on linkage groups I, II and III. The intraspecific linkage map spanned 534.5 cM with an average interval of 8.1 cM between markers. A total of 13 sequence-tagged microsatellite markers (STMS) were developed using two different approaches: (i) amplification using degenerate primers and (ii) cloning of inter-simple sequence repeat (ISSR)-amplified fragments (Choudhary *et al.*, 2006). STMS markers from chickpea generated a total of 92 new microsatellites of which 74 functional STMS primer pairs were developed (Sethy *et al.*, 2006). Two hundred and fifty STMS markers revealed eight linkage

groups covering a distance of 471.1 cM with an average marker density of 14.2 cM (Bharadwaj *et al.*, 2011). In an earlier report, a genetic linkage map was produced using 52 SSR primers resulting in eight linkage groups, where almost the whole of LG1, between markers H5A08 and TA8 (2.5 cM), was associated with various drought related traits (Rehman, 2009). Using another set of SSR markers developed, ICCM (ICRISAT chickpea microsatellite), a genetic map of 521 marker loci, spanning 2,602 cM with an average intermarker distance of 4.99 cM was developed (Nayak *et al.*, 2010). With the use of ISSR markers, a linkage map was generated that defined positions of 138 markers which spanned 630.9 cM with an average marker density of 4.57 cM (Gaur *et al.*, 2011b). Common markers in these and future maps with SSR primer pairs could lead to development of a high density genetic map of chickpea that could be used to identify tightly linked flanking marker genes of interest, which ultimately will be helpful in marker-assisted selection and positional cloning of agronomically important genes (Rehman, 2009). To enhance the density of genetic maps in chickpea, the use of a combination of several markers and other markers such as single nucleotide polymorphism (SNP) have been identified (Varshney *et al.*, 2007b; Rajesh and Muehlbauer, 2008; Nayak *et al.*, 2010). This will further enhance identification of markers tightly linked to genes controlling traits of interest and QTL detection and their application in MAS.

2.7.3 Identification of QTL related to chickpea yield and drought tolerance

Conventional breeding is time consuming and very dependent on environmental conditions. Marker-assisted breeding reduces the effect of environmental variation, which is a major hindrance in conventional breeding especially under drought conditions, during the selection process. Drought is controlled by multiple genes, hence, is a quantitative trait. Regions within genomes that contain genes associated with a particular quantitative trait are

known as quantitative trait loci (QTL) (Collard *et al.*, 2005). New molecular systems such as RAPD, AFLPs, SSR, diversity arrays technology markers (DArT) and SNP and statistical methods for detecting QTL and computer software for implementing the procedures have allowed the aggressive use of molecular markers for studying quantitative traits (Bernardo, 2008). Several molecular markers have been used to identify quantitative traits in chickpea (Winter *et al.*, 2000; Rehman, 2009; Hossain *et al.*, 2010; Imtiaz, 2010; Gaur *et al.*, 2011b; Gowda *et al.*, 2011). Knowledge of approximate location of a locus has been used as a starting point for fine mapping by non-QTL mapping approaches or studying candidate genes close to identified QTL which may be actual genes affecting a quantitative trait (Bernardo, 2008). The identified QTL in chickpea involve those associated with agronomic traits and yield and drought tolerance. The aim of a plant breeder is to identify QTL that increase yield under drought or at least increase yield stability under drought (Price *et al.*, 2002). Some of the important QTL detected included five QTL for harvest index on linkage groups (LGs) 1, 3, 4 and 8 explaining 84% of the total phenotypic variability, four QTL for flowering on LGs 1, 3, 4 and 6, four for maturity on LGs 1, 3 and 7 (Rehman, 2009). A QTL was mapped on linkage group (LG) 3A for both *Hg*/*hg* (growth habit) and flowering time (Cobos *et al.*, 2009) while two QTL for time to flowering were mapped on LG 1 and 2 (Lichtenzveig *et al.*, 2006). A marker TA-42 on LG 6 was associated with yields per plant and the same marker was associated with days to flowering on LG 4 under moisture stress during the late sowing season (Imtiaz, 2010). A marker TA47 was associated with QTL for four traits: plant spread (cm), number of branches/plant, number of pods/plant and yield/plant (g), while STMS13 was associated with QTL for plant height (cm), number of branches and days to maturity (Gowda *et al.*, 2011). One QTL for stomatal conductance was identified on LG 7 that explained 9% of total variability and three for canopy temperature differential on LGs 1, 3 and 6 which explained 39% of total phenotypic variability (Rehman, 2009). The SSR marker

(TAA 170) was identified for major QTL that accounted for 33% of the variation for root weight and root length (Chandra *et al.*, 2004), while flanking markers TAA 170 and ICCM 0249 were reported to contain QTL for several drought tolerance root traits contributing up to 36% phenotypic variation (Varshney, 2010). Recently nine markers were found in the *QTL hotspot* region that is responsible for drought tolerance root traits with 58.20% phenotypic variation (Varshney *et al.*, 2014b). Two putative QTL for drought tolerance score (DTS) were detected that explained 16% and 26% variation under rain-fed and stressed conditions, respectively(Imtiaz, 2010) Upon finding a QTL, there is need to introduce or pyramid these QTL through standard breeding procedures, into elite germplasm to develop improved cultivars (Bernardo, 2008). Applications of markers in breeding are expected to aid conventional methods and reduce the time taken to release developed lines.

CHAPTER THREE

3.0 Participatory rural appraisal (PRA) in chickpea growing areas in Bomet and Embu Counties in Kenya

3.1 Introduction

Chickpea is a relatively new crop in Kenya. The crop is grown on an estimated area of about 55,000 ha and production is approximately 15,000 tons to 18,500 tons (KARI, 2012). Recent efforts to introduce the crop in dry highlands as a relay crop has shown significant increase and adoption of new varieties with high yields of up to 1.8 tons/ha in arid to semi arid lands (ASALs) (Onyari *et al.*, 2010) and 3.2 tons/ha in dry highlands (Kimurto *et al.*, 2009). Chickpea is indeed a bonus crop in Kenya since after harvesting maize/wheat the land is normally left fallow awaiting the next cropping season (rainy season). Planting chickpea could give farmers a second crop (where only one crop would traditionally be grown) hence additional income, and nutrition (Muthisiya *et al.,* 1990). Engaging farmers in knowing production constraints during such cropping periods and understanding their varietal preferences would help in developing varieties that are adaptable to those farmers' conditions. This would also enhance chickpea adoption.

Involving farmers in breeding can be achieved in various ways namely: participatory plant breeding (PPB), farmer participatory varietal selection (FPVS) and participatory rural appraisal (PRA) among others. Involving farmers throught PPB and PVS helps to reduce the possibility of farmers being given unacceptable varieties (Kiiza *et al.*, 2012). Chickpea farmers in Naivasha and Bomet districts in Kenya, participated in selecting varieties based on their preferred traits, which included disease resistance, earliness, plant vigour, taste and high seed yield at harvest (Thagana *et al.*, 2009). PRA was also used in India and Zimbabwe to

identify reasons for crop establishment in maize, chickpea and upland rice (Harrisa *et al.*, 2001).

The PRA on the other hand engages participants in producing their own data, analyses and solutions (Cornwall and Pratt, 2011). PRA techniques have been applied in several crop fields to understand farmers' constraints and trait preferences. A PRA conducted in Zimbabwe on maize adaptation to drought, farmers had different views on the ranking of traits they consider of importance when selecting varieties for stress prone environments (Mhike *et al.*, 2012). In Kenya, a PRA conducted showed that the major constraints were drought and pest infestation and that varieties with multiple traits were preferred in common beans (Ojwang, 2010). In another PRA, farmers indicated that drought and lack of knowhow were the major concerns in maize production and farmers preferred drought tolerant lines in addition to high yielding, recovery after a dry spell and stay green traits (Leley, 2007). Other PRA studies conducted were on cassava production systems, contraints and farmer preference in Western Kenya (Were, 2011) and on finger millet contraints, variety diversity and preferences (Oduori, 2009). Surveys have also been used with similar objectives. In a survey conducted in Mbeere district to evaluate chickpea as an adaptation to agriculture system in Kenya, farmers indicated that they were planting chickpea as it was able to withstand dry and hot seasons (Kaloki, 2010). In another research, a survey was used to determine whether winter-sown chickpea technology disseminated in Syria had any impact on the livelihoods of small-scale farmers (Mazid *et al.*, 2013). According to these authors, understanding the criteria that farmers use to evaluate new crop varieties allows breeders to effectively set priorities and target different breeding strategies to different communities in the dry areas. It is for this reason that this study was conducted in chickpea growing areas in Kenya with the following objectives:

a) identify the major constraints in chickpea production

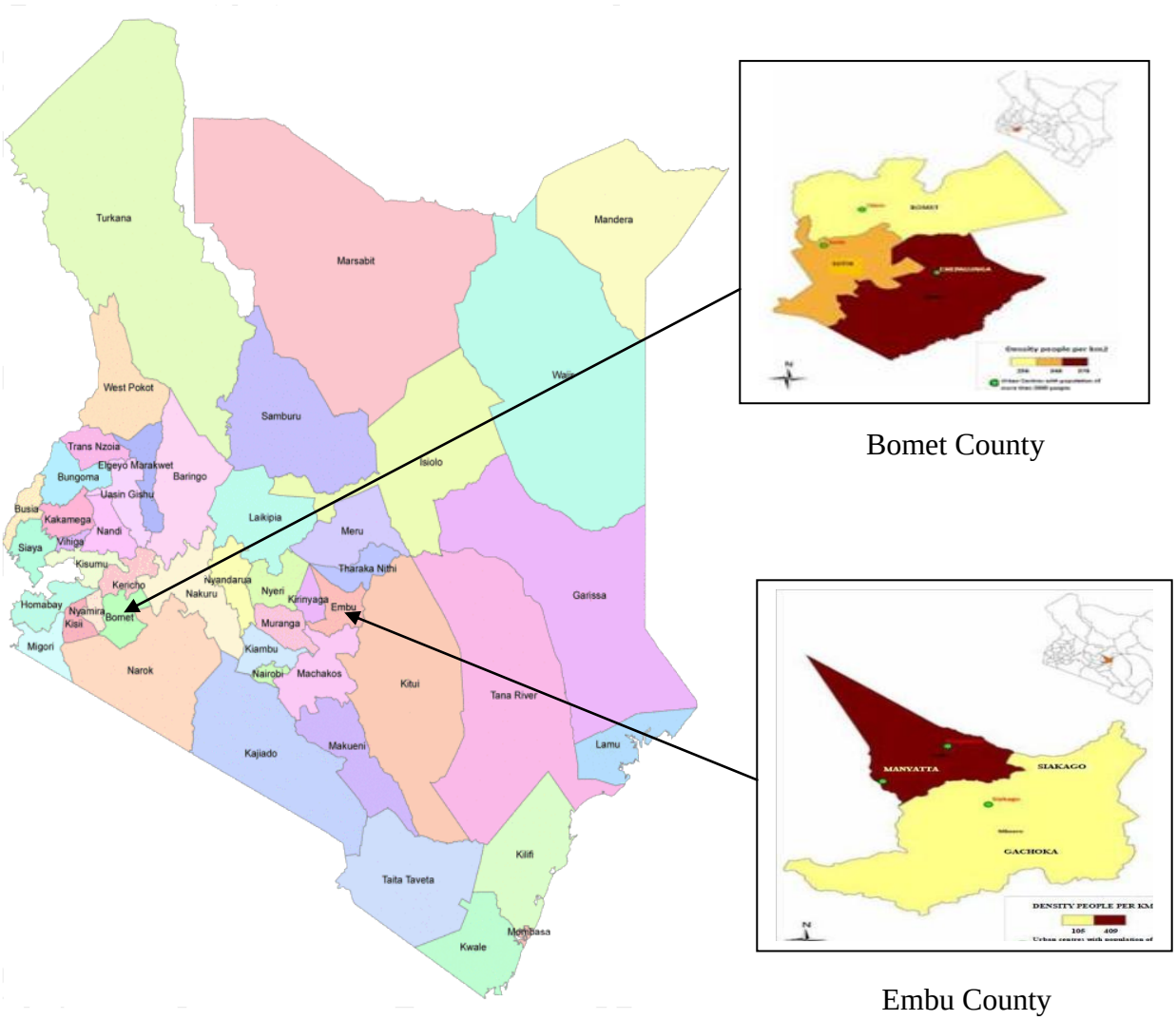
b) determine factors influencing varietal selection in chickpea

c) identify sources of seed and ways farmers access agricultural information

3.2 Materials and methods

3.2.1 Study area

The study area covered three chickpea growing areas namely: Bomet, Chepalungu and Mbeere South district (Figure 3.1). Bomet and Chepalungu districts are located in Bomet County. Bomet district is located at latitude 0° 47' 24" S and longitude 35° 21' 0" E while Chepalungu district lies in latitude 0° 55' 59" S and longitude 35° 12' 0" E. The altitude in the county ranges from 1,689 m to 2,328 m above sea level (asl) and represents dry highlands, while rainfall ranges between 1,000 mm to 1,400 mm per annum. The county receives bimodal rainfall with the long rains occurring from March to May and the short rains from August to October. Temperatures are in the range of 10 ºC to 27 ºC, with a mean monthly temperature of 18^{o}C (NEMA, 2009a).



Bomet County

Embu County

Figure 3.1: A map of Kenya showing locations of Bomet and Embu counties.

Source:http://commons.wikimedia.org/wiki/File:Map-showing-Counties-under the-new-kenyan-constitution..gif

Mbeere South district is located in Embu County. The district is located in latitudes 0° 20' 50" S and longitudes 37° 16' 56" E. The altitude ranges from 500 m - 1200 m asl. The extensive altitude range of the district influences the temperature, which ranges from 20 ºC to 32 ºC. August is usually the coldest month with an average monthly minimum temperature of 15 ºC, while March is the warmest month with an average monthly maximum temperature rising to 30 ºC. The district has two rainy seasons, the long rains falling between March and June, while the short rains are experienced from October to December. The rainfall, however, is not very reliable and ranges between (640 – 1100 mm) per year. Despite this, most parts of the district receive less than 500 mm of rainfall per year, giving the area a marginal status (NEMA, 2009b).

3.2.2 Sampling procedure

Three villages in Bomet district; Kiplabotwa, Cheboror and Olbobo and two villages in Chepalungu district (Bing'wa and Chemeng'wa) were sampled from Bomet County while in Mbeere South district, four villages (Ndia-Ndasa, Gategi, Maviani-Wovosyo and Maviani-Rurii) were sampled for the study. The choice of villages in Bomet County was randomly sampled while in Mbeere South district, it was based on presence of black cotton soils which could support chickpea growth for longer period of time. The identification of these villages was guided by agricultural extension officers. The total numbers of farmers involved in the study were 235 comprising of 103 male and 132 female (Table 3.1).

Table 3.1: Sites and number of farmers involved in the PRA study conducted in Bomet and Embu Counties in the year 2012

District	**Division**	**Sub location**	**Village**	**Number of farmers**
Bomet	Longisa	Kiplabotwa	Kiplabotwa	15
Chepalungu	Siongiroi	Bingwa	Bingwa	17
Bomet	Longisa	Kipreres	Cheboror	15
Chepalungu	Sigor	Kapsabul	Chemengwa	31
Bomet	Longisa	Olokyin	Olbobo	19
Mbeere South	Mwea	Karaba	NdiaNdasa	12
Mbeere South	Mwea	Gategi	Gategi	31
Mbeere South	Mwea	Karaba	Maviani -Wovosyo	75
Mbeere South	Mwea	Karaba	Maviani - Rurii	20
Total				**235**

3.2.3 Data collected

Data was collected using focus group discussions (FGD) comprising 235 farmers (103 males and 132 females) and from chickpea informants (those who were growing the crop at the time of study or those who used to grow it but stopped for one reason or another). Farmers were interviewed with the help of loosely structured questionnaire/checklist. These were meant to guide the discussions and provided the group sufficient opportunity to bring up their own issues and ideas. Male and female farmers were interviewed separately where possible, to allow the women to share freely, in small groups of 5 - 15 farmers. Secondary data were also obtained from the Ministry of Agriculture to support data from the field. The discussions covered various aspects of agriculture and farming under drought stress. Specifically, data were collected on farming systems and cropping calendar, chickpea production constraints, preferred chickpea traits and varieties, sources of chickpea seed and means of agricultural information on chickpea production among others. The study was organized with the help of village elders, local administration officers, extension officers from the Ministry of Agriculture and farmer groups including individual farmers. During all the discussions, the extension staff facilitated the process while enumerators took notes. The discussions were in the local languages.

3.2.4 Data analysis

The responses from farmers in all the study areas were compiled in Microsoft excel worksheets. The different criteria and rankings were combined into derived scores which represented the number of times a criterion was ranked according to De Groote *et al.* (2002) but with modifications. The criterion/rank received a value that was inversely proportional to the rank i.e. a rank of 1 received a score of 5 and a rank of 5 received a score of 1. These Mean Derived Scores (MDS) indicated the overall importance of the derived scores, ranging

from 0 (criterion not ranked) to 5 (criterion ranked first) (De Groote *et al.*, 2002) However, since responses differed from farmers in different locations, when a response was not mentioned in one location, a dash (-) was used to represent it. Also for responses that exceeded five, a score of one was used. The results were summarized in tables.

3.3 Results

3.3.1. Major crops grown by farmers and utilization of chickpea

Farmers in Bomet and Chepalungu districts planted maize and beans as the major crops. These were planted during the long rainfall season. Overall, chickpea was ranked 4th and 5th in Bomet and Chepalungu districts respectively and 2nd among the grain legumes (Table 3.2). The crop was used mainly as food in various ways such as boiling with maize and used as famous traditional food *'githeri'*. It was also made into flour and mixed with maize flour or wheat flour, from which they made *ugali* and *chapati*/cakes, respectively. Other uses included feed for livestock and poultry. In Mbeere South district the major crop was also maize followed by green grams. Chickpea was ranked 7th overall among the major crops grown and 5th among the legumes. The uses of chickpea in Mbeere South were similar to what was indicated by farmers in Bomet and Chepalungu districts. However, the farmers indicated that the crop was mainly used as commercial crop (Table 3.3).

Table 3.2: Ranking of crops grown by farmers in Bomet and Chepalungu Districts in the year 2012

	Bomet District					Chepalungu district			
Crops	**Kiplabotwa**	**Olbobo**	**Cheboror**	**MDS**	**Rank**	**Chepmeg'wa**	**Bing'wa**	**MDS**	**Rank**
Maize	1	1	1	5.0	1	1	1	5.0	1
Beans	3	2	2	3.7	2	2	2	4.0	2
Sweet potatoes	2	3	4	3.0	3	3	3	3.0	3
Sorghum	4	5	7	1.3	5	4	4	2.0	4
Pumkins	5	-	3	1.3	5	7	5	1.0	5
Finger millet	4	8	6	1.3	5	5	5	1.0	5
Chickpea	3	6	8	1.7	4	8	7	1.0	5
Fruit trees (assorted)	6	7	5	1.0	6	6	8	1.0	5
Vegetables	7	4	5	1.3	5	10	6	1.0	5
Tomatoes	3	7	9	1.7	4	6	9	1.0	5
Potatoes	4	9	5	1.3	5	9	9	1.0	5
Peas	8	-	10	0.7	7	-	10	0.5	6

Key: 1= High rank, 10= Low rank, - = No response, MDS - Mean Derived Score, A rank of 1 received a score of 5, 2 received 4, 3 received 3, 4 received 2 and 5 and above received a score of 1.

Table 3.3: Ranking of crops grown by farmers in Mbeere South District in the year 2012

	Ndia-Ndasa	Gategi	Maviani - Ririi	Maviani - Wavosyo	MDS	Rank
Maize	1	1	1	1	5.0	1
Green Grams	2	2	2	3	3.8	2
Beans	3	4	6	5	1.8	5
Cowpea	3	3	3	2	3.3	3
Pigeon Pea	3	5	4	4	2.0	4
Black Grams	4	7	5	3	1.8	5
Cassava	8	8	7	7	1.0	7
Sorghum	6	6	4	9	1.3	6
Chickpea 'saina'	5	6	7	6	1.0	7
Fruits trees (assorted)	9	9	8	6	1.0	7
Vegetables	9	9	9	7	1.0	7

1= Highest rank, 9= Lowest rank, MDS – Mean Derived Score; A rank of 1 received a score of 5, 2 received 4, 3 received 3, 4 received 2 and 5 and above received a score of 1.

3.3.2 Cropping calendar and farming systems

Farmers in the three districts presented the cropping calendar. Bi-modal rainfall was experienced in all the districts. In Bomet district the long rains occur from March to May. The short rains occur in the months of August to October (MOA, 2009). Mbeere South also receives a bi-modal rainfall with major season occurring in the months of March to June and minor season in October to December. The main chickpea planting season in Bomet and Chepalungu was in the month of July (ending), and harvested in October/November. In Mbeere South the planting was done twice a year. The main season was in May/June to coincide with late July cool season and harvested in August/September. The short rain planting season was in November/December to coincide with the January/February dry season.

The farming system commonly practiced by farmers in Bomet and Chepalungu districts was sole (mono) cropping followed by relay cropping. Sole cropping was practised when chickpea was planted as a main crop. Under relay cropping, chickpea was planted after harvesting the main crop, mainly maize. In Mbeere South district, intercropping was the most commonly practiced type of farming system. The crops intercropped with chickpea included maize, pigeon pea and sorghum. Relay cropping was not practiced in Mbeere South district (Table 3.4).

Table 3.4: Types of farming system as ranked by farmers in Bomet, Chepalungu and Mbeere South districts in the year 2012

	Bomet district		Chepalungu district		Mbeere South district	
Farming system	**MDS**	**Rank**	**MDS**	**RANK**	**MDS**	**RANK**
Sole (mono)	3.0	1	2.8	1	2.1	2
Intercropping	1.0	3	1.4	3	2.9	1
Relay cropping	2.0	2	2.2	2	-	-

Key: MDS-Mean Derived Score 1=Most common practice; 2=Common Practice; 3= Less common practice.

3.3.3 Constraints to chickpea production

The farmers in the three districts explained the problems encountered during the production of chickpea. Farmers in Bomet district ranked insect pests as the most important problem of chickpea followed by drought, bird damage, lack of seed and lack of early maturing varieties. In Chepalungu district, diseases were ranked as the most important followed by insect pests which tied in ranking with lack of early maturing varieties and lack of training, drought, bird damage and lack of markets. In Mbeere South district the most important constraint affecting chickpea production was lack of markets for the produce, followed by drought, pest infestations, diseases, difficulty in threshing and water logging (Table 3.5).

Table 3.5: Constraints to chickpea production in Bomet, Chepalungu and Mbeere South districts in the year 2012

	Bomet district		Chepalungu district		Mbeere South district	
Production constraints	**MDS**	**Rank**	**MDS**	**Rank**	**MDS**	**Rank**
Insect pests	3.8	1	2.5	2	3.6	3
Lack of training	0.2	10	2.5	2	-	-
Drought	2.8	2	1.5	3	3.9	2
Late maturity	1.7	5	2.5	2	-	-
Lack of seeds	1.8	4	0.3	7	-	-
Birds	2.3	3	1.3	4	-	-
Diseases	0.8	7	2.8	1	2.4	4
Lack of markets	1.5	6	0.8	5	4.4	1
Water logging	0.5	8	0.5	6	0.7	6
Threshing ability	0.3	9	0.5	6	0.9	5
Weeding	0.2	10	0.0	8	-	-
Poor timely planting	-	-	-	-	0.1	7

Key: MDS; Mean Derived Score 1=Highly Ranked; 8=Lower Rank, - = No response.

3.3.4 Chickpea preferred traits and variety ranking

The farmers were asked to list the preferred traits and rank them based on importance. The responses from farmers in Bomet district indicated that preferred varieties should be high yielding and drought tolerant as the main criteria of their choice. Other traits of importance were early maturity, pest tolerance and good taste. In Chepalungu district, the farmers' ranked high yielding, drought and earliness which tied in ranks as the major preferred traits. The other traits were disease tolerance, high germination percentage and good taste. Similarly in Mbeere South, high yield was ranked first, followed by drought tolerance, pest tolerance, early maturity, and disease tolerance (Table 3.6).

The specific varieties grown by farmers were also ranked based on five most preferred traits (Table 3.7). However, the latter was done only in Bomet (Kiplabotwa) and Chepalungu (Bing'wa and Chemeng'wa) districts since the farmers were aware of the specific varieties by name unlike in other villages in Bomet and Mbeere South Districts. The results indicated that the variety ICCV 97105, released as *Chania Desi I* was the most preferred variety because it had all the five traits except small seeds. The other three important varieties were ICCV 92944, released as *Chania Desi II* and ICCV 00108, released as *LDT 068* and ICCV 95423 released as *Saina K1*. The farmers in areas visited in Mbeere South were not able to distinguish the specific varieties they were planting. They generally distinguished them based on types, Desi or Kabuli. These farmers preferred the Desi types due to high yield, marketability and tolerance to both drought and pests.

Table 3.6: General criteria for preferred chickpea traits ranked by farmers in Bomet, Chepalungu and Mbeere districts in the year 2012

	Bomet		Chepalungu		Mbeere South	
Criteria	**MDS**	**Rank**	**MDS**	**Rank**	**MDS**	**Rank**
High yield	4.7	1	5.0	1	5.0	1
Drought tolerance	2.3	2	3.0	2	3.4	2
Earliness	2.2	3	3.0	2	2.0	4
Pest resistance	2.0	4	1.0	6	2.3	3
Disease resistance	1.2	6	1.8	3	1.3	5
Threshing ability	0.8	7	0.3	8	0.6	8
Good taste	1.7	5	1.3	5	0.9	7
Germination	0.7	8	1.5	4	-	-
Heavy seeds	0.7	8	0.5	7	-	-
Colour	0.3	8	0.3	8	0.6	8
Tolerant to water logging	0.2	10	-	-	1.1	6
Stability	0.3	9	-	-	-	-
Adaptation to intercrop	-	-	-	-	0.4	9
Soft testa	-	-	-	-	0.3	10

Key: MDS – Mean Derived Score 1=Highly Ranked; 9=Lower Rank, - = No response.

Table 3.7: Direct matrix ranking of specific chickpea varieties based on highest ranked traits and seed size in Bomet and Chepalungu districts in the year 2012

Variety/type	High yield	Drought tolerance	Early Maturity	Pest resistance	Disease resistance	Large seeds	Mean	Rank
Kiplabotwa – Bomet district								
ICCV 95423 (Kabuli)	3	3	4	4	4	1	3.2	4
ICCV 97105 (Desi)	1	1	1	2	2	4	1.8	1
ICCV 00108 (Desi)	4	4	3	1	3	3	3.0	3
ICCV 92944 (Desi)	2	2	2	3	1	2	2.0	2
Bing'wa – Chepalungu district								
ICCV 00305 (Desi)	5	4	4	3	5	1	3.7	4
ICCV 95423 (Kabuli)	1	2	5	4	2	2	2.7	2
ICCV 96329 (Kabuli)	4	5	3	5	5	3	4.2	5
ICCV 97105 (Desi)	1	1	1	1	1	5	1.7	1
ICCV 00108 (Desi)	3	3	2	2	3	4	2.8	3
Chemeng'wa – Chepalungu district								
ICCV 95423 (Kabuli)	1	3	3	5	2	2	2.7	3
ICCV 96329 (Kabuli)	3	4	4	3	4	1	3.2	4
ICCV 97105 (Desi)	1	1	1	1	1	4	1.5	1
ICCV 92944 (Desi)	2	2	2	2	3	3	2.3	2

Key: 1=Highest rank, 5= lowest rank.

3.3.5 Sources of seed

The major seed provider in Bomet and Chepalungu districts was International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi. Other seed providers were Egerton University, chickpea farmers and Kenya Agricultural Livestock and Research Organization (KALRO), Njoro. Farmers in Mbeere South relied mainly on other chickpea farmers, followed by local markets mainly Karaba, Nairobi and cereal dealers within their localities (Table 3.8). During the discussion farmers indicated that the seed prices ranged from Ksh. 70 ($ 0.85) for Desi and Ksh. 100 ($ 1.25) for Kabuli per kilogram. However, when it was not planting season the price ranged from Ksh. 45 ($ 0.56) per kilogram for Desi and Ksh. 65 ($ 0.81) per kilogram for Kabuli and these prices were similar in the three districts.

3.3.6 Ways through which farmers accessed agricultural information

The farmers indicated that they accessed agricultural information from the Ministry of Agriculture Livestock and Fisheries, Ministry of Forestry, Churches, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi, Kenya Agricultural and Livestock Research Organization (KALRO) formerly Kenya Agricultural Research Institute (KARI), Egerton University and Kenya Plant Health Inspectorate Services (KEPHIS) among others. In Bomet district, the most important means through which farmers received training was farm visits followed by field days, demonstrations and group training while in Chepalungu district farmers' ranked demonstrations as the most important means. This was followed by group training, farm visits and field days. Similarly, the same means were used in Mbeere South where field days was the most important followed by farm visits, demonstrations and group training (Table 3.9).

Table 3.8: Chickpea seed providers in Bomet, Chepalungu and Mbeere Districts

	Bomet		Chepalungu		Mbeere South	
	MDS	**Rank**	**MDS**	**Rank**	**MDS**	**Rank**
Chickpea farmers	3.0	3	3.0	3	5.0	1
ICRISAT, Nairobi	5.0	1	5.0	1	1.0	5
Egerton University	4.0	2	3.2	2	-	-
KALRO, Njoro	2.0	4	2.8	4	-	-
KEPHIS	-	-	0.4	5	-	-
Local market	-	-	-	-	4.0	2
Agro-dealers	-	-	-	-	2.4	4
Nairobi market	-	-	-	-	2.6	3

Key: 1= High rank 5= Low rank, - = No response, MDS= Mean Derived Score, KALRO (Kenya Agricultural and Livestock Research Organization), KEPHIS (Kenya Plant Health Inspection Services).

Table 3.9: Ways through which farmers obtained training on chickpea production in Bomet, Chepalungu and Mbeere South districts in the year 2012

	Bomet district		**Chepalungu district**		**Mbeere South district**	
	MDS	**Rank**	**MDS**	**Rank**	**MDS**	**Rank**
Farm visits	4.0	1	2.4	3	3.1	2
Field days	2.4	2	1.4	4	3.4	1
Demonstration	2.0	3	3.4	1	2.4	3
Group training	0.2	4	3.1	2	1.4	4

Key: 1= High rank, 4= Low rank, - = No response, MDS= Mean Derived Score.

In addition to training on chickpea production by various organizations and government ministries, farmers also attained general agricultural information through other means such as radio, television, bulletins, pamphlets and telephone calls. Farmers' in Bomet and Chepalungu relied on radio programs offered in vernacular languages such as 'KASS' FM, 'Chamgei' FM and 'Kitwek' FM with one famous programme "Kobotisiet abkasari" translated as "modern farming". Other means were face-to-face discussions with other farmers, television such as 'kilimo biashara' programme translated farming as a business aired by QTV and through telephone calls. Internet as a source of information was also mentioned (Table 3.10). Farmers in Mbeere South also relied mainly on radio stations offered in vernacular such as 'Wi-Mwaro' FM, 'Inooro' FM, 'Musyi' FM, 'Kameme' FM and 'Coro' FM. One of the famous programs being 'Mugambo wamuremi' translated as the voice of the farmer. The others were face-to-face discussions, television and also from the market especially agro-dealers. The farmers from the areas visited also reported that they relied on radio and television for information on weather forecast. This informs them on when to expect rains and hence timing when to plant. Others indicated that they also relied on radio to obtain information on market prices of several produce in different towns.

Table 3.10: Ways through which farmers accessed general information on agricultural production in Bomet, Chepalungu and Mbeere South district in the year 2012

	Bomet district		**Chepalungu district**		**Mbeere South district**	
	MDS	**Rank**	**MDS**	**Rank**	**MDS**	**Rank**
Radio	5.0	1	5.0	1	5.0	1
Face-to-face	3.4	2	3.7	2	3.3	3
Telephone	2.2	3	2.3	4	3.6	2
Barazas	1.6	5	1.0	5	0.6	6
Television	2.8	4	3.0	3	0.6	6
Internet	0.0	6	0.7	6	-	-
Market	-	-	-	-	1.3	4
Bulletins	-	-	-	-	0.7	5

1= High rank, 6= Low rank, MDS = Mean Derived Score, - = No response.

3.4 Discussions

Based on farmer rankings, chickpea was ranked as the second legume after common beans in both Bomet and Chepalungu districts and the fifth after green grams, beans, cowpeas, pigeonpeas and black grams in Mbeere District. Chickpea is grown not as a major crop but mainly as a relay crop after harvesting the main crop while in low lands such as Mbeere, it is grown during both seasons since these are drier areas and chickpea fits well with other dryland legumes such as cowpeas, green grams and pigeon peas. The ranking in the dry highlands (represented by Bomet and Chepalungu) was because farmers intercrop maize and beans and plant chickpea after harvesting the main crop. Chickpea, although it is relatively new, is gaining popularity in the highlands of Rift Valley such as Bomet, Koibatek and Nakuru (Rongai) as a relay crop. In addition, the crop is also extending to dry areas in the semi-arid regions such as Naivasha and Embu. Earlier research showed that chickpea was adapted to the lower altitude areas (Kibe and Onyari, 2007; Onyari *et al.*, 2010). Expansion of chickpea to other areas requires the intervention of farmers, scientists and other organizations (government ministries/institutions and non-governmental organizations). With the current high population growth rate coupled with unpredicted climate issues, food security is a key concern.

Farmers in Bomet and Chepalungu planted chickpea mainly as a sole crop and as a relay crop. As a relay crop, chickpea followed maize, millet or sorghum which are their main crops. The planting was done during the minor season mainly as sole crop. This has advantages, such as land that would have otherwise been left idle is utilized and also provides additional food/income to farmers. Researchers have stated that planting chickpea through rotations/relay in cereal-dominated farming systems leads to soil improvement in addition to other advantages (Mazid *et al.*, 2013) and increase in maize yield (Cheruiyot *et al.*, 2001).

Farmers rarely intercropped chickpea with maize or sorghum. The reason given was that maize/sorghum shaded chickpea resulting in excessive vegetative growth at expense of pods and also infections. Maize in Bomet and Chepalungu takes a longer time to mature due to cool environments as these are highlands and intercropping exposes chickpea to unsuitable conditions. Research done in Njoro, Kenya showed that intercropping common bean with maize/sunflower caused shading prolonging the vegetative stage and subjecting it to infestations (Tuey and Lelgut, 2002). Farmers in Mbeere South planted chickpea mainly as an intercrop with maize and sorghum. Chickpea was planted during the major rains and minor rain which was done the first weeks of May or June when rains subside and coincides with flowering in the month of June/July when temperatures are cool. Maize varieties in the semi-arid areas are mainly short duration varieties which take three to four months to mature hence coinciding with chickpea growth duration. Famers also indicated that in situations when rain fails, chickpea could survive and yield reasonable quantities compared to other legumes such as common beans. This is in agreement with results reported by Onyari *et al.*, (2010). Due to its ability to grow under stress environments, its yield provides the much needed food thus enhancing food security (Kaloki, 2010). Farmers also indicated that planting chickpea with maize improved yields of maize, similar to a report by Kaloki (2010). It is also a risk-minimization strategy as these crops respond differently to uncertain weather conditions, and especially to moisture stress (Pionetti, 2006).

Chickpea in Kenya is produced purely under rain-fed conditions where most farmers plant it during the short rains once the main crop is harvested. Farmers in Chepalungu and Bomet districts reported that pest infestations, drought and lack of early maturing varieties were the major constraints while farmers in Mbeere ranked lack of markets as a major constraint followed by drought and pest infestation. This difference in ranking was due to different needs and localities. Lack of markets for example was a major constraint in Mbeere

because most of the communities in the region were business oriented and they grew crops mainly for cash. Availability of markets strongly determines when and what to plant in this region. In other reports, market demand strongly influenced farmers' selection criteria (Witcombe *et al.*, 2006), however several factors determine price. Large seeded Kabuli varieties were shown to be highly priced (Shiferaw *et al.*, 2007; ICRISAT, 2009). This large seeded trait of Kabuli varieties can be utilized by researchers to improve small seed size of most Desi types.

Pest infestations were major constraints that cut across the three districts. Chickpea was planted during the short rains under rainfed conditions. These seasons are characterized by unreliable and unpredicted rainfall patterns and change in temperature due to dry periods. This probably increases the pest population mainly pod bores, *Helicoverpa armigera* (Hüb.). During the time when there is high rainfall, it was predicted to contribute in washing the noctuid eggs of *H. armigera* (Hüb.) from the plant lowering the population (Mulwa *et al.*, 2010). In common bean, it was reported that the bean fly is particularly serious during drought and late planted seasons compared to early sown beans (Byabagambi *et al.*, 1999; Sariah and Makundi, 2007; Kosgei, 2008). The yield loses due to *H. armigera* (Hüb,) alone was reported to range between 20 – 40% (Sharma *et al.*, 2005). Similarly, damage by pests such as maize leaf weevil, thrips, stem sawflies, flea beetles and cabbage aphids among others, were high under drought environment where their effect led to amplified yield losses (Popov *et al.*, 2006). These authors indicate that the aggressiveness of these pests was determined by increased need to extract water from weak plants. Birds' damage was also reported as a problem in Bomet and Chepalungu during the podding stage. Birds become serious during podding when pods are tender and easy to peck. Also chickpea during this season is probably the only crop, since it is planted after harvesting the main crop. This gives birds no alternative food.

Drought was also ranked as a major constraint in the districts. Chickpea was grown during the short rains under receding soil moisture. Drought has become a frequent occurrence in several parts of the country during certain dry periods of the year. Although chickpea is known for its drought tolerance compared to other crops, terminal drought reduces yield and can lead to total crop failure. Generally drought was reported to cause approximately 3.7 million tons amounting to 40 - 50% average yield loss (Varshney *et al.*, 2009) while terminal drought caused seed yield reduction between 58 - 95% in comparison to yield under irrigation in Australia (Leport *et al.*, 2006). In a survey done in Mbeere, farmers indicated that food shortage period occurs from December to February (Mergeai *et al.*, 2001). This is the time when such areas are receiving the short rains as indicated earlier and was also indicated as the time of planting chickpea.

Lack of early maturing variety was another constraint that caused yield reduction in Bomet and Chepalungu districts. When varieties take longer to mature, the crop will be at podding stage during the dry period of the short rains, hence they will not escape drought. During the short rains season, rainfall may either delay or stop early. This exposes the crop to both early stress and late drought (terminal). Reports on chickpea in Naivasha, Kenya, planted towards the end of the raining season had significantly decreased shoot biomass and number of pods (Onyari *et al.*, 2010). According to reports, early maturing varieties escape drought (Upadhyaya *et al.*, 2007). Significant advance in maturity date of chickpea in Canada was achieved by incorporating early flowering, double podding and other favorable alleles into the desirable genetic backgrounds (Anbessa *et al.*, 2007). International Center for Agricultural Research in the Dry Areas (ICARDA) considered traits like early seedling establishment, early growth vigour and canopy development, early flowering and maturity through which potential useful lines were identified (Mazid *et al.*, 2013). Such traits are important in adaptation to drought environment.

Disease infestation was also reported as an important constraint. The major disease was Ascochyta blight [*Ascochyta rabiei* L. (Pass.)] and cases of Fusarium wilt (*Fusarium oxysporum*). This was mainly a problem in the dry highlands due to cooler temperatures during the early months of chickpea production. According to Kimurto *et al.,* (2013b) chickpea losses due to Ascochyta blight could be up to 100% especially in dry highlands. In addition, clay soils in the highlands and black cotton soils in Mbeere South districts could also retain water longer, if excess rainfall occurred at later stage of crop growth. This resulted in root rots and blights.

Farmers in the three districts ranked high yielding, tolerance to drought and early maturing varieties as the main reasons for choice of varieties to plant. Other traits of importance were resistance to pests, diseases, water logging and good taste. Varieties that were high yielding under the planting conditions were preferred by farmers. During the short rains season, chickpea was exposed to harsh conditions such as drought reducing the yields. Varieties that could tolerate these conditions or mature early before the terminal drought sets in were preferred. Such varieties would also have less infestation by pests due to the short growth cycle when pest population pressure was still low. These traits are similar to traits that breeders test under controlled conditions or on-station research. Results from a survey done in Embu, Kenya, indicated that farmers were also interested in chickpea varieties that are large seeded, easy to thresh, drought and heat tolerant, easy to cook and have better taste (Kaloki, 2010). In a farmer participatory varietal selection (FPVS) conducted in Naivasha and Bomet districts in Kenya, farmers preferred chickpea varieities that were disease resistant, early maturing, high plant vigour, tasty and high seed yield (Thagana *et al.,* 2009). Seed size and uniformity were noted as important in determining market price especially for the Kabuli types (Davies *et al.,* 1999). Chickpea requires 152.4 – 254 mm of rainfall and/or irrigation water during the growing season and thus is well suited to dryland or limited-irrigation

production, however its exposure to terminal drought is one of the major constraints to increasing productivity (Kanouni *et al.*, 2012). In a research conducted in Zimbabwe on maize adaptation to drought, farmers indicated that they preferred maize that were high yielding, early maturing and drought tolerant (Mhike *et al.*, 2012). The current results obtained where farmers are more interested in high yielding and drought tolerant varieties could be attributed to the current issues of climate change. Rising temperatures, droughts, floods, desertification and weather extremes will severely affect agriculture, especially in the developing world (IPCC, 2009). Reports indicated that negative impacts of climate change on crop yields than positive impacts have been more common (IPCC, 2014). Farmers therefore have rich knowledge and experience in selection of varieties over years.

The choice of specific preferred varieties differed among the villages in Bomet and Chepalungu district. The difference could be attributed to different agro-ecological zones where most of these varieties were Desi type. Farmers indicated that the Desi types had many uses compared to Kabuli types in addition to less infestation by pod borers and disease. In addition, Desi varieties were shown to be more drought tolerant than the Kabuli types. Similar differences in choice of varieties across localities have been reported (Ojwang, 2010; Were, 2011; Kiiza *et al.*, 2012). Four varieties namely; ICCV 97105, ICCV 92944, ICCV 00108 were all being Desi types and common across the three locations and ICCV 95423 (Kabuli type) were released recently. Involving farmers in variety selection and identification, leads to knowing their choices under each target environment.

The farmers received training from non-governmental organizations and government institutions/ministries. Training was through field days, field visits and demonstrations. Chickpea production requires apprpriate management like any other crop, which includes timely planting, weeding, management of pests and diseases, and post-harvest handling processes. Farmers require this information for improved yields. Field days, for example, are

important forums where farmers openly discuss their challenges and argue on what dissatisfies them and visiting demonstration plots allow discussions and solutions suggested on major issues. Farmers could also be free to their fellow farmers especially those farmers who may have been trained earlier. Dissemination of information on winter-sown chickpea in Syria was achieved through field days, printing and distribution of extension materials or publications in addition to distribution of new varieties to chickpea producers, variety testing and demonstration fields by International Center for Agricultural Research in the Dry Areas (ICARDA) and the Syrian national programs (Mazid *et al.*, 2013). A survey conducted in Nigeria showed that the communication channels for dissemination of agricultural information to farmers included extension agent advisory visits, field days, demonstration/training, and agricultural shows (Adekunle *et al.*, 2004). Research on sweet potatoes indicated that training farmers on its production was one way of encouraging its adoption as those trained were likely to adopt than untrained (Witcombe *et al.*, 2006).

In addition to on-site training, general information on agricultural production was obtained by farmers through mass media and discussions with other farmers. Media is one source of information dissemination of new technologies and innovations on agriculture. Currently, this has been enhanced through most radio stations presenting in vernacular languages. Most of the agriculture related programmes presented by these stations invite experts and are also interactive as they provide listeners with opportunity to contribute to the presentations through the telephone sessions. In Kenya it was reported that 74.4% of farmers use radio while 37.8% use television to obtain agricultural information (Kituyi-Kwake and Adigun, 2008). Research indicated that in Sudan farmers preferred using radio as a means of obtaining agricultural information (Musa, 2011). According to the researchers, farmers rated the radio as very effective because it is available, has ability to offer agricultural information

that they need, inexpensive, easy to use, portable, accessible and battery operated. Through media, agricultural information is disseminated to all categories of farmers, old and young.

3.5 Conclusions

The findings of this study indicated that in order to develop high yielding varieties with qualities that address farmers' constraints and preference the breeders need to work closely with the farmers. In the Kenyan highlands chickpea was planted mainly as a relay crop while those in dry lowlands planted it as a sole crop or intercropped with other crops. Farmers in chickpea growing areas indicated that among the constraints, drought, pests attack, lack of market were ranked highest followed by lack of training and high quality seeds. Farmers were interested in chickpea varieties with the following traits: high yielding, drought tolerant, early maturing, resistant to pests and diseases and tolerant to water logging, especially in the black cotton soils of Eastern Kenya and clay soils of Chepalungu areas in the Rift Valley. Farmers gained knowledge on chickpea production and general agricultural information through field days, farm visits, and group trainings and demonstrations. They also received information through the mass media which is good for young and old farmers as the programmes were aired in local languages.

CHAPTER FOUR

4.0 Inheritance of drought tolerance root traits and yield components in chickpea

4.1 Introduction

Chickpea is grown without irrigation, planted in the post-rainy season and survives during the growing period on progressively declining residual soil moisture (Gaur *et al.*, 2008). This exposes the crop to drought especially terminal drought. Drought has been reported to cause 40 - 50% yield reduction globally (Varshney *et al.*, 2009). Given the current climate change and the increasing human population, there is urgent need to develop high-yielding chickpea varieties with improved drought tolerance (Krishnamurthy *et al.*, 2013).

Roots have been identified as important for drought adaptation in chickpeas. Research into chickpea diversity for root traits has led to the identification of two genotypes, ICC 4958 and ICC 8261, with prolific and large rooting systems (Kashiwagi *et al.*, 2005; Kashiwagi *et al.*, 2006). Polygenically controlled traits such as drought tolerance and yield are affected by both genetic and environmental factors. Kashiwagi *et al.* (2008a) reported that chickpea yields are highly prone to large genotype by environment (G × E) interactions in marginal environments. These genes have small effects contributing to phenotype and are not easily identifiable. Direct selection of such traits becomes difficult due to factors such as low heritability and instability due to G x E interactions. This has led to indirect selection in early generations through traits correlated with seed yield (Golparvar, 2011). Physiological traits like high root mass, smaller leaf area, osmotic adjustments, early growth vigor and maturity, and short-duration are easier to use (Saxena, 2003) in selecting genotypes for drought adaptation.

Genetic information regarding the inheritance of quantitative characters, especially the nature and magnitude of gene action governing the inheritance of a given trait, is important for successful breeding (Hinkossa *et al.*, 2013). Determination of genetic factors in selection therefore, becomes a primary aid and has been achieved using several procedures/models (Kearsey and Pooni, 1998; Farshadfar *et al.*, 2008). One of the best methods for the estimation of genetic parameters is generation mean analysis (GMA) in which epistatic effects could be estimated (Khodambashi *et al.*, 2012). In addition, heritability estimates play an important role for planning the breeding strategy. The heritability of a trait/character determines the extent to which it is transmitted from generation to generation and is a valuable tool when used in conjunction with other parameters in predicting genetic gain that follows the selection for that character (Ansari *et al.*, 2005).

In chickpea, additive gene effects were reported for pod and seed traits (length and width) while both additive and dominance gene effects were reported for pod thickness and width (Bicer and Sakar, 2010). Plant height at maximum flowering, plant weight just after harvest, pod weight per plant, number of pods and seeds per plant and seed weight per plant showed additive-dominance relationships (Deb and Khaleque, 2009). Generation mean analysis indicated that seed weight was controlled by additive gene effects, with significant additive x additive effect in Desi x Kabuli crosses (Sharma *et al.*, 2013). Significant gene interactions for days to flowering, days to maturity, 100 - seed weight, seed yield, pods per plant, seeds per plant and stem chloride concentration under controlled and saline conditions occur (Samineni *et al.*, 2011a). Further, 100 - seed weight has been shown to have positive correlation with yield (Noor *et al.*, 2003; Vaghela *et al.*, 2009; Kobraee *et al.*, 2010; Shamshi *et al.*, 2010) hence it is an important trait for indirect selection for yield in chickpea. The additive and additive × additive interaction effects play an important role in governing the

root length density and root dry weight which has been shown to provide adaptation to drought (Kashiwagi *et al.*, 2008a). Similarly, interactions govern shoot biomass and seed yield (Serraj *et al.*, 2004). Estimation of gene effects is important to breeders and geneticists in formulating the most advantageous breeding procedures for the improvement of the quantitative characters. In this context, the present research was undertaken with the following objective:

a) to determine inheritance of root traits and yield in chickpea.

4.2 Materials and methods

4.2.1 Parental materials

The genotypes used were ICCV 00108 (P_1) as female crossed to ICC 8261 and ICC 4958 as male/donor parent (P_2). The F_{1s} were selfed to obtain F_{2s}. The F_{1s} were also backcrossed to female and male parents to generate BC_1P_1 (F_1 x P_1) and BC_1P_2 (F_1 x P_2), respectively. ICCV 00108 is a pure line that was introduced from ICRISAT, India, and released for commercial production as *LDT 068*. It is a Desi type (pink flower) that is high yielding, medium maturity duration and widely adapted, medium seed size and it has some resistance to Ascochyta blight. However, it has a low root biomass. On the other hand, the male parents were released varieties in India and were introduced to Kenya as donor parents for drought improvement. They have shown resistance to drought in Kenya. ICC 4958 is a Desi type (pink flower) and drought tolerant breeding line. The line has root systems that are both more prolific and heavier compared to other well-adapted varieties (ICRISAT Plant material description No 33, 1992). This line has previously been crossed to ICC 1882 (low root length) to develop recombinant inbred lines (RILs) that were used in identification of *QTL-hotspot* region with SSR markers (Varshney *et al.*, 2013a). It is an early maturing

variety and large seeded (Gowda *et al.*, 2011). The donor parent ICC 8261 is a Kabuli type (white flower) that has a prolific root system but is late maturing.

4.2.2 Evaluation for root traits

The soil and sand were mixed in a ratio of 1:1, w/w in pots and placed under rain-out shelter at Egerton University. The pots were initially filled with water to 70% field capacity. Seeds of the six basic generations namely, F_1 (16 plants), F_2 (88 plants), BC_1P_1 (30 plants), BC_1P_2 (30 plants) and their parents, P_1 and P_2 (18 plants each), from ICCV 00108 (female) crossed to ICC 8261 (donor) and ICC 4958 (donor), were then planted. Water (1.5 litres per pot) was applied every two days after sowing until all the plants emerged, after which it was not applied. The shelter was usually covered to prevent rain water from entering and opened when there were no rains. Roots were sampled 40 days after planting. Shoots were removed and roots were washed gently under running water. After washing the soil-sand mixture until three quarters was removed, the remaining was washed in a sieve. The roots were then scanned using image analysis software (WinRhizo Regent Instrument Canada INC., Quebec, Cananda) for total root length.

4.2.2.1 Data collected

a) Total root length (TRL) (cm) – This was obtained from the WinRhizo analysis results.
b) Root length density (RLD) (cm cm^{-3}) – This was calculated as ratio of total root length to volume of the pot.
c) Shoot dry weight (SDW) (g) – Shoots separated from roots were oven dried at 80 ºC for 72 hours and their weights recorded. The SDW was used as an indicator of plant growth vigour.
d) Root dry weight (RDW) (g) – Scanned roots were oven dried at 80 ºC for 72 hours and their weights recorded.

e) Plan dry weight (PDW) (g) – This was obtained by summing the shoot dry weight and root dry weight.

f) Root to shoot ratio (R/S) – This was calculated as the ratio of root dry weight to shoot dry weight.

4.2.3 Field evaluation

4.2.3.1 Site description and field layout

The basic generations from ICCV 00108 (female) crossed to ICC 8261 (donor) and ICC 4958 (donor) were evaluated in the field for yield agronomic traits. These were planted at Cheptebo Africa Inland Church (AIC), in Kerio valley in Elgeyo Marakwet County. Cheptebo is located at 1º, 30´N and 35º, 30´E in Agro-ecological LM4 (Lower Midland) at an altitude of 900 m above sea level (asl). The average rainfall ranges between 265 - 510 mm; mean annual maximum and minimum temperatures of 22.6 ºC and 21.7 ºC respectively. The soils are Cambisols with lithosols, somewhat excessively drained, shallow to moderately deep, reddish brown, friable, rocky and stony (Jaetzold and Schmidt, 1983).

The design used was randomized complete block (RCBD) with two replications with blocks sizes differing based on the number of seeds planted. The plot size was 1m, single row, at spacing of 40 x 10 cm. The experiment was conducted under rainfed condition.

4.2.3.2 Data collected

Data was collected per plant with 10 plants for P_1, P_2 and F_1, 30 plants for BC_1P_1 and BC_1P_2 and 80 plants for F_2. Each plant was harvested separately and pods threshed. The number of seeds per plant were counted and 100-seed weight, as an estimate of seed size, was determined by the formula:

(weight of all seeds /number of seeds) x 100.

4.2.3.3 Data analysis

The generated root and field data were subjected to generation mean analysis to determine genetic variation associated with the traits under study. Generation mean analysis for field data were analysed according to Marther and Jinks (1982) models using macros developed by Cebalos (2007) analysed with SAS 9.3. Means of root traits were analysed following Haymans' model using SASQuant program (Gusmini *et al.*, 2007) as:

$$Y = m + \alpha [a] + \beta [d] + \alpha^2 [aa] + 2 \alpha \beta [ad] + \beta^2 [dd]$$

Where:

Y = The mean of one generation

m = The mean of all generations

a = The sum of additive effects

d = The sum of dominance effects

aa = The sum of additive x additive interaction (complementary) effects

dd = The sum of dominance x dominance interaction (duplicate) effects

ad = Sum of additive x dominance effects

α, β, $2\alpha\beta$ and β^2 are the coefficients of genetic parameters.

The genetic parameters [m], [a], [d], [aa], [dd], [ad]) were tested for significance using a t-test.

The adequacy of the additive x dominance model was determined by χ^2-test where significant χ^2 denoted the model was inadequate (Hayman, 1958) .

Variance estimates were determined according to Farshadfar *et al.* (2008) as indicated below:

$V_G = V_{F2}-V_E$,

$V_A = 2V_{F2}-V_{BC1P1}-V_{BC1P2}$

$V_D = V_{BC1P1}+V_{BC1P2}-V_{F2}-V_E$

$V_E = 0.25V_{P1}+0.25V_{P2}+0.5V_{F1}$

$V_P = V_G+V_E$

Where; V_G, V_A, V_D, V_E and V_P are genotypic, additive, dominance, environmental and phenotypic variances, respectively.

Broad-sense (h^2_b) and narrow-sense (h^2_n) heritability for the field data were estimated by formulae (Warner, 1952; Allard, 1960) as indicated:

$$h_b^2 = [\ V_{F2} - (\ V_{P1} + V_{P2} + V_{F1}\ ) / 3\ ] / V_{F2}$$

$$h_n^2 = [\ 2V_{F2} - (\ V_{BC1P1} + V_{BC1P2}\ )\ ] / V_{F2}$$

Where, V = variance of P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 generations.

Determination of gene factors was calculated according to (Wright, 1952) as follows:

$$[0.25(0.75-h+h^2)\ D^2]/V_{F2}-V_{F1}$$

Where D is the difference between observed parental means = (P_1-P_2) and h is the dominance ratio = (F_1-P_1)/D and V are variances for F_2 and F_1.

4.3 Results

4.3.1 Variations in root traits and yield components

The generations were highly significantly different ($P < 0.0001$) for total root length (cm) (TRL), root length density cm cm^{-3} (RLD), root dry weight (g) (RDW), shoot dry

weight (g) (SDW), root to shoot dry weight ratios and total plant dry weight (g) (PDW) (Table 4.1). The analysis of generations from ICCV 00108 x ICC 4958 for root traits could not be determined due to very few numbers of plants that survived.

The mean squares of generations for ICCV 00108 x ICC 4958 showed that 100-seed weight was highly significant ($P < 0.0001$) among the six basic generations (Table 4.2). However, the field evaluation for ICCV 00108 x ICC 8261 could not be presented due to very few plants in some of the generations.

Table 4.1: Mean squares for drought tolerance root traits for ICCV 00108 x ICC 8261

SOV	df	TRL(cm)	RLD (cm cm^{-3})	RDW(g)	SDW(g)	PDW(g)	R/S
Generations	5	2321029.41***	0.42***	0.02***	0.26***	0.16*	0.28***
Error	5	309591.02	0.06	0.350	0.020	0.08	4.07

*, ***=significant at $p < 0.05$, $p < 0.001$, ns= non-significant. SOV= source of variation, df= degrees of freedom, TRL= total root length, RLD= root length density, RDW= root dry weight, PDW= plant total dry weight, R/S= root to shoot ratio.

Table 4.2: Mean squares for 100-seed weight under rainfed condition for ICCV 00108 x ICC 4958

Source of variation	DF	100-seed weight (g) per plant
Replications	1	167.33
Generations	5	224.12***
Error	5	22.26

***= Significant at $P < 0.001$.

4.3.2 Mean performance of generations for drought tolerance root traits and 100-seed weight

Significant ($P < 0.001$) mean differences were observed for TRL (cm), RLD (cm cm^{-3}), RDW (g), SDW (g) PDW (g) and R/S ratio among the generations (Table 4.3). The RDW differed significantly ($P < 0.001$) between P_2 (ICC 8261) (0.49 g) and P_1 (ICCV 00108) (0.39 g). There was no significant difference between the parents for TRL, RLD, SDW, PDW and R/S ratio. However, parent one (P_1) differed significantly ($P < 0.05$) from the F_1 for SDW, TRL and RLD. The other traits did not differ significantly between P_1 and F_1. However, in all the traits, the F_1 had a lower mean than P_1. Parent two (P_2) differed significantly from the F_1 for all the traits and from BC_1P_1 for TRL, RLD, RDW, PDW and R/S. It also differed significantly from BC_1P_2 for TRL, SDW and PDW. The BC_1P_2 had higher RDW (0.40 g), TRL (1752.10 cm), and PDW (0.75 g) compared to the F_1 means for these traits. The means for BC_1P_1 were lower than the means for P_1 for all traits except for SDW.

The means for the six generations differed significantly ($P < 0.0001$) for 100-seed weight. Parent two (ICC 4958) had significantly higher mean seed weight (25.25 g) than parent one (ICCV 00108) (19.49 g). The mean of the F_1 (18.55 g) was lower than that of P_1 (19.49 g) and the overall mean (21.28 g), similarly BC_1P_1 had lower seed weight (19.62 g) compared to the overall mean (21.28g) but greater seed weight than that of the F_1 (18.55 g). The segregating populations, F_2 and BC_1P_2, had higher mean values of 23.21 g and 21.59 g, respectively (Table 4.4).

Table 4.3: Means of the root characters for drought tolerance for ICCV 00108 x ICC 8261

Generations	**TRL (cm)**	**RLD cm cm^{-3}**	**RDW (g)**	**SDW (g)**	**PDW (g)**	**R/S ratio**
P_1	1895.09	0.81	0.39	0.41	0.80	0.97
P_2	2096.26	0.89	0.49	0.46	0.95	1.04
F_1	1447.67	0.62	0.37	0.32	0.68	1.22
F_2	1650.23	0.71	0.44	0.39	0.82	1.23
BC_1P_1	1203.63	0.51	0.18	0.59	0.76	0.31
BC_1P_2	1752.10	0.75	0.40	0.35	0.75	1.19
Mean	**1674.163**	**0.703**	**0.378**	**0.420**	**0.793**	**0.993**
LSD (0.05)	**314.761**	**0.135**	**0.089**	**0.082**	**0.156**	**0.300**
Significance	***	***	***	***	*	***

Key: ***= significant at P < 0.0001, ns = non-significant, TRL= total root length, RLD= root length density, RDW= root dry weight, SDW= shoot dry weight, PDW= plant total dry weight and R/S= root to shoot ratio.

Table 4.4: Means for 100-seed weight for yield for ICCV 00108 x ICC 4958

Generations	100-seed weight (g) per plant
P_1	19.485
P_2	25.206
F_1	18.553
F_2	23.217
BC_1P_1	19.619
BC_1P_2	21.593
Mean	**21.28**
LSD (0.05)	**1.254**
Significance	***

***=significant at $P < 0.001$.

4.3.3 Gene effects for drought tolerance root traits and 100-seed weight

The Chi-square values were significant for RLD, RDW, SDW and R/S root traits and 100-seed weight. The genetic model fitted indicated that generation means [m] were significant ($p < 0.05$) for all root traits (Table 4.5). Additive effects were significant ($P < 0.001$) for TRL, RLD, RDW and R/S. Dominance effect was significant for RDW and R/S ratios. In addition, non - additive epistatic effects were present for RDW, SDW and R/S ratio where additive x additive and dominance x dominance gene effects were significant for RDW and R/S ratio while additive x dominance effect was significant for RDW, SDW and R/S ratio. There was duplicate epistatic interaction for RDW and R/S as indicated by the opposite signs for significant dominance [d] and dominance x dominance [dd] gene effects.

Generation means [m] for 100-seed weight was highly significant ($P < 0.0001$). The additive main effects and non - additive effects were significant for 100-seed weight. The observation also showed non - additive epistasis effects, where additive x additive effects were significant while additive x dominance ffects were not significant for 100-seed weight (Table 4.6).

Table 4.5: Estimates of gene effects (±SE) for root traits measured for ICCV 00108 x ICC 8261

Gene effects	**TRL(cm)**	**RLD cm cm^{-3}**	**RDW(g)**	**SDW(g)**	**PDW(g)**	**R/S**
m	1650.2±73.35*	0.70±0.02***	0.04±0.02**	0.39±0.02**	0.82±0.04**	1.23±0.08**
a	-548.5±116.01**	-0.23±0.05***	-0.22±0.04**	0.24±0.05**	0.02±0.07^{ns}	-0.88±0.08**
d	-1237±724.39^{ns}	-0.53±0.31^{ns}	-0.67±0.21**	0.20±0.22^{ns}	-0.47±0.39^{ns}	-1.69±0.62*
aa	-689.5±525.4^{ns}	-0.29±0.22^{ns}	-0.60±0.16**	0.31±0.17^{ns}	-0.28±0.29^{ns}	-1.91±0.48**
ad	-447.9±246.16^{ns}	-0.19±0.11^{ns}	-0.17±0.07*	0.27±0.07**	0.09±0.12^{ns}	-0.85±0.14**
dd	1664.7±1155.4^{ns}	0.71±0.49^{ns}	1.06±0.34**	-0.68±0.36	0.39±0.63^{ns}	3.35±0.93**
χ^2	****	**ns**	****	*****	**ns**	****
Epistasis	**-**	**-**	**Duplicate**	**Duplicate**	**-**	**Duplicate**

*, **, *** =significant at P < 0.05, P < 0.01 and P < 0.001, ns= non- significant, TRL (cm)= total root length, RLD= root length density, RDW (g)= root dry weight, SDW (g)= shoot dry weight, PDW (g)= total plant dry weight, R/S= root dry weight to shoot dry weight ratio.

Table 4.6: Estimates of gene effects (±SE) for 100-seed weight for ICCV 00108 x ICC 4958

Gene effects	**100-seed weight (g) per plant**
m	24.62±1.76***
a	2.86±0.35***
d	-8.33±1.83**
aa	-4.26±1.59*
ad	-2.04±2.32^{ns}
dd	-
$\chi 2$	*

*, **, *** =significant at P < 0.05, P < 0.01 and P < 0.001.

4.3.4 Heritability estimates for root traits and 100-seed weight

Estimates of additive component of variance for root traits were greater than dominance variance while environmental variance component was low. Broad-sense heritability was high for all traits ranging from 0.59 - 0.83. Narrow-sense heritability was also high for SDW (0.81) and even higher for TRL and RLD (1.47), RDW (1.34), PDW (1.32) and R/S ratio (1.77) (Table 4.7).

Additive variance for 100-seed weight (9.79) was higher than dominance variance (6.06) while the environmental variance was higher (12.29). The broad-sense heritability was moderate (0.56) while narrow-sense heritability was low (0.35) (Table 4.8).

The number of effective factors (number of genes) obtained for root traits were few (Table 4.7) while for 100-seed there were 4.9 genes which is approximately five genes (Table 4.8).

Table 4.7: Different components of genetic variances, heritability estimates and number of factors (genes) of root traits for ICCV 00108 x ICC 8261

Trait	**Genotypic variance (V_G)**	**Additive variance (V_A)**	**Dominance variance (V_D)**	**Environmental variance (V_E)**	**Phenotypic variance (V_P)**	**Broad-sense heritability (h^2b)**	**Narrow-sense heritability (h^2n)**	**Effective factors [Wright, (1921)]**
TRL (cm)	281386	696612	-415E3	192028	473413	0.59	1.47	0.3
RLD (cm cm^{-3})	0.05	0.13	-0.08	0.04	0.09	0.59	1.47	0.3
RDW (g)	0.02	0.05	-0.03	0.01	0.04	0.60	1.34	0.1
SDW (g)	0.02	0.02	-0.01	0.01	0.03	0.64	0.81	0.2
PDW (g)	0.08	0.16	-0.08	0.04	0.12	0.65	1.32	0.2
R/S ratio	0.45	0.95	-0.51	0.09	0.54	0.83	1.77	0.0

TRL (cm)= total root length, RLD= root length density, RDW (g)= root dry weight, SDW (g)= shoot dry weight, PDW (g)= total plant dry weight, R/S= root dry weight to shoot dry weight ratio.

Table 4.8: Genetic variances, heritability and minimum number of factors for 100-seed weight for ICCV 00108 x 4958

Estimates	**Formula**	**100-seed weight (g) per plant**
Genotypic variance (V_G)	V_{F2}-V_E	15.85
Additive variance (V_A)	$2V_{F2}$-V_{BC1P1}-V_{BC1P2}	9.79
Dominance variance (V_D)	V_{BC1P1}+V_{BC1P2}-V_{F2}-V_E	6.06
Environmental variance (V_E)	$0.25V_{P1}$+$0.25V_{P2}$+$0.5V_{F1}$	12.29
Phenotypic variance (V_P)	V_G+V_E	28.14
Heritability (h^2_b)	V_G/V_{F2}	0.56
Heritability (h^2_n)	V_A/V_{F2}	0.35
Minimum number of effective factors (Wright, 1952)	$[0.25(0.75-h+h^2)D^2]/(V_{F2}-V_{F1})$	4.9

V_{P1}, V_{P2}, V_{F1}, V_{F2}, V_{BC1P1}, V_{BC1P2} are the variances for P_1, P_2, F_1, F_2, and backcross to P_1 and P_2, respectively; D is difference between observed means (P_1-P_2); h is dominance ratio (F_1-P_1/D).

4.4 Discussions

Generations were significantly different for TRL (cm), RLD (cm cm^{-3}) RDW (cm), SDW (cm) and R/S ratio indicating the presence of genetic variability and the possibility for selection of these traits for drought tolerance. The mean root biomass was significantly higher in P_2 (ICC 8261) than for P_1 (ICCV 00108) indicating that ICC 8261 should have the ability to extract more moisture during dry season hence withstand drought conditions. Genotype ICC 8261 has been reported to have a large rooting system, deep rooting depth and large biomass allocation into the roots (Kashiwagi *et al.*, 2005). This was also indicated by large R/S ratio. The large R/S ratio is an indication of reduction in shoot biomass compared to root biomass which is an important trait during drought (Labidi *et al.*, 2009). Large biomass allocation into roots was exhibited by P_2 (1.04) and the F_1 (1.22) as shown by high R/S ratio compared to the mean value (0.993). This was also detected in the segregating F_2 and BC_1P_2 populations. However, the mean for the F_1 population was lower than P_1 for all root traits which is an indication of negative heterosis. Similar observations were reported in wheat where very short root (VSR) phenotype in the F_1 hybrid was reported to be controlled by a non-additive interaction between two alleles in a single gene locus (Li *et al.*, 2013). The mean for the F_2 population was also higher than that of the F_1 for all traits due to segregation. Similar high mean of F_2 population were obtained by Kashiwagi *et al.* (2008a). The backcrossing of F_1 to parent one (ICCV 00108) led to a reduction in most traits which could be an indication of inbreeding depression. However, SDW mean for BC_1P_1 (0.59) was higher than the better parent, ICC 8261 (0.46), which indicated the contribution of ICC 8261 to shoot production. Early shoot growth vigor was regarded as an important trait due to its contribution to terminal drought tolerance (Turner *et al.*, 2001).

The significant chi-square estimates for RLD, RDW, SDW, R/S and 100-seed weight indicated that additive-dominance model was insufficient to explain genetic variation among these traits. This indicated the presence of epistatic interactions. Six parameter model was used according to Haymam (1958). Additive gene effects [a] were significant for TRL and RLD. Additive gene effects are fixable and selection for such trait is effective. The negative sign associated with [a] showed that the sum of contributions by dispersed pair of genes were more than by associated pairs of genes. A large root length density (RLD) was reported as a crucial trait for drought tolerance adaptation indicating the capability for soil water exploitation (Kashiwagi *et al.*, 2008a). Additive gene effects were also significant for RDW, SDW and R/S ratio. Non- additive dominant gene effects were not significant for TRL and RLD. However, this was significantly negative for RDW and R/S. This is an indication that the predominance was towards the reducer trait. According to Khodambashi *et al.* (2012) negative dominance indicated reductive alleles involving dominant phenotype.

There were also non-additive gene interactions observed in some traits. There were significant negative [aa] interactions for RDW and R/S ratio. Mather and Jinks (1982) proposed the association or dispersion of genes in the parents based on signs associated with epistasis gene effects such as additive x additive and additive x dominance. A negative sign for any of these parameters is an indication of interaction between increasing and decreasing alleles, hence presence of dispersion in the parental genotypes. Therefore, negative and significant values of [aa] in this study showed the presence of alleles dispersion in the parents for RDW and R/S ratio. There were also significant observations of both [a] and [aa] interactions for RDW and R/S ratio. Findings conducted between two crosses (ICC 283 x ICC 8261 and ICC 4958 x ICC 1882) showed similar results for RDW in which [a] and [aa] gene effects were significant (Kashiwagi *et al.*, 2008a). In such cases, selection should not be done at an early generation to take advantage of additive genes effects. In addition, breeding

methods that result in or maintain high variation among plants/lines such as recurrent selection and single seed descent (SSD) should be used. Results by Kashiwagi *et al.* (2008a) showed that [a], [d] and [aa] were significant for SDW and the difference could be due to different genotypes used and environmental conditions. This large shoot biomass was reported to correlate well with high yields (Serraj *et al.*, 2004). Further, RDW and R/S showed duplicate gene effects as indicated by their opposite signs of dominance [d] and dominance x dominance [dd] gene effects, indicating the role of duplicate gene action. However, it was reported that [ad] and [dd] interactions were not advantageous in development of inbreds since they were non fixable by selection (Khodambashi *et al.*, 2012).

Mean for seed weight of F_1 (18.553 g) was lower than the mean for P_1 (19.485 g) which is also an indication of negative heterosis as was the case for roots. Similar results were obtained in chickpea for 100 - seed weight in two crosses (C-235 x Bittal-98 and C-235 x Dasht) (Bakhsh *et al.*, 2007). Also observations made in cross between Desi (ICC3996 – small seed) x Kabuli (S95362-large seed) showed that mean seed weight of F_1 were close to the small maternal (P_1) than the larger parental type for each cross (ICC3996 or Howzat) and reported this to the effect of the maternal parent that prevented full expression of the large size (Hossain *et al.*, 2010). Simillarly this was also reported in lentils between L-3685 and Lc74-1-5-1 for similar traits (Khodambashi *et al.*, 2012). However, results showed that backcrossing F_1 to P_1 lead to a higher mean (19.619 g) compared to the two generations. This is an indication that subsequent backcrosses to the P_1 (recurrent parent) restores the expression of seed weight.

The seed size represented by 100-seed weight showed that additive and additive x additive gene effects were significant. Similar results were obtained by Kumhar *et al.* (2013) under irrigated and stress environment. This is an indication that this trait is heritable and that selection method should maximize additive x additive epistasis effect by giving time before

applying selection. In addition, large population should be maintained to allow recombination to take place. Additive gene effects were obtained in all three crosses, ICC 5002 x ICC 17109 and ICC 7672 x ICC 11255 and ICC 17109 x ICC 11255, and additive x additive effects for Desi x Kabuli, ICC 5002 x ICC 17109 and ICC 7672 x ICC 11255 (Sharma *et al.*, 2013). Samineni *et al.* (2011) also reported significant additive x additive and additive x dominance gene effects under saline conditions. Significant negative dominant gene effects were shown to control 100-seed weight which is an indication that small seed is dominant over large seed. Hossain *et al.* (2010) reported that a mean closer to small maternal parent was an indication of complimentary interactions, where the gene controlling small size from the maternal parent prevents expression of the large size from the donor parent. Both dominant and additive effects were shown to control 100-seed weight (Farshadfar *et al.*, 2008). This was similar to reports by Kumhar *et al.* (2013) where dominance was not significant but negative in cross between ICC 17109 x ICC 11255, while the others were positively significant. This difference could be due to the different genotypes used and the allelic interactions that are involved. Report in cowpea indicated that seed size was controlled by dominant genes that were also negative (Edbadzor *et al.*, 2013). The 100-seed weight is an important trait as an indicator of yield. It was proposed as an indirect method of selection for high yield based on its correlation with yield (Khodadadi, 2013). Breeding for improved seed weight is a good measure of increase in yield.

Additive variances were greater for both root traits and 100-seed weight than the non-additive variance. This is an indication that individual selection and pedigree method will be useful for breeding of these traits. However, the negative variances of root traits indicate the influence of the lesser parent in the inheritance of these traits. Negative additive and dominance variance in some traits under control and saline environments was associated with the salt sensitive parent in chickpea (Samineni *et al.*, 2011a). Broad-sense heritability for all

the root traits was above 0.5 while narrow-sense heritability was even higher (more than one) in TRL, RDW, PDW and R/S ratio. Heritability is determined based on additive and environmental variance. High magnitudes of dominance and additive variances in addition to presence of epistasis probably caused the narrow-sense heritability to be more than one. Further, it was stated that exaggerated heritability could be due to epistasis and environmental influence (Coates and White, 1998). Similar higher heritability that ranged from 108 to 164% were reported in chickpea under saline and control environments (Samineni *et al.*, 2011a) and between 108% and 129% in rice (Kiani *et al.*, 2013). Heritability estimate for SDW was high while narrow-sense heritability for seed weight was low. However, there was high environmental influence on seed weight. 100-seed weight was calculated from seed yield per plant. Seed yield has been reported to be highly influenced by environmental variations (Kunkaew *et al.*, 2010).

Number of effective gene factors was low for root traits. In chickpea scanty information on number of genes controlling root traits has been reported based on field evaluation and calculations. With the use of markers one major QTL that explained 33% of the phenotypic variation was detected for root length and root biomass (Chandra *et al.*, 2004). Recently nine QTL clusters containing QTL for several drought tolerance traits have been identified (Varshney *et al.*, 2014b) and this is expected to improve breeding for root drought tolerance and speed up selection for complex quantitative traits related to drought tolerance. The number of genes obtained for 100-seed weight was five confirming results obtained by Sharma *et al.*, (2013). It was earlier reported that seed size in chickpea was determined by two genes (Upadhyaya *et al.*, 2006). The use of markers indicated five QTL found at different linkage groups (Cho *et al.*, 2002; Cobos *et al.*, 2007; Radhika *et al.*, 2007; Cobos *et al.*, 2009; Hossain *et al.*, 2010).

4.5 Conclusions

Roots trait(s) and yield components in chickpea are critical in adaptation to drought conditions. Root traits from the cross between ICCV 00108 x ICC 8261 were controlled mainly by additive [a] and additive x additive gene effects. However, non-additive; dominance [d] and epistatic (additive x dominance and dominance x dominance) gene effects were also present for RDW, SDW and R/S ratio. It was also found that additive, dominance and additive x additive gene effects determine the size of chickpea seed (100-seed weight). Additive component of variances were higher compared to dominance variances in all studied traits. Broad-sense heritability was high for root traits and seed weight while narrow-sense heritability was even higher for root traits except for SDW. However, the narrow-sense heritability for 100-seed weight for ICCV 00108 x ICC 4958 cross was low. Few genes were detected for root traits while five genes were found to control the 100-seed weight. Breeding for drought tolerance root traits and 100-seed weight is complex due to gene interactions. It is therefore, recommended that selection should be delayed to later generations preferably from F_4 generations. The choice of breeding method should be able to maintain heterozygous state to allow time for recombination. Methods such as recurrent selection and single seed descent could be used to improve these traits.

CHAPTER FIVE

5.0 Introgression of drought tolerance traits into adapted Kenyan chickpea varieties using marker assisted backcrossing (MABC)

5.1 Introduction

Drought has been the most important factor for yield instability in major chickpea production countries causing yield fluctuations (Tar'an *et al.*, 2013). Varshney *et al.*, (2009) reported that drought causes yield losses of approximately 3.7 million tons amounting to 40 - 50% crop losses in chickpea. Terminal drought has been reported as the major abiotic constraint in chickpea production (Kashiwagi *et al.*, 2005). It was reported to cause seed yield reduction between 58 – 95% (Leport *et al.*, 2006). Drought also causes impaired pollen viability and stigma functioning, reduced flowers and pods and their abortions and reduced secondary branches (Fang *et al.*, 2010). Significant decrease in shoot biomass and number of pods was also reported (Onyari *et al.*, 2010).

Two major strategies of managing drought are developing early maturing and drought tolerant varieties (Gaur *et al.*, 2008). Breeding for early maturing varieties that escape drought has been reported (Kumar and Rao, 2001; Anbessa *et al.*, 2007; Upadhyaya *et al.*, 2007; Gowda *et al.*, 2011). Although reports indicated that there was a positive association between long duration growth and yield potential (Caliskan *et al.*, 2008), early maturing varieties were shown to be better adapted under stress conditions (Grzesiak *et al.*, 1996). Root traits have been considered as the most important attributes that enables the plant to mine water efficiently from deep soil layers under drought (Vadez *et al.*, 2008). They play a role of dehydration avoidance as deep and prolific root systems are able to extract moisture from deeper layers even when the upper layer becomes dry (Serraj *et al.*, 2004; Kashiwagi *et*

al., 2005; Rehman, 2009). Root length density (RLD) and maximum root depth (RDp) were found to positively influence the seed yield under terminal drought environments (Ali *et al.*, 2005; Gaur *et al.*, 2008). Two lines ICC 8261 and ICC 4958 were identified to have largest RLD and most prolific and deep root systems (Kashiwagi *et al.*, 2005), and have been used in identification of root QTL conferring resistance to drought on linkage group 4 (CaLG04) (Varshney *et al.*, 2013a). The transfer of this QTL region from donor parents into widely adapted and commercial varieties is still low in chickpea.

Given that drought is a complex trait controlled by polygenes, application of modern breeding technologies such as use of molecular markers will lead to crop improvement and shorten breeding cycle. Several strategies such as marker assisted selection (MAS), marker assisted backcrossing (MABC), marker assisted recurrent selection (MARS) and genome-wide selection (GWS) may be used (Ribaut *et al.*, 2010). The MAS and MABC are the most utilized. Marker assisted backcrossing involves transfer of a target allele from a donor variety to a popular cultivar by repetitive backcrossing with the help of markers (Nayak *et al.*, 2010) and selection against donor introgressions across the rest of the genome (Tar'an *et al.*, 2013). The application of MABC has been successful in several crops such as introgression of drought tolerant QTL in pearl millet (Serraj *et al.*, 2005), stay green QTL in sorghum (Ngugi *et al.*, 2010) and transfer of four QTL for resistance to *Phytophthora capsici* into yellow wonder pepper variety (Thabius *et al.*, 2004). In chickpea, examples involving introgression of drought tolerance traits into adapted varieties are still few. The only reported cases are transferring of root trait from donor parent ICC 4958 into JG 11, an adapted Indian variety in ICRISAT, India (Varshney *et al.*, 2013a) and into an adapted Kenyan variety *Chania Desi I* (ICCV 97105) (Oyier, 2012). Chickpea adoption is gaining popularity in dry highlands as a relay crop planted during the short rain season. It is also expanding into the drylands of Eastern Kenya which usually receives unreliable and unpredicted rainfall that

exposes the chickpea commercial lines to terminal drought. It is for this reason that the study was carried out with the following objectives to:

a) identify polymorphic SSR and SNP markers among a set of chickpea genotypes,
b) introgress drought tolerance root traits into adapted Kenyan varieties using marker assisted backcrossing (MABC),
c) evaluate BC_2F_2 lines for yield components under drought stress environments, and
d) evaluate BC_2F_3 families for drought tolerant root trait(s).

5.2 Materials and methods

5.2.1 Selection of parents and markers

A total of 33 chickpea genotypes from various origins (India, Spain, Ethiopia and Kenya) were used in this study (Table 5.1). Eight simple sequence repeat (SSR) markers, linked to quantitative trait loci (QTL) for root and yield traits, were used to screen nine parents. This was done at ICRISAT, India. In addition, 30 parents (including six of the nine mentioned above) were genotyped with 1144 single nucleotide polymorphic (SNP) markers (Table 5.1). The genotyping services with SNP markers were outsourced from the Legume Genomics Centre (LGC), formerly KBioscience, United Kingdom. Leaf samples were harvested at 14 days after emergence and sent to LGC, UK. Selection of polymorphic SNP markers was done using Genotypic Data Management Systems (GDMS) version 2.0.7 (ICRISAT, 2014) which is in-built in Integrated Breeding Management System (IBMS) (Murray *et al.*, 2014). Three genotypes were screened with SSR markers only.

5.2.2 Development of progenies and their selections

Two recurrent parents were chosen based on polymorphic markers and also their adaptation to Kenya, while the donor parent is a recombinant inbred line (RIL) that was used

in drought tolerant QTL mapping as reported by Gaur *et al.* (2008) and Varshney *et al.* (2013a). The two recurrent parents *Chania Desi II* (ICCV 92944) and *LTD 068* (ICCV 00108) were each crossed to donor parent ICC 4958 to generate F_{1s}. Then the F_{1s} were subsequently crossed back to the recurrent parents to obtain backcross progenies. The hybridity in F_{1s} were checked with SSR markers (TAA170, GA24 and ICCM0249) linked to root QTL region. The three were markers used for foregroung selection to ensure the presence of QTL region. This was done using the GeneMapper software (Applied Biosystems, 2005 USA) by determining the presence of alleles from both parents (heterozygous plants). True F_{1s} were selected for the first generation of backcrossing with the recurrent parents as females, which was maintained throughout the backcrossing. The backcross progenies at BC_1F_1 were tested for heterozygosity using nine markers (TAA170, ICCM0249, NCPGR127, NCPGR21, CaM1903, TA130, TA11, TA113 and TA118). Five markers (ICCM0249, CaM0204, NCPGR21, TA113, and TA118) were used to screen BC_2F_1 for *Chania Desi II* x ICC 4958. Similarly, five other markers (NCPGR21, NCPGR127, TA11, TA113, and TA118) were used for screening BC_2F_1 for *LDT 068* x ICC 4958. The selected BC_2F_1 plants based on foreground SSR and background SNP markers (80 % and above recovery of recurrent parent) were selfed and the resulting BC_2F_2 families evaluated for yield traits. The percentage recovery of recurrent parent for background SNP markers was achieved using GDMS software program (ICRISAT, 2014). The seeds (BC_2F_3) were planted in pots under a rain-out shelter and evaluated for root traits.

Table 5.1: List of markers used in identifying polymorphism among the parental genotypes

S. No.	Genotype	Origin	Marker used
1	K031	India	SNP
2	*Chania Desi I* (ICCV 97105)	Kenya	SNP
3	D064	India	SNP
4	*LDT 068* (ICCV 00108)	Kenya	SNP/SSR
5	D013	India	SNP
6	K034	India	SNP
7	ICCV 10	India	SNP
8	Ngara local	Kenya	SNP
9	Acos Bubie RII	Ethiopia	SNP
10	D021	India	SNP
11	ICC 4958	India	SNP/SSR
12	C*hania Desi II* (ICCV 92944)	India	SNP/SSR
13	ICCV 92318	India	SNP/SSR
14	Natoli RI	Ethiopia	SNP
15	K038	India	SNP
16	Ejeri RII	Ethiopia	SNP
17	ICCV 08309	India	SNP
18	Arerti RI	Ethiopia	SNP

Table 5.1: Continued…. List of markers used in identifying polymorphism among the parental genotypes

S. No.	Genotype	Origin	Marker used
19	D012	India	SNP
20	ICCV 95423	India	SNP
21	*LDT 065* (ICCV 00305)	Kenya	SNP/SSR
22	ICC 8261	India	SNP/SSR
23	P-2245	Spain	SNP
24	RIL 33	Spain	SNP
25	Blancho Lechoso	Spain	SNP
26	Zoco	Spain	SNP
27	CRIL 1 -36	Spain	SNP
28	Cavir	Spain	SNP
29	CRIL -1 -94	Spain	SNP
30	WR 315	Spain	SNP
31	ICCV 93952	India	SSR
32	JG16	India	SSR
33	JG130	India	SSR

5.2.3 Genotyping with SSR markers

DNA extraction was done using Nucleospin® 96 plant II core kit (Ref: 740468.4). DNA was extracted from fresh leaves of parental genotypes and F_1s. DNA for backcross progenies (BC_1F_1 and BC_2F_1) on the other hand were extracted from dried leaf samples harvested at 14 days after emergence and oven dried at 37 ºC for three days. DNA quality and quantity was checked on 0.8% agarose gel dissolved in 10x TBE (Tris Boric EDTA) buffer. The DNA contents prepared contained, 1µl of DNA, 3 µl of sterilized water and 2 µl of orange dye, and it was checked against 20 ng of lambda DNA (1µl). This was run in gel electrophoresis (Owl D2 Wide – Thermo Scientific) at 100V for 1 hour. The gel was visualized on a trans-illuminator (Syngene gel documentation system).

The PCR was performed in 10 µl reaction volume. The PCR master mix contained 2 µl of 20 ng DNA, 1.0 µl of 10 x TBE buffer, 0.4 µl of 50 mM $MgCl_2$, 1.0 µl of 2 mM of dNTPs, 1.0 µl each of 2 pmol forward and reverse primers, 0.06 µl of Taq DNA polymerase (Fermentas) 50 µg , and 4.56 µl of sterile water. The SSR fragments were amplified in 384-well PCR machine (GeneAmp® PCR System 9700) using a touchdown program. The PCR programme consisted of initial denaturation at 94 ºC for 5 minutes, followed by the first 10 cycles consisting of denaturation at 94 ºC for 15 seconds, primer annealing at 60 ºC decreasing by 0.5 ºC for 30 seconds and primer extension at 72 ºC for 30 seconds followed by 40 cycles of the same denaturation, primer extension and primer annealing with a final extension step performed at 72 ºC for 20 minutes. The quality of PCR product using 2 µl of amplified DNA and 3x loading dye were mixed and checked on 1.2% agarose gel against 100 base pairs (bp) lambda DNA of 50 ng/µl and 100 ng/µl. The gel was run on 10x TBE buffer at a constant voltage of 100V for 30 minutes. The amplified PCR product was prepared for Applied Biosystems (ABI) electrophoresis. The ABI mixture contained 20 µl genescan 500

Liz, 800 µl Hi-Di formamide and 400 µl of water where 10 µl of this mixture was added to 2 µl of amplified PCR product and dispensed in 96-well plate. This was then separated by capillary electrophoresis using ABI Prism 3730 DNA Sequencer and analyzed using GeneMapper® software (Applied Biosystems, 2005 USA) to identify the segregating plants at every F_1 stage (F_1, BC_1F_1 and BC_2F_1).

5.2.4 Genotyping with SNP markers

The F_1, backcross progenies and parents were planted in rain-out shelter at Egerton University. The leaves were harvested at 14 days after emergence and oven dried at 37 ºC for three days. They were then placed in tubes and shipped to LGC genomics. The principles and procedure of DNA assay was performed according to Karspar protocol available at http://www.kbioscience.co.uk/reagents/KASP. The genotyping results from LGC were were used to determine polymorphic markers among the parents and these markers were used for foreground selection of the progeniesin order to select those with high percentage (80% and above) recovery of the recurrent parents using GDMS software program (ICRISAT, 2014).

5.3 Evaluation of BC_2F_2 population for yield traits and BC_2F_3 families for root traits

5.3.1 Field evaluation of BC_2F_2 population for yield traits under rainfed condition

5.3.1.1 Experimental sites

The selected BC_2F_1 plants were selfed to obtain BC_2F_2 families. These were planted at Kenya Agricultural Livestock and Research Organization (KALRO) - Perkerra, in Marigat, under rainfed conditions. KALRO - Perkerra is located in Baringo County in the Rift valley province. Perkerra centre lies at 0.5º N and 36º E in Lower midland 5 agro-ecological zone (LM5) approximately 1067 m asl. The area receives a bimodal mean annual rainfall of 650 mm with the first rainy season between April and June; and second season between

November and early December. The area has mean annual maximum and minimum temperature of 32.4°C and 24.6°C, respectively. The mean annual temperature is 25 ºC with the hottest season (37.7 ºC) occurring between January and April. Soils are volcanic fluvisols of sandy/silty clay loam texture, slightly acidic to slightly alkaline, highly fertile with adequate P, K, Ca, Mg but low in N and carbon (Jaetzold and Schmidt, 1983).

5.3.1.2 Experimental layout

The experimental design used was RCBD with two replications at a spacing distance of 40 cm between rows and 10 cm within rows in 1.5 m length. Numbers of rows differred between 2 to 10 rows depending on number of seeds planted. The experiment was conducted under rainfed conditions except at the start of flowering, when one slot of furrow irrigation was applied up to 70% field capacity.

5.3.1.3 Data collected

Data was collected from 30 plants selected randomly per family on the number of seeds per plant and seed weight per plant (g) that was obtained by measuring the total seeds per plant using a weighing balance (Model: TANITA TLD-610). 100 - seed weight (g) was determined as using the formulae:

100 - seed weight = (weight of all seeds / number of seeds) x 100

5.3.1.4 Data analysis

The means from each of the families were used for carrying out analysis of variance (ANOVA) using PROC GLM in SAS 9.3. Treatment means between two genotypes were compared using the least significant differences (LSD) at $P < 0.05$ test. The model used was:

$Y_{ij} = \mu + t_i + r_j + e_{ij}$

Where: Y_{ij} = Observation of treatments; μ = Overall mean; t_i = i^{th} mean family effect; r_j = j^{th} replication; e_{ij} = error term.

Correlations estimates were computed using Pearson's correlation of SAS 9.3.

Broad-sense heritability estimates were obtained using the following formula:

$$h^2_b = \sigma_g^2 / \sigma_p^2$$

Where:

$\sigma_g^2 = (MS\ families - MS\ error)/r$ =genotype variance

$\sigma_p^2 = \sigma_g^2 + MS\ error/r$ = phenotype variance

MS families = Family mean squares

MS error = Error mean squares

5.3.2 Root evaluation of BC_2F_3 families

5.3.2.1 Experimental layout

The root evaluation of BC_2F_3 families and their recurrent parents *Chania Desi II* (ICCV 92944) and *LDT 068* (ICCV 00108) with the donor parent ICC 4958 was carried out under a rain-out shelter at Egerton University, Njoro, Kenya. Similar procedure as explained under materials and methods in chapter four, section 4.2.2 was adopted. The design used was RCBD with two replications.

5.3.2.2 Data collected

The fresh roots were scanned using image analysis software (WinRhizo Regent Instrument Canada INC., Quebec, Canada) for total root length. The data collected were:

a) Total root length (TRL) (cm) – This was obtained from the WinRhizo analysis results
b) Root length density (RLD) (cm cm^{-3}) – This was calculated as ratio of total root length to volume of the pot

c) Shoot dry weight (SDW) (g) – Shoots separated from roots were oven dried at 80 ºC for 72 hours and their weights recorded. The SDW was used as an indicator of plant growth vigour

d) Root dry weight (RDW) (g) – scanned roots were oven dried at 80 ºC for 72 hours and their weights recorded.

e) Root to shoot ratio (R/S) – this was calculated as the ratio of root dry weight to shoot dry weight.

f) Length to root dry weight ratio (LWR) (cmg^{-1}) – this was calculated as total root length/root dry weight

5.3.2.3 Data analysis

Analysis was done using PROG GLM with SAS 9.3. Similar model and analysis as shown in section 5.3.1.4 was adopted. Means between two families were separated by the least significant difference test (LSD) at $P < 0.05$.

5.4 Results

5.4.1 Selection of parents and polymorphic markers for the populations

The recurrent parents ICCV 92944, released as *Chania Desi II* and ICCV 00108, released as *LDT 068*, with the donor parent, ICC 4958 were selected for improvement for drought tolerance. The two recurrent parents have wide adaptation in Kenya but affected by terminal drought since they are mostly planted during the short duration rains. From the eight SSR markers (CaM1903, ICCM0249, GA24, NCPGR127, NCPGR21, STMS11, TA130 and TA170), four markers (CaM1903, ICCM0249, NCPGR127 and NCPGR21) were also polymorphic for *LDT 068* x ICC 4958 population and two markers (NCPGR127 and NCPGR21) also polymorphic between *Chania Desi II* x ICC 4958. Markers that failed to

amplify parental DNA were not used when screening progenies for selection of segregating plants.

During the screening of BC_1F_1, additional SSR markers identified to be linked to QTL associated with root drought tolerance traits were added making a total of nine markers (CaM204, ICCM0249, NCPGR127, NCPGR21, CaM1903, TA130, TA11, TA113 and TA118). Five markers (ICCM0249, CaM0204, NCPGR21, TA113, and TA118) were polymorphic for *Chania Desi II* x ICC 4958 crosses. Similarly five other markers (NCPGR21, NCPGR127, TA11, TA113, and TA118) were polymorphic for *LDT 068* x ICC 4958. These markers were used to screen BC_2F_1 and results obtained showed that three markers (ICCM0249, CaM204, NCPGR127) were polymorphic for *Chania Desi II* x ICC 4958 and four markers (NCPGR21, NCPGR127, TA11 and ICCM0249) were also polymorphic for *LDT 068* x ICC 4958. Two of the markers (ICCM0249 and NCPGR127) were common in the two crosses. The SNP markers screened also showed low polymorphism among the 30 parents in which 18 and 14 markers were polymorphic between *Chania Desi II* (ICCV 92944) x ICC 4958 and *LDT 068* (ICCV 00108) x ICC 4958, respectively (Table 5.2).

Table 5.2: List of polymorphic SNP markers for *Chania Desi II* x ICC 4958 and *LDT 068* x ICC 4958

S. NO.	***Chania Desi II* x ICC 4958**	S. NO.	***LDT 068* x ICC 4958**
1	CKAM0005	1	CKAM0005
2	CKAM0042	2	CKAM0020
3	CKAM0343	3	CKAM0042
4	CKAM0411	4	CKAM0662
5	CKAM0804	5	CKAM0833
6	CKAM0833	6	CKAM1256
7	CKAM1175	7	CKAM1387
8	CKAM1256	8	CKAM1431
9	CKAM1387	9	CKAM1443
10	CKAM1443	10	CKAM1548
11	CKAM1548	11	CKAM1850
12	CKAM1797	12	CKAM1933
13	CKAM1850	13	CKAM1963
14	CKAM1886	14	CKAM1971
15	CKAM1894		
16	CKAM1933		
17	CKAM1963		
18	CKAM1971		

CKAM: Chickpea Kaspar Microsatellites.

5.4.2 Development of progenies and selection of heterozygous plants

The population was developed to backcross two (BC_2F_1) and advanced by selfing to BC_2F_3. Segregating plants were selected from F_1 lines and backcross F_1 populations (Table 5.3). Based on foreground markers the numbers of plants selected were: 7, 8 and 7 for F_1, BC_1F_1 and BC_2F_1, respectively for *Chania Desi II* x ICC 4958. For *LDT 068* x ICC 4958 there were 8, 17 and 19 plants for F_1, BC_1F_1 and BC_2F_1 respectively (Table 5.4). In addition, the BC_2F_1 segregating plants with 80% and above recovery of the recurrent parent were determined GDMS software (ICRISAT, 2014) and the selected plants were selfed, where 6 plants were selected for *Chania Desi II* x ICC 4958 and 16 plants for *LDT 068* x ICC 4958. Although seven F_1 plants were obtained for *Chania Desi II* x ICC 4958, four plants died before maturity with some failing to germinate possibly due to seed sterility or partial seed fertility and/or environmental effects. The naming convention adapted represents the plant number that was selected, where P followed by the first numeral represented the female plant number and subsequent numerals represented the plant number that was selected after each cycle of crossing.

Table 5.3: Allele size for F_1 and backcross progenies for selecting heterozygous plants generated using GeneMapper from ABI product

		F_1 population		
Cross	**Plant number**	**Marker Type**	**Allele size**	
			Allele 1	**Allele 2**
Chania Desi II x ICC 4958	EUC-03-F_1-P6-1	ICCM 0249	168	192
	EUC-03- F_1-P6-2	ICCM 0249	168	192
	EUC-03- F_1-P11-2	ICCM 0249	168	192
	EUC-03- F_1-P12-1	ICCM 0249	168	192
	EUC-03- F_1-P18-1	ICCM 0249	168	192
	EUC-03_1-P22-1	ICCM 0249	168	192
	EUC-03_1-P28-1	ICCM 0249	168	192
LDT 068 x ICC 4958	EUC-04- F_1-P6-1	ICCM 0249	192	208
	EUC-04- F_1-P6-2	ICCM 0249	192	208
	EUC-04- F_1-P27-1	ICCM 0249	192	208
	EUC-04- F_1-P39-1	ICCM 0249	192	208
	EUC-04- F_1-P40-2	ICCM 0249	192	208
	EUC-04- F_1-P52-1	ICCM 0249	192	208
	EUC-04- F_1-P52-2	ICCM 0249	192	208
	EUC-04- F_1-P53-2	ICCM 0249	192	208

Table 5.3: Continued… Allele size for F_1 and backcross progenies used in selecting heterozygous plants generated using GeneMapper from ABI product

BC_1F_1 population				
	Plant number	**Marker**	**Allele 1**	**Allele 2**
Chania Desi II x ICC 4958	EUC-03- BC_1F_1-P6-1-1	ICCM0249	185	209
	EUC-03- BC_1F_1-P6-1-2	TA118	182	194
	EUC-03- BC_1F_1-P6-1-3	TA113	201	210
	EUC-03- BC_1F_1-P6-1-3	ICCM0249	185	209
	EUC-03- BC_1F_1-P6-2-1	TA113	201	210
	EUC-03- BC_1F_1-P6-2-1	ICCM0249	185	209
	EUC-03- BC_1F_1-P6-2-2	TA113	201	210
	EUC-03- BC_1F_1-P6-2-2	TA118	182	194
	EUC-03- BC_1F_1-P6-2-2	ICCM0249	185	209
	EUC-03- BC_1F_1-P6-2-3	TA113	201	210
	EUC-03- BC_1F_1-P6-2-3	TA118	182	194
	EUC-03- BC_1F_1-P6-2-3	ICCM0249	185	209
	EUC-03- BC_1F_1-P6-2-4	ICCM0249	185	209
	EUC-03-BC_1F_1-P22-1-2	TA118	182	194
	EUC-03- BC_1F_1-P22-1-2	CaM204	285	299
	EUC-03- BC_1F_1-P22-1-2	ICCM0249	185	209
LDT 068 x ICC 4958	EUC-04- BC_1F_1-P27-1-3	NCPGR127	215	217
	EUC-04- BC_1F_1-P39-1-1	NCPGR127	215	217

Table 5.3: Continued… Allele size for F_1 and backcross progenies used in selecting heterozygous plants generated using GeneMapper from ABI product

	Plant number	**Marker**	**Allele 1**	**Allele 2**
LDT 068 x ICC 4958	EUC-04- BC_1F_1-P40-1-4	TA118	185	194
	EUC-04- BC_1F_1-P40-1-5	TA118	185	194
	EUC-04- BC_1F_1-P52-1-1	TA118	188	194
	EUC-04- BC_1F_1-P52-1-2	TA118	188	194
	EUC-04- BC_1F_1-P52-1-3	NCPGR21	134	150
	EUC-04- BC_1F_1-P52-1-3	TA11	227	233
	EUC-04- BC_1F_1-P52-1-3	TA118	188	194
	EUC-04- BC_1F_1-P52-1-4	NCPGR21	134	150
	EUC-04- BC_1F_1-P52-1-4	TA118	188	194
	EUC-04- BC_1F_1-P52-2-2	TA11	227	233
	EUC-04- BC_1F_1-P53-1-1	TA118	185	194
	EUC-04- BC_1F_1-P53-1-2	TA118	185	194
	EUC-04- BC_1F_1-P53-2-2	TA118	185	194
	EUC-04- BC_1F_1-P6-2-2	NCPGR21	134	150
	EUC-04- BC_1F_1-P6-2-2	TA118	188	194
	EUC-04- BC_1F_1-P6-2-3	NCPGR21	134	150
	EUC-04- BC_1F_1-P6-2-3	TA11	227	233

Table 5.3: Continued… Allele size for F_1 and backcross progenies used in selecting heterozygous plants generated using GeneMapper from ABI product

Cross	Plant number	Marker	Allele 1	Allele 2
LDT 068 x ICC 4958	EUC-04- BC_1F_1-P6-2-5	NCPGR21	134	150
	EUC-04- BC_1F_1-P6-2-5	TA11	227	233
	EUC-04- BC_1F_1-P6-2-5	TA118	188	194
	EUC-04- BC_1F_1-P7-1-4	TA118	185	194
		BC_2F_1 population		
Chania Desi II x ICC 4958	EUC-03-BC_2F_1-P6-2-2-2	ICCM0249	185	208
	EUC-03- BC_2F_1-P6-1-3-9	ICCM0249	185	208
	EUC-03- BC_2F_1-P22-1-2-7	ICCM0249	185	208
	EUC-03- BC_2F_1-P22-1-2-1	ICCM0249	185	208
	EUC-03- BC_2F_1-P22-1-2-3	ICCM0249	185	208
	EUC-03- BC_2F_1-P6-2-1-5	ICCM0249	185	208
	EUC-03- BC_2F_1-P6-1-3-3	ICCM0249	284	299
	EUC-03- BC_2F_1-P22-1-2-7	NCPGR127	215	219
LDT 068 x ICC 4958	EUC-04- BC_2F_1-P52-1-3-3	TA11	228	234
	EUC-04- BC_2F_1-P52-1-3-6	TA11	228	234
	EUC-04- BC_2F_1-P6-2-3-3	TA11	228	234
	EUC-04- BC_2F_1-P6-2-5-1	TA11	228	234
	EUC-04- BC_2F_1-P52-1-1-2	ICCM0249	185	208
	EUC-04- BC_2F_1-P52-1-1-3	ICCM0249	185	208
	EUC-04- BC_2F_1-P52-1-2-1	ICCM0249	185	208

Table 5.3: Continued… Allele size for F_1 and backcross progenies used in selecting heterozygous plants generated using GeneMapper from ABI product

	Plant number	**Marker**	**Allele 1**	**Allele 2**
LTD 068 x ICC 4958	EUC-04- BC_2F_1-P27-1-3-3	NCPGR21	134	150
	EUC-04- BC_2F_1-P27-1-3-4	NCPGR21	134	150
	EUC-04- BC_2F_1-P27-1-3-7	NCPGR21	134	150
	EUC-04- BC_2F_1-P39-1-1-4	NCPGR21	134	150
	EUC-04- BC_2F_1-P52-1-3-5	NCPGR21	134	150
	EUC-04- BC_2F_1-P52-1-4-1	NCPGR21	134	150
	EUC-04- BC_2F_1-P52-1-4-4	NCPGR21	134	150
	EUC-04- BC_2F_1-P52-1-4-5	NCPGR21	134	150
	EUC-04- BC_2F_1-P52-1-4-7	NCPGR21	134	150
	EUC-04- BC_2F_1-P53-2-2-1	NCPGR21	134	150
	EUC-04- BC_2F_1-P53-2-2-2	NCPGR21	134	150
	EUC-04- BC_2F_1-P52-1-3-1	NCPGR127	215	217

Table 5.4: Summary of the F_1s and backcross progenies selected by foreground SSR and background SNP markers

Cross	**F_1**	**BC_1F_1**	***BC_2F_1**
Chania Desi II x ICC 4958	7	8	7
LDT 068 x ICC 4958	8	23	19
TOTAL	15	31	26

*Foreground and background selection was done with SSR and SNP markers, respectively.

5.4.3 Field evaluation of BC_2F_2 under rainfed condition

5.4.3.1 Variability among families for yield traits

The seed weight per plant and 100-seed weight were significantly ($P < 0.05$) different between families for *Chania Desi II* x ICC 4958 but there was no significant difference for number of seeds/plant (Table 5.5). However, *LDT 068* x ICC 4958 families were not significantly different for the three yield traits measured (Table 5.6).

5.4.3.2 Distribution, mean performance and heritability estimates of the families for yield traits

a) Distribution of the families for 100-seed weight

The distribution of parents and families for *Chania Desi II* (CCV 92944) x ICC 4958 is presented in Figure 5.1. ICC 4958 had heavier seeds than *Chania Desi II*. Two families, EUC-03-BC_2F_2-P22-1-2-1 and EUC-03-BC_2F_2-P22-1-2-3 had seeds larger than the donor parent (ICC 4958). The families EUC-03-BC_2F_2-P6-1-2-2 and EUC-03-BC_2F_2-P6-2-1-5 had some plants with heavier seeds than the donor parent. One family, EUC-03-BC_2F_2-P22-1-2-7, had lighter seeds than the recurrent parent. Five families (EUC-03-BC_2F_2-P22-1-2-1, EUC-03-BC_2F_2-P6-2-2-2, EUC-03-BC_2F_2-P6-2-1-5, EUC-03-BC_2F_2-P22-1-2-7 and EUC-03-BC_2F_2-P22-1-2-3) showed wide variation compared to two families (EUC-03-BC_2F_2-P6-1-3-3 and EUC-03-BC_2F_2-P6-1-3-9) and the parents.

Table 5.5: Mean squares for yield traits: mean seed weight, 100-seed weight and number of seeds per plant of BC_2F_2 families for *Chania Desi II* (ICCV 92944) x ICC 4958

Source	df	Seed weight (g)/plant	100 - seed weight (g)	Number of seeds/plant
Replication	1	1.10	1.99	18.28
Families	8	25.08*	20.93**	406.49^{ns}
Residual	8	6.38	1.56	288.21

df= degrees of freedom, ns= non – significant.

Table 5.6: Mean squares for yield traits: mean seed weight, 100 seed weight and number of seeds per plant of BC_2F_2 families for *LDT 068* (ICCV 00108) x ICC 4958

Source	df	Seed weight (g)/plant	100 seed weight (g)	Number of seed/plant
Replication	1	63.73	0.45	786.02
Families	13	28.34 ^{ns}	11.16 ^{ns}	590.27 ^{ns}
Residual	13	18.20	5.10	246.88

Df= degrees of freedom, ns= non – significant.

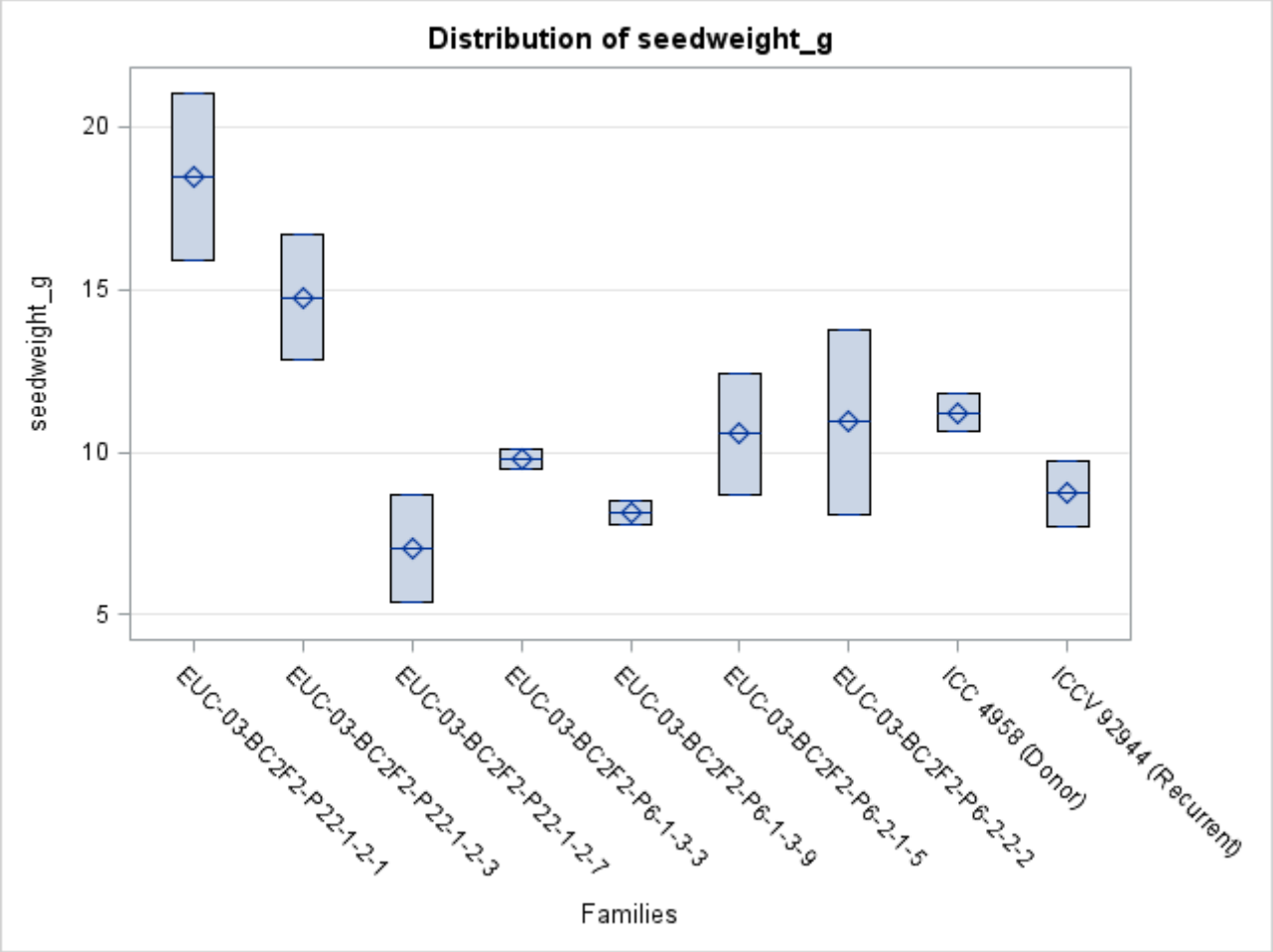


Figure 5.1: Distribution of seed weight (g)/plant of BC_2F_2 families for *Chania Desi II* (ICCV 92944) x ICC 4958.

b) Means of the families and heritabilities for seed yield traits

The family means were significantly different for seed weight (g) per plant and 100 - seed weight for the cross *Chania Desi II* x ICC 4958 (Table 5.7). The mean seed weight per family ranged from 7.04 g/plant to 18.46 g/plant with an overall mean of 11.07 g/plant while 100-seed weight ranged from 16.20 g to 25.56 g with an average of 19.26 g. Five families (EUC-03-BC_2F_2-P22-1-2-1, EUC-03-BC_2F_2-P22-1-2-3, EUC-03-BC_2F_2-P6-2-2-2, EUC-03-BC_2F_2-P6-2-1-5 and EUC-03-BC_2F_2-P6-1-3-3) had between 9.80 - 18.47 g seed weight per plant compared to the recurrent parent (8.74 g). There were no significant differences among the families for *LDT 068* x ICC 4958, however, five families attained between 21 g and 24 g of 100-seed weight compared to recurrent parent (20 g) (Table 5.8).

High heritability was observed for 100-seed weight (0.925) and seed weight per plant (0.746) for *Chania Desi II* x ICC 4958. However, low heritability for number of seeds per plant (0.29) was obtained (Table 5.6). Moderate heritability was obtained for 100-seed weight (0.543) and number of seeds per plant (0.582) but a low heritability was detected for seed weight per plant (0.358) for *LTD 068* x ICC 4958 (Table 5.8).

Table 5.7: Mean yield traits characteristics including seed weight, 100-seed weight and number of seeds per plant of BC_2F_2 families for *Chania Desi II* (ICCV 92944) x ICC 4958

Families	Seed weight (g)/plant	100 - seed weight (g)	Number of seeds/plant
EUC-03-BC_2F_2-P22-1-2-1	18.467	23.270	74.643
EUC-03-BC_2F_2-P22-1-2-3	14.755	17.723	81.489
ICC 4958 (Donor parent)	11.208	25.560	41.194
EUC-03-BC_2F_2-P6-2-2-2	10.924	17.997	51.449
EUC-03-BC_2F_2-P6-2-1-5	10.566	20.759	48.478
EUC-03-BC_2F_2-P6-1-3-3	9.797	17.292	56.659
Chania Desi II (recurrent)	8.744	17.908	46.704
EUC-03-BC_2F_2-P6-1-3-9	8.161	16.670	48.067
EUC-03-BC_2F_2-P22-1-2-7	7.041	16.202	41.756
Mean	**11.073**	**19.264**	**54.493**
LSD (0.05)	**5.824**	**2.879**	**36.149**
Heritability (h^2_b)	**0.746**	**0.925**	**0.290**
P-value	**0.035**	**0.0007**	**0.319**
CV (%)	**22.81**	**6.48**	**31.15**

Table 5.8: Mean yield traits characteristics of seed weight, 100 seed weight and number of seeds per plant of BC_2F_2 families for *LDT 068* (ICCV 00108) x ICC 4958

Families	Seed weight (g)/plant	100 - seed weight (g)	Number of seeds/plant
EUC-04-BC_2F_2-P52-1-3-1	17.13	18.96	70.15
EUC-04-BC_2F_2-P52-1-3-3	15.40	18.37	82.44
EUC-04-BC_2F_2-P52-1-2-5	14.98	22.18	61.04
LDT 068 (Recurrent parent)	14.11	20.00	64.65
EUC-04-BC_2F_2-P52-1-1-3	12.53	21.65	52.69
ICC 4958 (Donor parent)	11.18	25.56	41.35
EUC-04-BC_2F_2-P27-1-3-3	11.11	17.55	64.33
EUC-04-BC_2F_2-P52-1-4-7	8.51	21.65	27.32
EUC-04-BC_2F_2-P52-1-3-6	8.06	21.12	37.00
EUC-04-BC_2F_2-P6-2-5-2	8.00	18.16	43.81
EUC-04-BC_2F_2-P52-1-1-2	7.56	24.24	34.15
EUC-04-BC2F1-P39-1-1-4	7.37	19.91	37.58
EUC-04-BC2F1-P27-1-3-4	5.94	18.15	32.43
EUC-04-BC2F1-P6-2-3-3	5.72	20.50	31.39
Mean	**10.543**	**20.570**	**48.597**
LSD (0.05)	**9.216**	**4.880**	**33.945**
Heritability ((h^2_b))	**0.358**	**0.543**	**0.582**
p-value	**0.217**	**0.086**	**0.064**
CV (%)	**40.465**	**10.981**	**32.332**

5.4.3.3 Correlation estimates for yield traits

There was a strong positive significant correlation ($p < 0.0001$, $r = 0.849$) between seed weight (g)/plant and number of seeds per plant and a moderate but non-significant correlation ($p > 0.05$, $r = 0.448$) with 100-seed weight for *Chania Desi II* x ICC 4958 families (Table 5.9). However, a non-significant negative correlation ($P > 0.05$, $r = -0.017$) was detected between 100-seed weight (g) and number of seeds per plant in this cross. Seed weight (g)/plant was significantly positively correlated ($p < 0.0001$, $r = 0.899$) with number of seeds per plant and a low positive non-significant correlation ($p > 0.05$, $r = 0.031$) between seed weight and 100-seed weight for *LTD 068* x ICC 4958. A negative non-significant correlation ($p > 0.05$, $r = -0.233$) was observed between seed weight (g)/plant and number of seeds/plant (Table 5.10).

Table 5.9: Genetic correlation estimates for yield traits: seed weight, 100-seed weight and number of seeds per plant of BC_2F_2 families for *Chania Desi II* x ICC 4958

	Seed weight (g/plant)	**100 seed weight (g)**	**Number of seeds/plant**
Seed weight (g)/plant	-		
100 seed weight (g)	0.448^{ns}	-	
Number of seeds/plant	0.849***	-0.017^{ns}	-

Table 5.10: Genetic correlation estimates for yield traits: seed weight, 100-seed weight and number of seeds per plant of BC_2F_2 families for *LDT 068* x ICC 4958

	Seed weight (g)/plant	**100-seed weight (g)**	**Number of seeds/ plant**
Seed weight (g)/plant	-		
100-seed weight (g)	0.031^{ns}	-	
Number of seeds/plant	0.899***	-0.233^{ns}	-

5.4.4 Root evaluation of BC_2F_3 families

5.4.4.1 Variability for root traits among families

Root dry weight (RDW), shoot dry weight (SDW), plant dry weight (PDW) and root to shoot ratio (R/S) differed significantly ($p < 0.05$) among families from *Chania Desi II* x ICC 4958 (Table 5.11). Total root length (TRL), root length density (RLD), rooting depth (RDp) and root length to root dry weight ratio (LWR) did not differ significantly. For *LDT 068* (ICCV 00108) x ICC 4958 there were no significant difference among families for the traits measured except R/S ratio (Table 5.12).

Table 5.11: Mean squares for root traits of BC_2F_3 families for *Chania Desi II* (ICCV 92944) x ICC 4958

Source	df	RDp(cm)	TRL(cm)	RLD (cm cm^{-3})	RDW(g)	SDW(g)	PDW(g)	R/S	LWR (cmg^{-1})
Replications	1	19.92	831120.5	0.152	0.00	0.28	0.26	0.04	14379393.6
Families	140	51.57^{ns}	186493.4^{ns}	0.034^{ns}	0.01*	0.06***	0.10**	0.01*	2124007.7^{ns}
Residual	140	40.27	167931.6	0.032	0.01	0.04	0.06	0.01	2133145.7

*, **, *** probability values significant at P < 0.05, 0.01 and 0.001; RDp= rooting depth, TRL= total root length, RLD= root length density, RDW = root dry weight, SDW= shoot dry weight, PDW= total plant dry weight, R/S= root to shoot ratio and LWR= length to root dry weight ratio.

Table 5.12: Mean squares for root traits of BC_2F_3 families for *LDT 068* (ICCV 00108) x ICC 4958

Source	df	RDp (cm)	TRL(cm)	RLD(cm cm-3)	RDW(g)	SDW(g)	PDW(g)	R/S	LWR (cmg-1)
Replication	1	3635.66	5523277.05	0.010	0.09	0.32	0.75	0.00	2.8E+07
Families	219	61.12^{ns}	233574^{ns}	0.043^{ns}	0.01^{ns}	0.05^{ns}	0.08633^{ns}	0.05*	1801024^{ns}
Residual	219	55.78	192948	0.035	0.01	0.04	0.07083	0.035	1471320

*, probability values significant at P < 0.05; RDp= rooting depth, TRL= total root length, RLD= root length density, RDW= root dry weight, SDW= shoot dry weight, PDW= plant dry weight, R/S= root to shoot ratio and LWR= length to root dry weight ratio.

5.4.4.2 Mean performance and heritability estimates of root traits

Families, EUC-03-BC_2F_3-P6-2-2-2-8, EUC-03-BC_2F_3-P22-1-2-7-8 and EUC-03-BC_2F_3-P22-1-2-7-13 from *Chania Desi II* (ICCV 92944) x ICC 4958 had the highest TRL, RLD, and RDW compared to the parents (Table 5.13). The root traits did not differ significantly except R/S ratio for *LDT 068* (ICCV 00108) x ICC 4958 (Table 5.14). However, most families had higher TRL, RLD, RDW, SDW and R/S ratio than their parents. The Families EUC-04-BC_2F_3-P52-1-3-6-2, EUC-04-BC_2F_3-P39-1-1-1-9 and EUC-04-BC_2F_3-P52-2-2-2-15 had between 21.7 - 23.4 m total root length compared to parents *LDT 068* (13.6 m) and ICC 4958 (16.7 m). The overall means for most of these root traits were higher for *Chania Desi II* x ICC 4958 compared to *LDT 068* x ICC 4958 except for LWR. Among the parents, the recurrent parents had the lowest TRL compared to their families. The recurrent parent *LDT 068* was 30% less than donor parent while *Chania Desi II* was 20% less in length.

The families, EUC-03-BC_2F_3-P22-1-2-1, EUC-03-BC_2F_3-P6-2-2-2, EUC-03-BC_2F_3-P6-2-1-5 and EUC-03-BC_2F_3-P6-1-3-3 had both better roots traits and higher seed weight per plant in addition to 100-seed weight compared to the recurrent parent *Chania Desi II*. Similarly, the families, EUC-04-BC_2F_3-P52-1-1-3, EUC-04-BC_2F_3-P52-1-4-7 and EUC-04-BC_2F_3-P52-1-3-6 from *LDT 068* and ICC 4958, had both high root and seed traits. However, there was a lot of variation in this population. Heritability estimates were low for most traits across the two populations (Table 5.13 and 5.14). The highest heritability obtained was in PDW for *Chania Desi II* x ICC 4958 that was 0.40.

Table 5.13: Mean root characteristics of BC_2F_3 families for *Chania Desi II* (ICCV 92944) x ICC 4958

Genotypes	RDp (cm)	TRL (cm)	RLD (cm cm^{-3})	RDW (g)	SDW (g)	PDW (g)	R/S	LWR (cmg^{-1})
EUC-03-BC_2F_3-P6-2-2-2-8	49.00	2386.35	1.02	0.50	1.06	1.56	0.47	4817.08
EUC-03- BC_2F_3-P22-1-2-7-8	41.73	2012.20	0.86	0.42	0.96	1.38	0.44	4705.89
EUC-03- BC_2F_3-P22-1-2-1-13	36.50	1970.44	0.84	0.47	0.80	1.27	0.60	4187.88
EUC-03- BC_2F_3-P22-1-2-7-41	48.73	1966.10	0.84	0.31	1.20	1.51	0.27	6294.64
EUC-03- BC_2F_3-P6-2-2-2-10	39.50	1940.93	0.83	0.39	0.87	1.27	0.46	4926.14
EUC-03- BC_2F_3-P6-2-1-5-1	45.50	1887.90	0.81	0.34	1.12	1.46	0.32	5477.33
EUC-03- BC_2F_3-P6-1-3-9-2	47.50	1856.88	0.80	0.40	0.85	1.25	0.47	4693.76
EUC-03- BC_2F_3-P22-1-2-7-29	45.73	1855.56	0.79	0.36	0.67	1.03	0.52	5117.69
EUC-03- BC_2F_3-P6-2-1-5-27	31.00	1850.98	0.79	0.39	1.19	1.57	0.33	4782.89
EUC-03- BC_2F_3-P6-2-1-5-11	43.00	1843.71	0.79	0.34	0.93	1.27	0.36	5487.23
EUC-03- BC_2F_3-P6-2-1-5-12	39.00	1821.09	0.78	0.44	0.97	1.41	0.45	4431.00
EUC-03- BC_2F_3-P22-1-2-7-13	38.73	1813.77	0.78	0.34	1.00	1.34	0.34	5308.46
Mean	**39.065**	**1406.469**	**0.60**	**0.273**	**0.812**	**1.085**	**0.342**	**5343.649**
LSD (0.05)	**13.195**	**853.065**	**0.365**	**0.167**	**0.393**	**0.514**	**0.165**	**3040.479**
Heritability (h^2_b)	**0.219**	**0.100**	**0.060**	**0.000**	**0.333**	**0.400**	**0.000**	**-**
p-value	**0.065**	**0.256**	**0.263**	**0.025**	**0.000**	**0.001**	**0.022**	**0.501**
CV (%)	**16.244**	**29.136**	**29.140**	**29.376**	**23.233**	**22.736**	**23.163**	**27.332**

Key: RDp= rooting depth, TRL= total root length, RLD= root length density, RDW= root dry weight, SDW= shoot dry weight, PDW= total plant dry weight, R/S= root to shoot ratio and LWR= length to root dry weight ratio.

Table 5.23: Continued… Mean root characteristics of BC_2F_3 families for *Chania Desi II* (ICCV 92944) x ICC 4958

Genotypes	**RDp (cm)**	**TRL(cm)**	**RLD (cm cm^{-3})**	**RDW(g)**	**SDW(g)**	**PDW(g)**	**R/S**	**LWR(cmg^{-1})**
EUC-03- BC_2F_3-P6-1-1-3-12	36.00	1810.28	0.77	0.34	0.78	1.11	0.44	5358.52
EUC-03- BC_2F_3-P6-1-3-9-23	44.00	1810.12	0.77	0.39	1.02	1.41	0.38	4701.47
EUC-03- BC_2F_3-P22-1-2-3-18	33.00	1809.35	0.77	0.26	0.90	1.16	0.29	8751.91
EUC-03- BC_2F_3-P6-2-2-2-14	46.50	1806.45	0.77	0.36	0.82	1.18	0.43	5166.20
EUC-03- BC_2F_3-P6-2-1-5-20	49.00	1799.28	0.77	0.36	1.05	1.41	0.34	5025.92
EUC-03- BC_2F_3-P22-1-2-3-21	37.50	1776.42	0.76	0.34	1.05	1.38	0.31	5401.14
EUC-03- BC_2F_3-P6-2-1-5-4	42.00	1774.78	0.53	0.42	1.21	1.63	0.34	4221.88
EUC-03- BC_2F_3-P6-1-1-3-29	32.00	1756.39	0.72	0.33	1.04	1.36	0.31	5404.26
Chania Desi II (Recurrent)	35.85	1232.00	0.76	0.25	0.68	0.92	0.36	5087.71
ICC 4958 (Donor)	41.32	1685.30	0.75	0.33	1.02	1.34	0.33	5197.37
Mean	**39.065**	**1406.469**	**0.60**	**0.273**	**0.812**	**1.085**	**0.342**	**5343.649**
LSD (0.05)	**13.195**	**853.065**	**0.365**	**0.167**	**0.393**	**0.514**	**0.165**	**3040.479**
Heritability (h^2_b)	**0.219**	**0.100**	**0.060**	**0.000**	**0.333**	**0.400**	**0.000**	**-**
p-value	**0.065**	**0.256**	**0.263**	**0.025**	**0.000**	**0.001**	**0.022**	**0.501**
CV (%)	**16.244**	**29.136**	**29.140**	**29.376**	**23.233**	**22.736**	**23.163**	**27.332**

Key: RDp= rooting depth, TRL= total root length, RLD= root length density, RDW= root dry weight, SDW= shoot dry weight, PDW= total plant dry weight, R/S= root to shoot ratio and LWR= length to root dry weight ratio.

Table 5.14: Mean root characteristics of BC_2F_3 families for *LDT 068* (ICCV 00108) x ICC 4958

Genotypes	**RDp (cm)**	**TRL (cm)**	**RLD (cm cm^{-3})**	**RDW (g)**	**SDW (g)**	**PDW (g)**	**R/S**	**LWR (cmg^{-1})**
EUC-04-BC_2F_3-P52-1-3-6-2	36.00	2344.05	1.00	0.33	0.54	0.87	0.61	7124.76
EUC-04- BC_2F_3-P39-1-1-1-9	41.52	2175.33	0.93	0.42	1.20	1.62	0.36	4976.75
EUC-04- BC_2F_3-P52-2-2-2-15	47.48	2166.91	0.93	0.31	0.66	0.97	0.46	7259.08
EUC-04- BC_2F_3-P52-1-1-3-3	52.52	1983.80	0.85	0.28	0.59	0.86	0.48	6881.96
EUC-04-BC2F3-P39-1-1-4-12	41.52	1981.52	0.85	0.35	0.80	1.15	0.44	5452.48
EUC-04- BC_2F_3-P52-1-4-7-2	37.51	1980.73	0.85	0.36	0.89	1.25	0.41	5473.24
EUC-04- BC_2F_3-P53-2-2-2-14	36.52	1918.45	0.82	0.30	0.73	1.03	0.42	6097.25
EUC-04- BC_2F_3-P53-2-2-2-15	40.52	1901.26	0.81	0.28	0.61	0.88	0.46	6673.04
EUC-04- BC_2F_3-P27-1-3-23	28.00	1863.44	0.80	0.27	0.77	1.04	0.35	6702.11
EUC-04- BC_2F_3-P6-2-2-3-11	27.52	1823.57	0.78	0.33	1.05	1.38	0.32	5328.81
EUC-04- BC_2F_3-P52-2-2-2-12	37.48	1817.86	0.78	0.28	0.76	1.04	0.36	6615.84
EUC-04- BC_2F_3-P52-1-3-6-5	33.50	1799.78	0.77	0.30	0.91	1.22	0.33	5941.79
Mean	**32.380**	**1216.850**	**0.520**	**0.206**	**0.620**	**0.825**	**0.353**	**6028.541**
LSD (0.05)	**17.192**	**1013.933**	**0.434**	**0.182**	**0.481**	**0.614**	**0.430**	**2801.308**
Heritability (h^2_b)	**0.087**	**0.172**	**0.186**	**0.000**	**0.200**	**0.179**	**0.300**	**0.183**
p-value	**0.266**	**0.096**	**0.096**	**0.306**	**0.067**	**0.089**	**0.025**	**0.084**
CV (%)	**23.051**	**36.099**	**36.099**	**38.403**	**33.613**	**32.249**	**53.033**	**20.040**

Key: RDp= rooting depth, TRL= total root length, RL= root length density, RDW= root dry weight, SDW= shoot dry weight, PDW= total plant dry weight, R/S= root to shoot ratio and LWR= length to root dry weight ratio.

Table 5.14: Continued… Mean root characteristics of BC_2F_3 families for *LDT 068* (ICCV 00108) x ICC 4958

Genotypes	RDp (cm)	TRL (cm)	RLD (cm cm^{-3})	RDW (g)	SDW (g)	PDW (g)	R/S	LWR (cmg^{-1})
EUC-04- BC_2F_3-P53-2-2-2-7	40.52	1757.00	0.75	0.24	0.13	0.37	1.59	7031.88
EUC-04 BC_2F_3-P52-2-2-2-18	32.48	1756.91	0.75	0.26	0.65	0.91	0.38	7088.38
EUC-04- BC_2F_3-P27-1-3-4-21	33.50	1751.47	0.75	0.22	0.49	0.70	0.44	8130.38
EUC-04- BC_2F_3-P39-1-1-4-3	32.00	1742.48	0.75	0.31	0.84	1.15	0.37	5657.39
EUC-04- BC_2F_3-P52-1-1-3-11	31.50	1731.88	0.74	0.32	0.68	1.00	0.50	5629.84
EUC-04- BC_2F_3-P39-1-1-4-1	46.48	1719.91	0.74	0.27	1.03	1.30	0.26	6468.46
EUC-04- BC_2F_3-P27-1-3-4-19	29.50	1716.03	0.73	0.23	0.72	0.96	0.33	7345.23
EUC-04- BC_2F_3-P53-2-2-2-17	36.52	1708.90	0.73	0.28	0.53	0.80	0.53	6014.24
LDT 068 (ICCV 00108) (Recurrent)	33.80	1365.87	0.58	0.23	0.78	1.01	0.31	5968.87
ICC 4958 (Donor)	37.38	1695.49	0.73	0.28	0.87	1.15	0.33	6252.41
Mean	**32.380**	**1216.850**	**0.520**	**0.206**	**0.620**	**0.825**	**0.353**	**6028.541**
LSD (0.05)	**17.192**	**1013.933**	**0.434**	**0.182**	**0.481**	**0.614**	**0.430**	**2801.308**
Heritability (h^2_b)	**0.087**	**0.172**	**0.186**	**0.000**	**0.200**	**0.179**	**0.300**	**0.183**
P-value	**0.266**	**0.096**	**0.096**	**0.306**	**0.067**	**0.089**	**0.025**	**0.084**
CV (%)	**23.051**	**36.099**	**36.099**	**38.403**	**33.613**	**32.249**	**53.033**	**20.040**

Key: RDp= rooting depth, TRL= total root length, RLD= root length density, RDW= root dry weight, SDW= shoot dry weight, PDW= total plant dry weight, R/S= root to shoot ratio and LWR= length to root dry weight ratio.

5.4.4.3 Correlation estimates of root traits

There was a moderate positive correlation between rooting depth (RDp) and all the traits except LWR which was negatively correlated for *Chania Desi II* x ICC 4958 (Tables 5.15). TRL had a significantly positive correlation ($p < 0.001$, $r = 1.000$) with RLD and strong significant positive association with RDW ($p < 0.001$, $r = 0.771$), SDW ($p < 0.001$, $r = 0.601$) and PDW ($p < 0.001$, $r = 0.706$). However, it had low positive significant correlation with R/S ratio and LWR. Root length density (RLD) was positively and significantly correlated with all traits and showed a strong positive correlation with RDW, PDW, SDW, as was the case with TRL. Root dry weight (RDW) was significantly positively correlated with SDW ($p < 0.001$, $r = 0.620$), PDW ($p < 0.001$, $r = 0.791$), and moderately correlated ($p < 0.001$, $r = 0.574$) with R/S ratio. Shoot dry weight was strong and significantly positively correlated ($p < 0.001$, $r = 0.971$) with PDW and weak but negatively correlated ($p < 0.001$, $r = -0.254$) with R/S ratio. Total plant dry weight (PDW) was negatively correlated with both R/S ratio and LWR. R/S ratio on the other hand had weak negative significant correlation ($p < 0.001$, $r = -0.397$) with LWR. Similar correlations trends were observed for *LTD 068* x ICC 4958 (Table 15.6). From the results, positive significant correlation of more than $r = 0.50$ were obtained between SDW and TRL, RLD and RDW from the two populations.

Table 5.15: Genetic correlations among the root traits of BC_2F_3 families for *Chania Desi II* x ICC 4958

	RDp (cm)	**TRL (cm)**	**RLD (cm cm^{-3})**	**RDW (g)**	**SDW (g)**	**PDW (g)**	**R/S**	**LWR (cmg^{-1})**
RDp (cm)	-							
TRL (cm)	0.432***	-						
RLD (cm cm^{-3})	0.432***	1.000***	-					
RDW (g)	0.409***	0.771***	0.771***	-				
SDW (g)	0.356***	0.601***	0.601***	0.620***	-			
PDW (g)	0.402***	0.706***	0.706***	0.791***	0.971***	-		
R/S	0.156**	0.326***	0.326***	0.574***	-0.254***	-0.022^{ns}	-	
LWR (cmg^{-1})	-0.062^{ns}	0.172**	0.172**	-0.414***	-0.094^{ns}	-0.200***	-0.397***	-

Key: RDp= rooting depth, TRL= total root length, RDW=: root dry weight, SDW= shoot dry weight, PDW= total plant dry weight, R/S= root to shoot ratio, LWR= length to root dry weight ratio and RLD= root length density.

Table 5.16: Genetic correlations among the root traits of BC_2F_3 families for *LDT 068* x ICC 4958

	RDp (cm)	**TRL (cm)**	**RLD (cm cm^{-3})**	**RDW (g)**	**SDW (g)**	**PDW (g)**	**R/S**	**LWR (cmg^{-1})**
RDp (cm)	-							
TRL (cm)	0.614***	-						
RLD (cm cm^{-3})	0.614***	1.000***	-					
RDW (g)	0.561***	0.869***	0.869***	-				
SDW (g)	0.385***	0.542***	0.542***	0.669***	-			
PDW (g)	0.464***	0.675***	0.675***	0.813***	0.977***	-		
R/S	0.087^{ns}	0.274***	0.274***	0.279***	-0.266***	-0.128*	-	
LWR (cmg^{-1})	0.077^{ns}	0.193***	0.193***	-0.261***	-0.254***	-0.275***	-0.024^{ns}	-

Key: RDp: rooting depth, TRL: total root length, RDW: root dry weight, SDW: shoot dry weight, PDW: total plant dry weight, R/S: root to shoot ratio, LWR: length to root dry weight ratio and RLD: root length density.

5.5 Discussions

Low levels of polymorphism were observed in the local and introduced chickpea parents and among families. Three SSR markers (ICCM0249, CaM0204, NCPGR127) showed polymorphism for *Chania Desi II* x ICC 4958 while four markers (NCPGR21, NCPGR127, TA11 and ICCM0249) revealed polymorphism for *LDT 068* x ICC 4958 in BC_2F_1 progenies. Two polymorphic markers (NCPGR127 and ICCM0249) were common for the two populations. These markers were within the '*QTL – hotspot'* region on linkage group 4. This linkage group (CaLG04) harbors several drought-related traits including root traits that contribute up to 58.20% of phenotypic expression (Varshney *et al.*, 2013a; Varshney *et al.*, 2014b). Seven markers were earlier identified on CalG04 (Varshney *et al.*, 2014b) and these have been used to track the QTL region. Recently 15 markers associated with root dry weight, root length density, root surface area, root volume, and rooting depth were identified, out of which two markers, NCPGR7 (SSR) and DR-237 (SNP), were reported to be associated with more than one trait and the markers could be associated with co-localized QTL (Thudi *et al.*, 2014b). This will be helpful in chickpea improvement as more than one desirable trait can be introgressed from the same region simultaneously and tracked by the same markers.

The low genetic variation among populations could be due to continuous selection for desirable traits and intercrossing lines with closely related traits in developing superior genotypes. The genetic diversity of chickpea is an important resource in breeding. Using diverse lines in breeding allows recombination which sometimes results in transgressive segregants with beneficial traits that can be selected for high yielding lines with desirable trait combinations (Upadhyaya *et al.*, 2007).

Limited polymorphism was also observed in the cultivated chickpea (Gaur *et al.*, 2012). The observation could be due to limited tools available to detect polymorphism

(Varshney *et al.*, 2007a). Low polymorphism has also been reported to be due to monophyletic descendant from its wild progenitor *C. reticulatum* in the Fertile Crescent (Abbo *et al.*, 2003). In addition, farmers' adopting new high yielding varieties and abandoning landraces could also partly account for low genetic diversity. This has implications such as increased vulnerability to biotic and abiotic stresses (Kuruma *et al.*, 2010). Inter-mating between lines or inter-varietal crossing has also been one reason for low polymorphism in chickpea (Chaturvedi and Nadarajan, 2010). Continuous search for high yielding varieties has led to low diversity due to frequent crosses resulting in narrow genetic variation within populations. Chickpea is a self-pollinated crop with less than 1% out crossing rate (Singh *et al.*, 2008), hence there is minimal gene contamination from other chickpea in open fields as is the case in cross pollinated crops. Such low diversity was also reported in crops such as cowpeas (Kuruma *et al.*, 2010). This narrow genetic variation in cultivated chickpea limits molecular marker development and QTL for certain traits (Coram *et al.*, 2007) due to lack of polymorphism of markers among genotypes. Wild relatives with traits of interest may be useful in breeding programmes to increase diversity in cultivated chickpea. Although the utilization of wild relatives has some drawbacks, such as crossing ability barriers (Gaur *et al.*, 2012), this could be overcome with modern breeding strategies such as mutation breeding and with the recent completion of chickpea genome sequencing (Varshney *et al.*, 2013c) offering more opportunities for such studies.

The families obtained from the *Chania Desi II* x ICC 4958 cross varied significantly ($P < 0.05$) in seed weight/plant and 100-seed weight while there was no significant difference in the traits for *LTD 068* x ICC 4958. Higher mean seed weight/plant and number of seed/plant was also obtained with the former cross compared to the latter, indicating differences in response of introgressed traits. This could also mean that trait(s) may be easier to identify in later generations (BC_2F_4 onwards). In *Chania Desi II* x ICC 4958, five families

out of 7 had seed weight/plant between 9.79 - 18.47 g which is greater than the recurrent parent (8.74 g). The donor parent produced the highest 100-seed weight (25.56 g) compared to the recurrent parent (17.09 g) and the overall mean (19.26 g). This is an indication that those families with heavier seeds than the recurrent parent probably inherited the trait(s) from the donor parent. Variation for seed yield among some families (EUC-03-BC_2F_2-P6-1-3-3 and EUC-03-BC_2F_2-P6-1-3-9) was low indicating that these lines could be stablized after few generations of selfing.

There was a very high positive correlation between seed weight/plant with number of seeds/plant indicating that the trait could be used to predict seed weight/plant and consequently yield. Indirect selection of the trait could probably result in selection for high yielding genotypes. Similar results were obtained by other authors (Sidramappa *et al.*, 2008; Kobraee *et al.*, 2010; Shamshi *et al.*, 2010; Biabani *et al.*, 2011). Similarly, seed yield was highly correlated with 100-seed weight (Talebi and Rokhzadi, 2013). The correlation between seed yield and 100-seed weight (indicator of seed size) will be useful in selection for large seeds. Large chickpea seeds was reported to fetch higher market prices (Shiferaw *et al.*, 2007). However, negative correlations were obtained between 100-seed weight (g) and number of seeds per plant. This is because average seed size is reduced as number of seeds increases. Similar association were reported in which 100-seed weight was negatively correlated with number of pods/plant in chickpea (Malik *et al.*, 2009).

High yield is most important to the chickpea farmer and to the breeder. It has many traits as its component such as biomass, harvest index, 100 - seed weight and number of seeds/pod. In addition, environment plays a great role in yield expression. Yield is a complex trait and therefore, direct selection for yield in early generation is not efficient due to low heritability and environmental influences (Gaur *et al.*, 2012). Research in common beans also showed that seed yield was highly influenced by environment (Kunkaew *et al.*, 2010).

Heritability is an important factor in trait selection. The three traits (seed weight (g) per plant, 100 - seed weight and number of seeds per plant) had heritabilities ranging from 0.290 to 0.925. Seed weight per plant had the highest heritability of 0.925. This agrees with earlier reports (Sidramappa *et al.*, 2008; Thakur and Sirohi, 2008; Farhatullah and Khan, 2011). In addition, heritability is a function of additive variance due to additive gene effects, which is fixable. Such high heritability could result in good genetic gain through selection.

Root traits play an important role in drought tolerance of chickpea and other legumes under terminal drought. In this study root traits varied significantly among the families. Genetic variation in root traits has been reported in various recombinant inbred lines (RILs) (Serraj *et al.*, 2004; Kashiwagi *et al.*, 2005; Kashiwagi *et al.*, 2006; Rehman, 2009). Among the root traits, root dry weight (RDW), shoot dry weight (SDW), plant dry weight (PDW) and root to shoot ratio (R/S) were significantly different among families of the cross *Chania Desi II* x ICC 4958, while R/S ratio was significant for *LDT 068* x ICC 4958. Total root length (TRL), RLD, RDp and LWR were not significant the latter cross. Overall means for most of these root traits were higher for *Chania Desi II* crosses compared to those for *LDT 068* crosses. The BC_2F_3 families that had mean total root length values higher than the two parents were; EUC-03-BC_2F_3-P6-2-2-2-8, EUC-03-BC_2F_3-P22-1-2-7-8 and EUC-03-BC_2F_3-P22-1-2-1-13 for *Chania Desi II* cross and EUC-04-BC_2F_3-P52-1-3-6-2, EUC-04-BC_2F_3-P39-1-1-1-9 and EUC-04-BC_2F_3-P52-2-2-2-15 for *LDT 068* x ICC 4958. These families had improved total root length of between 40 - 50% compared to the recurrent parents. This is an indication of successful improvement in root traits and such families may be used as donor parents to improve other lines once they are stable at later generations. Root length and rooting depth are important traits for drought avoidance mechanism. Chickpeas roots grow deeper to extract moisture from lower soil profiles under rainfed compared to irrigated conditions and avoid drought (Kumar *et al.*, 2012). Total root length and rooting depth

influenced distribution of roots in the soil profile and the amount of water absorbed in wheat (Manschadi *et al.*, 2006).

Root length density (RLD) and root dry weight (RDW) for most of the top 20 families was higher than those of the parents indicating better absorption of water from soil and also increased biomass accumulation. Root dry weight is a good indicator of root biomass accumulation which is also important in water absorption. RLD represents the root's capability for soil water exploitation, while RDW shows its biomass (Kashiwagi *et al.*, 2008a). Research conducted under rainfed condition indicated that genotypes had increased root biomass compared to those under irrigated conditions (Kumar *et al.*, 2010). Earlier report indicate that water deficit affect the distribution of root weight density (RWD) and root length density (RLD) at various depths, providing increased water absorption capacity in deeper soil layers to cope with drought (Rehman, 2009). This is an indication that root biomass is increased in the deeper soil layers to extract more water.

Root length to root dry weight ratio (LWR) which is an important parameter for estimating changes in root densities were not significantly different among the BC_2F_3 families from the two crosses. This is consistent with findings of Serraj, *et al.*, (2004) and Ali *et al.*, (2005). However, significantly lower LWR was reported in related studies showing an increment in LWR with depth compared to upper soil layers in stressed environments which was attributed to the production of many fine roots (Rehman, 2009). These fine roots are associated with increased water absorption, however, under dry conditions, such roots are not common (Krishnamurthy *et al.*, 1998) probably, as a result of drying up due to lack of water.

A high root to shoot ratio is an indication of root growth. More photosynthates going to roots leads to high root growth hence increased water absorption. Among the 20 best families in this study, some had high R/S ratios. These included EUC-03- BC_2F_3-P22-1-2-1-13 (0.60) and EUC-03- BC_2F_3-P2-1-2-7-29 (0.52) for *Chania Desi II* x ICC 4958 and EUC-

04- BC_2F_3-P53-2-2-2-7 (1.59) and EUC-04- BC_2F_3-P52-1-3-6-2 (0.61) for *LDT 068* x ICC 4958 compared to their recurrent parents. High R/S ratio results from inhibition of shoot growth compared to root growth which is an adaptation mechanism under drought stress. In chickpea root to shoot ratio has been used as an indicator of drought tolerance (Labidi *et al.*, 2009). In maize, it was predicted as a suitable criterion for classifying genotypes into drought tolerant or susceptible through exhibiting desiccation tolerance (Shaddad *et al.*, 2013).

Moderate heritability of 0.333 was obtained for SDW for *Chania Desi II* x ICC 4958 and 0.200 for *LTD 068* x ICC 4958. Moderate heritability of 0.400 for plant dry weight (PDW) was obtained in *Chania Desi II* x ICC 4958. The low heritability of these root traits was from *LTD 068* x ICC 4958. Heritability estimates are good indicators of inheritance of traits. Trait(s) with high heritability have high chances of being passed to subsequent generations and selection for such trait is promising. Low heritability of 0.27 for root dry weight was obtained by Serraj *et al.*, (2004). They also reported a heritability of 0.49 for SDW, which is slightly higher than the results obtained in this study. This could be due to differences in genotypes used and environment under study. The low heritability values are an indication that root traits are governed by polygenic genes which also implies high environmental influence on such traits. Low heritability was also observed in other traits that are polygenically controlled and these are highly influenced by environment indicating low to moderate genetic advance would be possible (Arshad *et al.*, 2001; Thakur and Sirohi, 2008). Indirect selection of quantitative traits with low heritability has relied on selection of highly heritable traits that have high genetic correlation with the quantitative trait(s).

There was a positive correlation between RLD and all other root traits as was the case with rooting depth (RDp) with all the other traits except with LWR. Root length to root dry weight ratio (LWR) is a parameter that determines the changes in root densities. High rooting depth and large root biomass are important traits for adaptation in drought environment as

this allows extraction of moisture from deeper soil depths compared to those with shallow rooting depth. Root biomass and rooting depth were recognized as main drought avoidance mechanism traits (Turner *et al.*, 2001; Kashiwagi *et al.*, 2005). Deep and prolific root systems are expected to contribute more in heavy and adequately deep soils (Kashiwagi *et al.*, 2005). In addition, recent findings showed that the chickpea root system is known to be well adapted to growth under receding soil moisture due to large numbers of thin xylem tubes that are effective and require less energy for soil moisture absorption (Purushothaman *et al.*, 2013). TRL and RDW have shown significant positive correlation and that TRL was an important criterion for selection of drought resistant genotypes (Ganjeali and Kafi, 2007). The correlations between SDW and TRL, RLD and RDW in both crosses in this study were more than 50%. This makes SDW useful for indirect selection of root traits whose measurement is expensive, labour intensive and a difficult task especially under field conditions. Thus indirect selection based on traits that have high correlation and are easy to measure will result in progress in development of varieties for drought tolerance. Similarly a linear relationship was observed between root dry weight and shoot dry weight at 35 days after sowing (Serraj *et al.*, 2004). Similar findings also showed that SDW was significantly positively correlated (approximately 70%) with several important root traits such as RDW, RL (root length), and RLD but had a low correlation with RD (rooting depth) (0.36) (Nayak *et al.*, 2010). Research in spring wheat showed that shoot dry weight was positively associated with rooting depth, root dry weight, total root length and root length density (Narayanan and Prasad, 2014).

New molecular technologies using molecular marker(s) tightly linked to the trait of interest improve breeding efficiency (Bharadwaj *et al.*, 2011). The most popular method is marker assisted backcrossing which involves introgression of one or more traits from a donor into an adapted line. From the analysis, more than 20 BC_2F_3 families had mean RDW, SDW, RDp, TRL higher than the recurrent parents (*Chania Desi II* and *LDT 068*) and donor parent

ICC 4958. Some of these families (EUC-03-BC_2F_3-P22-1-2-1, EUC-03-BC_2F_3-P6-2-2-2, EUC-03-BC_2F_3-P6-2-1-5 and EUC-03-BC_2F_3-P6-1-3-3) showed good yield traits such as seed weight/plant and 100-seed weight. Their yield performance was higher than the recurrent parent and they inherited the large seed trait from ICC 4958 in addition to root traits. This offers an additional advantage to farmers as large seed sizes fetch higher prices. Deep rooting systems are associated with high seed yield in chickpea (Kashiwagi *et al.*, 2006; Kumar *et al.*, 2010). This probably indicates that selection for improved root traits could lead to improved yields. This was confirmed by the identification of *"QTL-hotspot"* on linkage group 4 (CaLG04) that harbors several drought related traits and yield traits QTL including root traits that contribute up to 58.20% phenotypic variation (Varshney *et al.*, 2013a). Introgression of root traits into ICCV 97105 released as *Chania Desi I* from donor parent ICC 4958 (Oyier, 2012) and that of the QTL root region from ICC 4958 into JG 11, an adapted Indian variety, showed that the families had high root length density (RLD) and root dry weight biomass (RDW), compared to both parents (Varshney *et al.*, 2013a). Similar successes were also reported for other traits in other crops (Thabius *et al.*, 2004; Serraj *et al.*, 2005; Ngugi *et al.*, 2010).

The successful application of marker selection in chickpea and other crops is an indication of effective and efficient improvement of varieties with the aid of molecular tools especially with traits that are quantitatively inherited. Application of markers also shortens breeding cycle as there is less environmental influence on selection, which requires several repeated field trials. In this study, it was observed that with the use of markers it was possible to identify families with improved yield and root traits at BC_2F_2 and BC_2F_3 respectively. It is also possible to directly select a trait of interest based on tightly linked markers and which is more effective than indirect selection based on correlated traits. In the current study, it was possible to select F_1 and backcross F_1 (BC_1F_1 and BC_2F_1) plants that were heterozygous

based on markers linked to root QTL region (foreground selection). The completion of sequencing of the chickpea genome (Varshney *et al.*, 2013c) will result in improvement of chickpea breeding in terms of time, efficiency and effectiveness. Furthermore, the recent combination of genome wide association study (GWAS) and candidate gene sequencing approaches have led to the identification of marker trait associations (MTAs) for drought and several drought responsive genes (Thudi *et al.*, 2014b). This will lead to the development of superior drought tolerant varieties with the help of molecular techniques hence lessen the number of years it takes to release a variety.

5.6 Conclusion

Low levels of polymorphism were detected in the chickpea parents screened with SSR and SNP markers. Three markers (ICCM0249, CaM0204, NCPGR127) were polymorphic for *Chania Desi II* X ICC 4958 while four markers (CaM1903, ICCM0249, NCPGR127 and NCPGR21) were polymorphic for *LDT 068* x ICC 4958 in BC_2F_1 generation. These markers were within the '*QTL-hotspot'* region. The number of heterozygous BC_2F_1 plants selected were seven for *Chania Desi II* x ICC 4958 and 19 for *LDT 068* x ICC 4958. This indicated successful introgression of the QTL region in genetic background of recurrent parents. Evaluation of selfed BC_2F_2 families for yield traits and BC_2F_3 for various roots traits showed significant variation for the traits with some families showing early improvement in both root and yield traits. The means of the best 20 performing families were better than their parents for root traits. For *Chania Desi II* x ICC 4958, EUC-03-BC_2F_3-P6-2-2-2-8, EUC-03-BC_2F_3-P22-1-2-7-8, EUC-03-BC_2F_3-22-1-2-7-13 and EUC-03-BC_2F_3-P6-1-3-9-2 had higher TRL, RLD, RDp, RDW SDW and PDW in comparison to the recurrent parent. Families, EUC-04-BC_2F_3-P52-1-4-7-20, EUC-04-BC_2F_3-P52-1-1-3-3 and EUC-04-BC_2F_3-P52-1-3-6-5 for *LDT 068* x ICC 4958 had mean root performance better than the recurrent parent. These families also had bigger seed size (100 - seed weight) than the recurrent parent. This improvement

could be an early expression of some of the measured traits, which is usually difficult to select for due to the complexities of drought related genes and environmental influence. Shoot dry weight (SDW) had strong positive associations with root traits and could be used for indirect selection of these traits. Families identified to be better than the parents need to be evaluated further alongside checks for possible release of the best lines. In addition, families better than ICC 4958 could also be identified as donor parents. Breeding for tolerance to drought is the most effective and economical means of improving and stabilizing yield in drought prone areas.

CHAPTER SIX

6.0 Performance of chickpea genotypes and identification of quantitative trait loci (QTL) for yield related traits under drought conditions

6.1 Introduction

Chickpea seeds are a rich source of protein (24.63%), fat (5.62%), carbohydrates (64.60%), ash (3.30%) and fiber (1.85%) (Abu-Salem and Abou-Arab, 2011). Kenya's chickpea yields are still low. Average yield of 1.8 tons/ha was reported under the long rainy seasons (Onyari *et al.*, 2010) and 0.545 tons/ha during the short rainy seasons under low altitude areas in low altitude areas (Thagana *et al.*, 2009). Currently, several varieties have been introduced in Kenya and evaluated for drought tolerance, resistance to pod borers as well as Ascochyta (Kimurto *et al.*, 2009; Mulwa *et al.*, 2010; Kimurto *et al.*, 2013a; Kimurto *et al.*, 2013b). Some of them have been released (http://www.kephis.org/images/docs/updated-variety-list%202014.pdf). However, more high yielding varieties for recommendation to varied agro-ecological zones are needed.

Yield is a complex trait controlled by many genes each contributing small effects. Its expression is also highly affected by environment and genotype x environment interactions. These factors affect direct selection for yield and hence slow progress in development of varieties that are high yielding. Indirect selection using highly correlated traits for yield has been one option to overcome this low progress. However, some of these correlated traits are also controlled by many genes. Quantitative trait loci (QTL) have been defined as regions within genomes that contain genes associated with a particular quantitative trait (Collard and Mackill, 2008). The use of current technologies such as molecular markers that are closely linked to the QTL trait of interest makes it possible to track these traits with the help of marker assisted approaches (Ribaut *et al.*, 2010). The prerequisite to this success is the

identification of these QTL. QTL cannot be identified phenotypically but it is possible with the help of DNA markers that are tightly linked.

QTL detection is achieved using various methods such as single-marker analysis, simple interval mapping (SIM) but composite interval mapping (CIM) is more precise and effective (Collard and Mackill, 2008). Several software have been used in QTL detection such as mapmaker (Lincoln *et al.*, 1993), MapManager QTX (Manly *et al.*, 2001), PLABQTL (Utz and Mechinger, 1996), WinQTL Cartographer (Wang *et al.*, 2012b) and IciMapping (ICIM) (Wang *et al.*, 2012a). Several markers have been used in chickpea to map and detect QTL linked to important quantitative traits. Currently the common markers are simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs).

In chickpea, SSR markers have been used in identifying reliable QTL for example, drought tolerance (Varshney *et al.*, 2013a; Varshney *et al.*, 2014b) and osmotic adjustment (Courtois *et al.*, 2003)**.** In related studies, five QTL for harvest index were detected on linkage groups (LGs) 1, 3, 4 and 8 explaining 84% of the total phenotypic variability (Rehman, 2009), a marker TA-42 on LG 6 was associated with yield per plant (Imtiaz, 2010) and marker TA47 on LG 4 was reported to be associated with number of pods/plant and yield/plant (g) (Gowda *et al.*, 2011). Identification of QTL linked to yield and its related traits are anticipated to be utilized for marker assisted selection (MAS) in chickpea improvement. The objectives of this study were to:

a) determine the performance of chickpea genotypes for yield and its related traits under irrigated, rainfed and across environments, and
b) identify quantitative trait loci (QTL) associated with yield and yield related traits under irrigated, rainfed and across environments.

6.2 Materials and methods

6.2.1 Parental plant materials

The parental materials used were two inbred lines developed by ICRISAT, India but evaluated for adaptation in Kenya. These were ICCV 05107 (male parent) and ICCV 94954 also called JG 130 (female). The ICCV 05107 (Desi type) is an intermediate yielding variety developed from a cross between ICC 4958 (Desi type with large rooting system) and ICCV 92311 (Kabuli type, early maturing variety and large seeds). The donor parent, ICCV 94954 was developed from a cross between ICCV 42 and BG 256; both are Desi type and high yielding. The pedigree ICCV 42 has resistance to Fusarium wilt while BG 256 has both Ascochyta and Fusarium wilt resistance.

6.2.2 Development of population

The two parents, ICCV 94954 and ICCV 05107 were crossed in ICRISAT, India to generate F_1 (ICCV 94954 x ICCV 05107) and selfed to produce F_2. The F_2 population was advanced by single seed decent (SSD) to generate F_3 families. The purpose of using SSD was to advance fast generations and ensure a random sample from F_2 is retained. Seed multiplication was done at $F_{3:4}$ and the $F_{3:5}$ families were evaluated in Kenya.

6.2.3 Genotyping of parents and F_3 families

Screening for polymorphic markers between the two parents, ICCV 94954 and ICCV 05107 was done using 72 simple sequence repeat (SSR) markers. The F_3 families were genotyped with the polymorphic markers. DNA extraction protocol (Chakraborti *et al.*, 2006) was used and DNA quantification, quality check and normalization to 5 ng/µl was done on agarose gel (0.8%) using lambda DNA standard (MBI Fermentas, USA) according to Upadhyaya *et al.* (2008). The PCR amplification and genotyping protocol was done according to Varshney *et al.* (2009). This genotyping was performed at Applied Genomics

Laboratory, ICRISAT, Patancheru, Hyderabad, India. Data scoring was done with GeneMapper software version 4.0 (Applied Biosystems, 2005). Deviations from expected ratios were tested using the chi square test.

6.3 Evaluation of $F_{3:5-6}$ families for yield and related traits

6.3.1 Site description and field layout

Field evaluation of $F_{3:5}$ families were carried out at three sites, Koibatek Agricultural Training Centre (KATC), Muserech and Kenya Agricultural and Livestock Research Organization (KALRO) - Perkerra, Marigat, giving five environments. The three sites are located in extensive Baringo County in the Rift Valley, Kenya. KATC and Muserech both lie in latitude 1^{o} 35' S, longitude 36^{o} 66' E, altitude 1890 m in uppper midland four agro-ecological zone (UM4) with low agricultural potential. The average annual rainfall is 767 mm; mean annual minimum and maximum temperatures are 10.9 ^{o}C and 28.8 ^{o}C respectively. The soils are vitric andosols with moderate to high soil fertility, well-drained deep loam to sandy loam soil (Jaetzold and Schimdt, 1983). However, Muserech is in the lower region of Eldama Ravine towards the dry areas of Mogotio. It is in the transition zone between the Eldama Ravine and Mogotio and the area receives unpredicted and unreliable rainfall. Kenya Agricultural Livestock and Research Organization - Perkerra - Marigat description is as in the previous chapter five, section 5.3.1.1.

The experimental design was laid out in a 19 x 10 alpha lattice design, at a spacing of 40 cm x 10 cm, single row each with ten seeds replicated twice. The treatments applied were irrigated and non-irrigated (rainfed) in two sites KATC and KALRO-Perkerra while Muserech was planted under rainfed conditions. Plants were irrigated after planting until all plants emerged. The rainfed conditions were maintained without irrigation but one slot of irrigation up to 70% field capacity was applied at 50% flowering after which no irrigation

was applied until maturity. The irrigated conditions were achieved by applying and maintaining water at or near 70% field capacity. This was done by gravimetric method where furrow irrigation was applied and soil sampling was done. The soil was collected in air tight container with known weight (tare) and weighed using weighing balance (Model: TANITA TLD-610). The soil sample was then dried at 105° C for 24 hour and reweighed. The percentage soil water content on dry mass or gravimetric content, Pw was determined using the formulae:

$$Pw = \frac{\text{WSW} - \text{DSW}}{\text{DSW}} * 100$$

Where WSW = wet sample weight (g), DWS = dry sample weight (g). This was done on every 15th day after irrigation and when moisture was below 70% the plants were irrigated to maintain the 70% field capacity.

6.3.2 Description of the environments

Environment comprised location and water regime. Therefore, the experiment was conducted under five environments as indicated below:

a) Koibatek Agricultural Training Centre (KATC) + irrigation = Environment one
b) Koibatek Agricultural Training Centre (KATC) + non irrigation (rainfed) = Environment two
c) Kenya Agricultural Livestock and Research Organization (KALRO) - Perkerra + irrigation = Environment three
d) Kenya Agricultural Livestock and Research Organization (KALRO) - Perkerra + non irrigation (rainfed) = Environment four
e) Muserech + non irrigated (rainfed) = Environment five

6.3.3 Data collected

Data were collected on morphological and phenological traits according to Upadhyaya *et al.,* (2007) as described below:

a) Crop growth vigor was rated on a scale of 1 to 5 (1=Very good, 2=Good, 3=Average, 4= poor and 5= very poor) based on visual early growth vigor (plant height and shoot biomass). This was done 15 days after emergence (DAE).

b) Days to 1st flower - This was calculated from the date of emergence to date when first flower appears in a single plant or more per genotype.

c) Days to 1st podding - This was calculated by determining the number of days from the date of emergence to when 1st pod emerged in a plant or plants per genotype.

d) Days to 50% flowering – This represented the number of days from emergence to when 50% of chickpea showed fully opened flowers in a row.

e) Days to 75% podding- This was taken as days from date of emergence to when 75% of the plants were at podding stage.

f) Plant height (cm) – This was measured at 50% flowering by taking five readings in centimeter on each row excluding border plants and averaging before analysis.

g) Days to maturity - Days from emergence to physiological maturity was recorded by calculating the difference from date of emergence to the date when 90% of the plants attain physiological maturity (when 90% of the leaves per row turned brown).

h) Above ground biomass -At physiological maturity, above ground plants were harvested and dried in an electric oven at 60 ºC to constant dry weight for 48 hours. The dry plants were weighed using electronic weighing balance (Model: TANITA TLD-610). This was converted to kgha^{-1} using the formula;

10,000 m^2 x biomass per plot (kg) /area of experimental plot (m^2).

i) 100-seed weight - After drying, the pods were shelled and 100 seeds were counted and weighed using electric weighting balance, same model as indicated above.

j) Grain yield - Dry grain yield per plot at harvest was weighed and converted into kilograms per hectare using the formulae:

Grain yield = 10 000 m^2 x yield per plot /area of experimental plot (m^2).

k) Harvest index - This was determined using the formula:

HI = dry grain weight/total above ground biomass dry weight.

6.3.4 Data analysis

The analysis of the phenotypic data was achieved using PROC GLM of SAS software version 9.2. Means between two genotypes were separated by least significant difference (LSD) at $p < 0.05$. The model used was:

$$Y_{ijkl} = \mu + t_i + r(l)_{jl} + b(r)_{jk} + l_l + (cxl)_{il} + e_{ijkl}$$

Where:

Y_{ijkl} = Observation of treatments; μ = Overall mean; t_i = effect of i^{th} genotypes; $r(l)_{jl}$ = jl^{th} effect of j^{th} replication within environment; $b(r)_{jk}$ = effect of jk^{th} block within replication; l_i = effect of l^{th} environment, $(cxl)_{il}$ = effect of il^{th} interaction between genotypes and environment; e_{ijkl} = error term.

Expected mean squares were calculated based on the ANOVA (Table 6.1) and broad-sense heritability estimates were determined.

Table 6.1: ANOVA table for estimation of expected mean squares

Source	**DF**	**Mean Square**	**Expected Mean Squares**
Environments	l-1	M_{ev}	$\sigma_e^2 + grb\sigma_{ev}^2 + rb\sigma_{gev}^2 + gb\sigma_{r(ev)}^2$
Rep(Env)	l(r-1)	$M_{r(ev)}$	$\sigma_e^2 + bg\sigma_{r(ev)}^2 + lg\sigma_{b(r)}^2$
Block(Rep)	r(b-1)	$M_{b(r)}$	$\sigma_e^2 + lg\sigma_{b(r)}^2$
Genotypes	g-1	M_g	$\sigma_e^2 + lrb\sigma_g^2 + rb\sigma_{gev}^2$
Env*Genotypes	(l-1)*(g-1)	M_{gev}	$\sigma_e^2 + rb\sigma_{gev}^2$
Error	(r-1)(lgb -1)	M_e	σ_e^2

Key: r = number of replications, b = number of blocks, l = number of environments and g = number of genotypes.

Heritability in the broad-sense was determined based on the formula derived from expected mean squares as shown:

$$h^2b = \frac{\sigma_g^2}{\left\{\left(\frac{\sigma_e^2}{lrb}\right) + \left(\frac{\sigma_{gev}^2}{l}\right) + \sigma_g^2\right\}}$$

Where:

$\sigma_e^2 = Me$

$\sigma_{gev}^2 = \frac{Mgev - Me}{rb}$

$\sigma_g^2 = \frac{Mg - Mgev}{rbl}$

6.4 Identification of quantitative trait loci (QTL) for yield and related traits

6.4.1 Linkage map construction

A linkage map was constructed based on genotypic data from the polymorphic markers using IciMapping. The Microsoft excel workbook file was prepared containing general information, genotype and anchor markers according to Wang *et al.* (2012a). The following parameters were used:

a) Grouping was achieved using logarithmic of odds (LOD) of 2.5 where any two markers with a LOD higher than the threshold were grouped together.
b) Ordering option nnTwoOpt: nearest neighbor was used for tour construction, and two-opt was used for tour improvement.
c) Rippling option SARF (sum of adjacent recombination fractions) was used to fine tune the ordered chromosomes.

Linkage map was generated using 'QTL mapping input file' outputting options. Chi-square values and probability were generated by pairwise distance (cm) outputting options.

6.4.2 QTL detection

QTL detection was done using IciMapping (Inclusive Composite Interval Mapping) version 3.2 (Wang *et al.,* 2012a) based on the linkage map generated, genotypic and the phenotypic data. The phenotypic data used was plant height, days to maturity, grain yield ($kgha^{-1}$), above ground biomass ($kgha^{-1}$), harvest index (HI) and 100-seed weight (g). The IciMapping data/file was supplied with the following information with the options chosen bracketed: i) General information i.e indicator (mapping), mapping population type (selfing), mapping function (Kosambi), marker space (positions), marker space unit (centiMorgan) (cM), number of chromosomes (8), size of mapping population (188) and number of traits (6 traits listed were used); ii) Chromosome information indicating the linkage group (LG) and

the number of markers on each LG; iii) Linkage map specifying the marker, linkage group and distance; iv) genotype data; v) phenotype data. This information, prepared in one excel workbook, was uploaded to ICIM software. The missing phenotypes were set to be deleted and ICIM additive and dominance mapping (ICIM-ADD) was used for QTL detection and estimation of additive and dominance effects. Scanning of genome was every 1 cM with probability stepwise regression of 0.001 by 1000 permutations of data which maintained the chromosome type 1 error of 0.05. Kosambi's functions were used to convert recombination percentage to centiMorgan (cM) map unit distances.

6.5 Results

6.5.1 Evaluation of $F_{3:5-6}$ genotypes for yield and yield related traits

a) Yield and yield related traits across five environments (rainfed + irrigated conditions in 3 locations)

The ANOVA results across environments were highly significant ($P < 0.0001$) for all the traits. Genotypes and genotypes x environments were significant for all other traits except plant vigour (EGRV) (Table 6.2).

Table 6.2: Mean square values for yield, yield traits and phenological traits across the five environments (irrigated, rainfed conditions and three locations) for $F_{3:5}$ families

Source	df	EGRV	DFFL	DFPD	DFTFL	DAP-75	DM	PLHGT	BYHA	SDWT	SYHA	HI
Env	4	94***	11224***	14618***	10892***	29659.7***	21024***	4836***	225956592***	116***	16532143***	1.32***
Rep(Env)	4	0.31^{ns}	24.53^{ns}	38.73*	47.04***	27.56*	9.24^{ns}	39.03*	1395662^{ns}	9.69^{ns}	224188^{ns}	0.00^{ns}
Block(Rep)	18	0.49^{ns}	12.12^{ns}	14.83^{ns}	9.71^{ns}	15.97^{ns}	5.02^{ns}	13.88^{ns}	1393617^{ns}	4.68^{ns}	152145^{ns}	0.00^{ns}
Genotype	187	0.66^{ns}	73.20***	71.19***	57.95***	49.82***	47.97***	31.49***	1398694**	27***	177872*	0.01*
G x E	748	0.66^{ns}	37.11***	35.26***	31.69***	29.27***	30.94***	21.20***	1214668**	15.35***	153330*	0.01**
Error	937	0.62	12.26	13.21	10.54	11.37	13.41	13.09	1000222	5.53	13456.1	0.008

*, ** and ***= significance level at $p < 0.05$, 0.01 and 0.001, EGRV= Plant growth vigor, DFFL= days to first flower emergence, DFPD= days to first pod emergence, DFTFL= days to 50% flowering, DAP-75= days to 75% podding, DM= days to maturity, PHLGH= plant height, BYHA= above ground biomass ($kgha^{-1}$), SDWT= 100-seed weight, SYHA= yield in $kgha^{-1}$ and HI= harvest index.

The genotype means were significantly different for all traits except EGRV (Table 6.3). The overall mean for yield was 777.73 kgha^{-1}. Based on the overall mean, 90 genotypes were above this mean while 140 genotypes performed better than the better parent, ICCV 05107 (689.24 kgha^{-1}). Among these genotypes, four had greater than 1100 kgha^{-1} and these were; ICCX-060045-F3-P174-BP, ICCX-060045-F3-P76-BP, ICCX-060045-F3-P4-BP and ICCX-060045-F3-P146-BP. Overall, 11 genotypes had between 1000 -1200 kgha^{-1}. Also, these genotypes had high biomass ranging from 3000 - 3500 kgha^{-1}. Genotype ICCX-060045-F3-P146-BP had consistently higher yield (1100 tons/ha) and above ground biomass (3500 kgha^{-1}). The seed weight of genotype ICCX-0600453-P23-BP (28 g) was heavier than the best parent, ICCV 05107 (26g), while ICCX-060045-F3-P19-BP was equivalent to that of the best parent. This corresponded to 7.01% increase in seed weight from the best parent. It was noted that those genotypes with high yield also had high above ground biomass and heavier seeds in addition to high harvest indices. The maturity period was between 81.3 – 87.8 days which was a one week difference. The other phenological traits difference was between 5 – 6 days.

Table 6.3 Mean values of best 20 genotypes and the parents across the five environments for yield and yield related traits for $F_{3:5}$ families

Genotypes	EGRV (days)	DFFL (days)	DFPD (days)	DFTFL (days)	DAP-75 (days)	DM (days)	PLHGT (cm)	BYHA ($kgha^{-1}$)	SDWT(g)	SYHA ($kgha^{-1}$)	HI
ICCX-060045-F3-P174-BP	2.00	47.10	52.30	58.40	69.70	84.80	27.71	2977.39	22.70	1157.64	0.41
ICCX-060045-F3-P76-BP	1.80	44.80	51.30	58.10	69.20	84.00	26.97	2864.84	25.84	1117.20	0.41
ICCX-060045-F3-P4-BP	1.60	45.60	52.50	58.90	69.80	87.80	26.97	2669.13	24.70	1107.73	0.42
ICCX-060045-F3-P146-BP	1.50	44.30	50.40	56.10	67.60	81.80	26.20	3581.88	24.83	1102.06	0.31
ICCX-060045-F3-P134-BP	2.10	44.70	50.70	58.00	67.10	84.20	24.98	2663.40	24.60	1072.90	0.41
ICCX-060045-F3-P139-BP	1.70	41.10	46.80	54.30	65.60	81.30	29.43	2702.93	20.92	1057.10	0.40
ICCX-060045-F3-P23-BP	1.50	46.60	53.00	59.10	70.10	85.60	30.01	2537.09	28.07	1055.13	0.43
ICCX-060045-F3-P46-BP	1.90	41.70	48.40	54.50	66.80	82.10	25.27	2724.15	25.29	1051.88	0.38
ICCX-060045-F3-P208-BP	2.10	44.70	50.40	57.00	69.50	83.70	28.82	2575.83	24.71	1043.43	0.41
ICCX-060045-F3-P91-BP	1.80	44.70	51.60	57.60	68.00	84.20	25.87	2900.35	25.94	1027.47	0.37
ICCX-060045-F3-P19-BP	2.00	41.80	48.70	55.20	65.70	82.80	28.32	2547.53	26.02	1011.10	0.41
Mean	**1.85**	**43.62**	**49.81**	**56.02**	**67.21**	**83.49**	**26.47**	**2233.88**	**23.51**	**777.73**	**0.37**
LSD (0.05)	**0.159**	**0.708**	**0.735**	**0.656**	**0.682**	**0.74**	**0.731**	**202.181**	**0.475**	**23.45**	**0.02**

EGRV; Plant growth vigor, DFFL; days to first flower emergence, DFPD; days to first pod emergence, DFTFL; days to 50% flowering, DAP-75; days to 75% podding, DM; days to maturity, PLHGT; plant height, BYHA; above ground biomass ($kgha^{-1}$), SDWT, 100-seed weight, SYHA; yield in $kgha^{-1}$; HI; harvest index.

Table 6.3: Continued… Mean values of best 20 genotypes across the five environments for yield and yield related traits for $F_{3:5}$ families

Genotypes	EGRV (days)	DFFL (days)	DFPD (days)	DFTFL (days)	DAP-75 (days)	DM (days)	PLHGT (cm)	BYHA ($kgha^{-1}$)	SDWT(g)	SYHA ($kgha^{-1}$)	HI
ICCX-060045-F3-P280-BP	1.50	46.40	52.00	58.20	68.10	83.80	28.71	2806.26	24.82	997.85	0.37
ICCX-060045-F3-P188-BP	1.50	45.30	51.80	56.10	67.90	83.50	27.28	2549.19	22.74	992.27	0.37
ICCX-060045-F3-P159-BP	1.80	45.20	50.30	56.00	66.80	83.50	29.66	3046.68	23.10	980.58	0.34
ICCX-060045-F3-P3-BP	1.60	44.80	51.40	56.30	67.30	84.90	24.46	2426.98	24.52	968.57	0.42
ICCX-060045-F3-P31-BP	1.60	42.20	48.90	55.80	67.20	82.20	25.55	2668.39	24.36	968.48	0.41
ICCX-060045-F3-P246-BP	1.80	43.10	49.20	54.60	66.20	83.70	26.30	2805.60	20.91	950.31	0.36
ICCX-060045-F3-P179-BP	1.40	47.20	52.00	58.00	69.50	86.80	28.55	2579.03	23.37	948.19	0.40
ICCX-060045-F3-P90-BP	1.80	41.30	48.80	54.10	66.30	84.10	26.77	2254.50	24.50	947.03	0.41
ICCX-060045-F3-P62-BP	1.90	40.80	47.40	53.80	65.20	82.50	25.38	3237.22	22.79	943.29	0.36
ICCV 05107 (donor)	1.85	42	48.2	54.45	65.1	82.55	27.02	2141.46	26.23	689.24	0.34
ICCV 94954 (recurrent)	1.95	41.5	47.65	54.4	65.85	81.9	24.23	1860.07	23.98	603.82	0.34
Mean	**1.85**	**43.62**	**49.81**	**56.02**	**67.21**	**83.49**	**26.47**	**2233.88**	**23.51**	**777.73**	**0.37**
LSD (0.05)	**0.159**	**0.708**	**0.735**	**0.656**	**0.682**	**0.74**	**0.731**	**202.181**	**0.475**	**23.45**	**0.02**

EGRV; plant growth vigor, DFFL; days to first flower emergence, DFPD; days to first pod emergence, DFTFL; days to 50% flowering, DAP-75; days to 75% podding, DM; days to maturity, PLHGT; plant height, BYHA; above ground biomass ($kgha^{-1}$), SDWT, 100-seed weight, SYHA; yield in $kgha^{-1}$; HI; harvest index.

Yield was significantly positively correlated ($P < 0.001$, $r = 0.14$) with SDWT and HI ($P < 0.001$, $r = 0.28$) but the correlation was low. It was also significantly correlated ($P < 0.001$, $r = 0.75$) with BYHA (Table 6.4). However, phenological traits (EGRV, DFFL, DFPD, DFTFL and DAP-75) were negatively correlated with seed yield. Days to maturity (DM) was positively correlated ($r = 0.24$) with SYHA. Seed weight (SDWT) was significantly positively correlated ($P < 0.001$, $r = 0.14$) with PLHGT. Similarly, HI was significantly positively correlated ($P < 0.001$, $r = 0.19$) with PLHGT. However, these correlations were low. Above ground biomass had relatively low positive correlation ($P < 0.001$, $r = 0.38$) with DM. There were highly significant positive correlations amongst the phenological traits (Table 6.4).

Table 6.4: Correlation among yield, yield related traits and phenological traits across the five environments (irrigated and rainfed conditions)

	SYHA ($kgha^{-1}$)	**SDWT (g)**	**HI**	**BYHA ($kgha^{-1}$)**	**EGRV (days)**	**DFFL (days)**	**DFPD (days)**	**DFTFL (days)**	**DAP-75 (days)**	**DM (days)**	**PLHGT (cm)**
SYHA	-										
SDWT	0.14***	-									
HI	0.28***	0.02ns	-								
BYHA	0.75***	0.13ns	-0.32***	-							
EGRV	-0.13***	-0.05*	-0.19***	-0.00ns	-						
DFFL	-0.17***	-0.04ns	-0.25***	0.03ns	0.35***	-					
DFPD	-0.19***	-0.03ns	-0.22***	0.00ns	0.36***	0.95***	-				
DFTFL	-0.16***	-0.04ns	-0.25***	0.05*	0.38***	0.92***	0.95***	-			
DAP-75	-0.15***	-0.04ns	-0.29***	0.09***	0.38***	0.86***	0.90***	0.91***	-		
DM	0.24***	-0.09***	-0.25***	0.38***	0.12***	0.38***	0.35***	0.46***	0.42***	-	
PLHGT	0.018ns	0.14***	0.19***	-0.016ns	-0.12***	-0.04ns	0.01ns	-0.072**	-0.012ns	-0.36***	-

*, ** and ***= significance level at $p < 0.05$, 0.01 and 0.001, EGRV= Plant growth vigor, DFFL= days to first flower emergence, DFPD= days to first pod emergence, DFTFL= days to 50% flowering, DAP-75= days to 75% podding, DM= days to maturity, PLHGT= plant height, BYHA= above ground biomass ($kgha^{-1}$), SDWT= 100-seed weight, SYHA= yield in $kgha^{-1}$ and HI= harvest index.

b) Response of yield and yield related traits under irrigated conditions

Based on results across environments, environment x genotype interaction were significant and therefore data were analyzed separately for each environment. Environment was highly significant $P < 0.0001$ under irrigated conditions. Genotypes were significant across the two sites except for EGRV. There was significant genotype by environment interaction for most traits excluding EGVR, BHYA, SHYA and HI (Table 6.5).

Genotypes were significantly different ($P < 0.05$) for all traits except EGRV (Table 6.6). The 20 top performing genotypes had high mean yields compared to the parents. Six genotypes had yield greater than 1.3 tons/ha. These were: ICCX-060045-F3-P174-BP, ICCX-060045-F3-P146-BP, ICCX-060045-F3-P23-BP, ICCX-060045-F3-P62-BP, ICCX-060045-F3-P46-BP and ICCX-060045-F3-P134-BP. Among these six genotypes, four ICCX-060045-F3-P146-BP, ICCX-060045-F3-P174-BP, ICCX-060045-F3-P46-BP and ICCX-060045-F3-P134-BP yielded twice compared to the better parent, ICCV 05107, across the environments. These genotypes also had above ground biomass greater than 3.1 tons/ha. Two genotypes, ICCX-060045-F3-P23-BP and ICCX-060045-F3-P50-BP had seeds heavier than the better parent (26 g) where the former gneotyp (ICCX-060045-F3-P23-BP) also recorded heavier seeds across environments. The HI of the best 20 genotypes ranged from 0.29 - 0.44. Differences among genotypes for 50% flowering were 11 days while days to maturity among all genotypes differed by 7 days. However, the maturity difference among the best 20 performing genotypes was 2 days.

Table 6.5: Mean squares for yield and yield related traits and phenological traits under irrigated condition in Koibatek ATC (KATC) and KALRO-Perkerra

Source	df	EGRV	DFFL	DFPD	DFTFL	DAP-75	DM	PLHGT	BYHA	SYHA	SDWT	HI
Env	1	69.26***	9206***	5540***	5503***	21517***	20525***	2695***	5.5E+08***	1.4E+07***	4.70ns	3.056***
Rep(Env)	1	0.58ns	4.15 ns	6.48 ns	7.24 ns	6.67ns	7.24 ns	5.68 ns	3630473 ns	434728ns	20.77 ns	0.001 ns
Block(Rep)	18	0.70 ns	10.54 ns	9.94 ns	8.21ns	11.76 ns	10.17 ns	12.08 ns	1305032 ns	171013 ns	6.55 ns	0.005ns
Genotypes	187	0.89 ns	48.24***	49.30***	42.67***	37.98***	47.36***	25.42***	2112402**	216341*	18.29***	0.014*
Env*Gen	187	0.92 ns	32.59***	33.50***	32.57***	29.79***	36.05***	26.09***	1579667ns	152058 ns	18.51***	0.012 ns
Error	364	0.82	10.56	12.401	10.84	13.10	19.66	13.07	1490097	167002	8.97	0.011

*, ** and ***= significance level at p < 0.05, 0.01 and 0.001, EGRV= Plant growth vigor, DFFL= days to first flower emergence, DFPD= days to first pod emergence, DFTFL= days to 50% flowering, DAP-75= days to 75% podding, DM= days to maturity, PLHGT= plant height, BYHA= above ground biomass ($kgha^{-1}$), SDWT= 100-seed weight, SYHA= yield in $kgha^{-1}$ and HI= harvest index.

Table 6.6: Means of genotypes for yield, yield related and phenological traits under irrigated condition in KATC and KALRO-Perkerra

Genotypes	**EGRV (days)**	**DFFL (days)**	**DFPD (days)**	**DFTFL (days)**	**DAP-75 (days)**	**DM (days)**	**PLHGT (cm)**	**BYHA ($kgha^{-1}$)**	**SYHA ($kgha^{-1}$)**	**SDWT (g)**	**HI**
ICCX-060045-F3-P174-BP	2.25	49.75	55.00	61.50	75.50	89.75	29.40	3592.71	1502.11	26.20	0.44
ICCX-060045-F3-P146-BP	1.50	48.25	54.50	60.25	75.50	88.25	28.63	4861.28	1355.16	25.49	0.29
ICCX-060045-F3-P23-BP	1.75	51.00	58.00	63.00	76.50	89.50	33.88	3148.44	1314.07	28.09	0.44
ICCX-060045-F3-P62-BP	2.00	44.25	53.50	59.50	73.25	86.00	30.98	4987.69	1302.90	25.62	0.37
ICCX-060045-F3-P46-BP	1.50	41.50	49.75	55.25	72.25	82.75	27.90	3609.56	1290.39	24.35	0.36
ICCX-060045-F3-P134-BP	2.50	46.25	54.50	62.25	71.50	85.50	28.23	3342.56	1271.73	23.97	0.40
ICCX-060045-F3-P234-BP	2.25	46.25	52.75	58.25	75.25	87.25	29.08	2858.56	1241.72	25.94	0.40
ICCX-060045-F3-P76-BP	1.75	46.50	55.00	62.25	76.50	86.25	29.93	2807.06	1232.31	25.67	0.44
ICCX-060045-F3-P50-BP	1.25	49.25	57.50	60.25	76.00	90.00	28.55	4293.55	1203.64	27.17	0.31
ICCX-060045-F3-P71-BP	1.50	43.00	51.00	57.75	71.25	83.50	28.05	3425.56	1163.14	25.43	0.36
ICCX-060045-F3-P208-BP	2.25	42.00	48.75	56.00	73.75	83.25	31.40	3049.15	1151.61	26.11	0.40
ICCX-060045-F3-P19-BP	2.50	41.75	51.50	58.50	71.75	86.25	29.75	2484.06	1112.19	25.92	0.45
ICCX-060045-F3-P139-BP	1.50	40.50	47.50	54.50	70.00	80.75	32.98	3133.45	1100.25	21.07	0.36
Means	**1.996**	**46.101**	**53.73**	**59.513**	**73.909**	**86.513**	**27.567**	**2291.158**	**739.633**	**23.39**	**0.36**
LSD (0.05)	**0.184**	**0.659**	**0.714**	**0.668**	**0.734**	**0.899**	**0.733**	**247.592**	**82.88**	**0.608**	**0.02**

EGRV= Plant growth vigor, DFFL= days to first flower emergence, DFPD= days to first pod emergence, DFTFL= days to 50% flowering, DAP-75= days to 75% podding, DM= days to maturity, PLHGT= plant height, BYHA= above ground biomass ($kgha^{-1}$), SDWT= 100-seed weight, SYHA= yield in $kgha^{-1}$ and HI= harvest index.

Table 6.6: Continued…. Means of genotypes for yield, yield related and phenological traits under irrigated condition in KATC and KALRO-Perkerra

Genotypes	**EGRV (days)**	**DFFL (days)**	**DFPD (days)**	**DFTFL (days)**	**DAP-75 (days)**	**DM (days)**	**PLHGT (cm)**	**BYHA ($kgha^{-1}$)**	**SYHA ($kgha^{-1}$)**	**SDWT (g)**	**HI**
ICCX-060045-F3-P91-BP	1.75	48.25	58.00	63.50	76.25	89.75	27.83	3463.00	1094.39	24.59	0.32
ICCX-060045-F3-P55-BP	2.75	51.25	59.00	62.25	76.75	90.00	24.70	2427.25	1091.79	22.85	0.52
ICCX-060045-F3-P164-BP	1.00	44.50	54.50	59.00	72.75	84.25	26.28	3557.55	1086.45	23.44	0.36
ICCX-060045-F3-P207-BP	1.00	41.00	48.00	53.75	69.25	80.75	28.58	3154.56	1079.27	26.71	0.35
ICCX-060045-F3-P183-BP	1.75	45.00	52.50	58.75	73.50	86.25	25.70	3401.20	1063.37	23.60	0.31
ICCX-060045-F3-P4-BP	1.75	51.00	60.25	65.25	79.25	93.50	24.53	2374.06	1062.81	23.96	0.45
ICCX-060045-F3-P280-BP	1.50	48.00	54.75	59.75	73.75	85.50	27.88	2996.94	1055.20	24.89	0.37
ICCV 05107 (donor)	1.50	44.13	51.50	57.13	71.38	84.25	28.95	2091.72	592.07	26.80	0.33
ICCV 94954 (recurrent)	1.88	41.88	49.38	55.25	69.75	82.25	24.72	1882.17	514.22	24.55	0.30
Means	**1.996**	**46.101**	**53.73**	**59.513**	**73.909**	**86.513**	**27.567**	**2291.158**	**739.633**	**23.39**	**0.36**
LSD (0.05)	**0.184**	**0.659**	**0.714**	**0.668**	**0.734**	**0.899**	**0.733**	**247.592**	**82.88**	**0.608**	**0.02**

EGRV= Plant growth vigor, DFFL= days to first flower emergence, DFPD= days to first pod emergence, DFTFL= days to 50% flowering, DAP-75= days to 75% podding, DM= days to maturity, PLHGT= plant height, BYHA= above ground biomass ($kgha^{-1}$), SDWT= 100-seed weight, SYHA= yield in $kgha^{-1}$ and HI= harvest index.

Most traits were significantly positively correlated among each other, few were not correlated while others were negatively correlated (Table 6.7). There was highly significant positive correlation ($P < 0.001$, $r = 0.73$) between SYHA and BYHA. The SYHA was significantly positively correlated ($P < 0.001$, $r = 0.24$; $P < 0.001$, $r = 0.16$) with SDWT and HI respectively, but weakly correlated. There was significantly low positive correlation ($P < 0.001$, $r = 0.22$) between seed weight (SDWT) and BYHA but with non-significant correlation ($P>0.05$, $r = 0.04$) with HI. Harvest index (HI) was significantly negatively correlated ($P < 0.001$, $r = 0.45$) with BYHA. Seed weight and harvest index were negatively correlated with EGRV, DFFL, DFPD, DFTFL, DAP-75 and DM. On the other hand, BYHA was significantly ($P < 0.001$) positively correlated with these traits ranging from $r = 0.08$ - 0.40. There was highly significant positive correlation amongst the phenological traits (EGRV, DFFL, DFPD, DFTFL, DAP-75 and DM).

.

Table 6.7: Correlation among traits under irrigated conditions in two sites, Koibatek ATC (KATC) and KALRO-Perkerra

	SYHA ($kgha^{-1}$)	**SDWT (g)**	**HI**	**BYHA ($kgha^{-1}$)**	**EGRV (days)**	**DFFL (days)**	**DFPD (days)**	**DFTFL (days)**	**DAP-75 (days)**	**DM (days)**	**PLHGT (cm)**
SYHA	-										
SDWT	0.24***	-									
HI	0.16***	-0.04ns	-								
BYHA	0.73***	0.22***	-0.45***	-							
EGRV	0.04ns	-0.07ns	-0.09*	0.08*	-						
DFFL	0.11**	-0.05ns	-0.29***	0.28***	0.23***	-					
DFPD	0.10*	-0.02ns	-0.21**	0.24***	0.23***	0.92***	-				
DFTFL	0.09*	-0.01ns	-0.24***	0.24***	0.24***	0.90***	0.94***	-			
DAP-75	0.18***	-0.04 ns	-0.40***	0.40***	0.27***	0.86***	0.81***	0.83***	-		
DM	0.16***	-0.03ns	-0.34***	0.37***	0.26***	0.85***	0.80***	0.80***	0.91***	-	
PLHGT	0.15***	0.17***	0.12***	0.13**	-0.18***	-0.23***	-0.20***	-0.24***	-0.32***	-0.29***	-

*, ** and ***: significance level at $p < 0.05$, 0.01 and 0.001, EGRV= Plant growth vigor, DFFL= days to first flower emergence, DFPD= days to first pod emergence, DFTFL= days to 50% flowering, DAP-75= days to 75% podding, DM= days to maturity, PLHGT= plant height, BYHA= above ground biomass ($kgha^{-1}$), SDWT= 100-seed weight, SYHA= yield in $kgha^{-1}$ and HI= harvest index.

c) Response of yield and yield related traits under rainfed (non-irrigated) conditions

There were highly significant differences ($P < 0.05$) among the environments, genotypes and G x E (Table 6.8). Genotypes differences were significant for most traits except EGRV and BYHA. There were significant G x E interactions for all traits except EGRV indicating the role of environment in their expression.

Mean yield for genotypes under rainfed condition (non-irrigated) were significantly different for SYHA (Table 6.9). Six genotypes (ICCX-060045-F3-P188-BP, ICCX-060045-F3-P111-BP, ICCX-060045-F3-P4-BP, ICCX-060045-F3-P118-BP, ICCX-060045-F3-P113-BP and ICCX-060045-F3-P61-BP) attained yield greater than 1100 $kgha^{-1}$ compared to the better parent (754 $kgha^{-1}$). From the 20 top high yielding genotypes, six were among the high yielding genotypes across environments. These were; ICCX-060045-F3-P188-BP, ICCX-060045-F3-P4-BP, ICCX-060045-F3-P159-BP, ICCX-060045-F3-P76-BP, ICCX-060045-F3-P179-BP and ICCX-060045-F3-P91-BP. These genotypes had atleast 10% yield more than the better parent. However, the yield performances under rainfed conditions were lower than under irrigated conditions. Seed weight (SDWT) was significantly different among genotypes with highest genotype weighing 27.07 g. This was lower than the SDWT of the best genotype under irrigated conditions (28.09 g), translating to 7% weight loss. However, four genotypes (ICCX-060045-F3-P76-BP, ICCX-060045-F3-P176-BP, ICCX-060045-F3-P128-BP and ICCX-060045-F3-P91-BP) had seed weight ranged from 25.95 to 27.07 g heavier than the better parent, ICCV 05107 (25.85 g). On average, days to maturity among genotypes under irrigated and rainfed conditions were 5 days different with some genotypes maturing early under rainfed than irrigated. Four genotypes matured earlier under rainfed compared to the earliest maturing genotypes under irrigated conditions.

Table 6.8: Mean squares for yield and yield related traits and phenological traits under rainfed conditions in KATC, Muserech and KALRO-Perkerra

Source	df	EGRV	DFFL	DFPD	DFTFL	DAP-75	DM	PLHGT	BYHA	SYHA	SDWT	HI
Env	2	139***	13938***	16692.***	11281***	20174***	25975***	7564***	1.8E+08***	2.5E+07***	220***	1.09***
Rep(Env)	2	0.26^{ns}	46.9*	73.91**	90.07***	50.59*	14.62^{ns}	74.04**	751771^{ns}	159357^{ns}	2.51^{ns}	0.00^{ns}
Block(Rep)	18	0.24^{ns}	11.82^{ns}	16.17^{ns}	11.47^{ns}	18.42*	7.13^{ns}	9.89^{ns}	702963^{ns}	94695^{ns}	3.91^{ns}	0.01^{ns}
Genotypes	187	0.43^{ns}	56.51***	52.92***	42.54***	38.71***	31.61***	27.95***	747280^{ns}	126537**	32.32***	0.01*
Env*Gen	374	0.52^{ns}	41.16***	37.32***	32.89***	30.34***	28.39***	18.21***	900579**	146221**	9.71***	0.01***
ERROR	555	0.489	13.439	13.798	10.377	10.142	9.344	13.271	691449	113968	3.267	0.006

*, ** and ***= significance level at $p < 0.05$, 0.01 and 0.001 respectively, EGRV= Plant growth vigor, DFFL= days to first flower emergence, DFPD= days to first pod emergence, DFTFL= days to 50% flowering, DAP-75= days to 75% podding, DM= days to maturity, PLHGT= plant height, BYHA= above ground biomass ($kgha^{-1}$), SDWT= 100-seed weight, SYHA= yield in $kgha^{-1}$ and HI= harvest index.

Table 6.9: Means of yield and yield related traits and phenological traits under rainfed conditions in KATC, Muserech and KALRO-Perkerra

Genotypes	**EGRV (days)**	**DFFL (days)**	**DFPD (days)**	**DFTFL (days)**	**DAP-75 (days)**	**DM (days)**	**PLHGT (cm)**	**BYHA ($kgha^{-1}$)**	**SYHA ($kgha^{-1}$)**	**SDWT (g)**	**HI**
ICCX-060045-F3-P188-BP	1.50	46.00	51.33	56.17	65.50	83.83	27.90	3091.35	1310.35	23.42	0.44
ICCX-060045-F3-P111-BP	1.67	40.83	47.00	53.17	61.17	82.67	26.60	2572.42	1172.58	17.95	0.48
ICCX-060045-F3-P4-BP	1.50	42.00	47.33	54.67	63.50	84.00	28.60	2865.83	1137.67	25.19	0.40
ICCX-060045-F3-P118-BP	1.83	38.00	42.67	50.17	59.83	79.17	28.33	2269.58	1126.88	24.18	0.49
ICCX-060045-F3-P133-BP	2.00	43.00	48.67	55.17	64.17	82.33	29.18	2571.88	1121.38	24.60	0.44
ICCX-060045-F3-P61-BP	1.67	37.67	44.67	50.33	59.33	78.33	28.03	2455.80	1101.57	22.66	0.46
ICCX-060045-F3-P278-BP	1.50	42.67	48.50	54.17	63.33	81.17	27.70	2813.43	1092.35	25.00	0.38
ICCX-060045-F3-P230-BP	1.83	37.00	41.67	49.00	59.00	78.67	23.67	2700.83	1085.46	25.56	0.38
ICCX-060045-F3-P95-BP	1.17	41.83	47.67	53.33	63.00	78.33	25.92	2936.78	1078.72	24.91	0.39
ICCX-060045-F3-P175-BP	1.33	43.83	49.00	55.83	64.50	83.50	28.50	2839.78	1060.26	25.34	0.38
ICCX-060045-F3-P260-BP	1.67	47.50	52.00	58.50	67.33	87.67	28.67	2661.67	1047.64	24.17	0.40
Mean	**1.746**	**41.969**	**47.196**	**53.696**	**62.747**	**81.488**	**25.735**	**2195.693**	**803.124**	**23.590**	**0.373**
LSD ($p < 0.05$)	**0.141**	**0.741**	**0.751**	**0.651**	**0.644**	**0.618**	**0.736**	**168.102**	**68.247**	**0.365**	**0.016**

EGRV= Plant growth vigor, DFFL= days to first flower emergence, DFPD= days to first pod emergence, DFTFL= days to 50% flowering, DAP-75= days to 75% podding, DM= days to maturity, PLHGT= plant height, BYHA= above ground biomass ($kgha^{-1}$), SDWT= 100-seed weight, SYHA= yield in $kgha^{-1}$ and HI= harvest index.

Table 6.9: Continued… Means of yield and yield related traits and phenological traits under rainfed conditions in KATC, Museresh and KALRO-Perkerra

Genotypes	**EGRV (days)**	**DFFL (days)**	**DFPD (days)**	**DFTFL (days)**	**DAP-75 (days)**	**DM (days)**	**PLHGT (cm)**	**BYHA ($kgha^{-1}$)**	**SYHA ($kgha^{-1}$)**	**SDWT (g)**	**HI**
ICCX-060045-F3-P159-BP	2.17	45.00	49.83	55.50	64.67	83.00	30.50	3344.72	1044.30	23.36	0.34
ICCX-060045-F3-P76-BP	1.83	43.67	48.83	55.33	64.33	82.50	25.00	2903.35	1040.46	25.95	0.39
ICCX-060045-F3-P5-BP	1.33	44.67	50.33	56.50	64.17	82.67	28.10	2309.97	1040.27	23.32	0.44
ICCX-060045-F3-P139-BP	1.83	41.50	46.33	54.17	62.67	81.67	27.07	2415.92	1028.34	20.83	0.43
ICCX-060045-F3-P179-BP	1.67	45.17	49.67	56.17	65.33	84.83	28.38	2876.88	1024.68	24.19	0.37
ICCX-060045-F3-P176-BP	2.00	38.17	43.83	51.67	61.00	80.50	30.00	2689.02	1008.21	26.40	0.37
ICCX-060045-F3-P128-BP	1.50	41.83	46.83	53.50	62.83	82.33	28.73	2710.30	1002.70	27.07	0.41
ICCX-060045-F3-P27-BP	1.67	42.83	46.83	54.67	63.83	80.50	23.37	2461.02	989.17	22.93	0.40
ICCX-060045-F3-P91-BP	1.83	42.33	47.33	53.67	62.50	80.50	24.57	2525.25	982.85	26.84	0.39
ICCV 05107 (donor)	2.08	40.58	46.00	52.67	60.92	81.42	25.74	2174.62	754.02	25.85	0.35
ICCV 94954 (recurrent)	2.00	41.25	46.50	53.83	63.25	81.67	23.91	1845.34	663.56	23.59	0.36
Mean	**1.746**	**41.969**	**47.196**	**53.696**	**62.747**	**81.488**	**25.735**	**2195.693**	**803.124**	**23.590**	**0.373**
LSD ($p < 0.05$)	**0.141**	**0.741**	**0.751**	**0.651**	**0.644**	**0.618**	**0.736**	**168.102**	**68.247**	**0.365**	**0.016**

EGRV= Plant growth vigor, DFFL= days to first flower emergence, DFPD= days to first pod emergence, DFTFL= days to 50% flowering, DAP-75= days to 75% podding, DM= days to maturity, PLHGT= plant height, BYHA= above ground biomass ($kgha^{-1}$), SDWT= 100-seed weight, SYHA= yield in $kgha^{-1}$ and HI= harvest index.

There was no significant correlation between SYHA and SDWT under rainfed conditions. However, there was significant positive correlation ($P < 0.001$, $r = 0.389$) between SYHA and HI. A significant positive correlation ($P < 0.001$, $r = 0.356$) between SYHA and DM was observed. There was a highly positive significant correlation ($P < 0.001$, $r = 0.812$) between SYHA and BYHA. Phenological traits were significantly negatively correlated with yield traits. Correlations between the various phenological traits (EGRV, DFFL, DFPD, DFTFL, DAP-75 and DM) were positive among themselves (Table 6.10).

Table 6.10: Correlation among phenological and yield traits under rainfed conditions in KATC, Muserech and KALRO-Perkerra

	SYHA (kgha^{-1})	**SDWT (g)**	**HI**	**BYHA (kgha^{-1})**	**EGRV (days)**	**DFFL (days)**	**DFPD (days)**	**DFTFL (days)**	**DAP-75 (days)**	**DM (days)**	**PLHGT (cm)**
SYHA	-										
SDWT	0.055ns	-									
HI	0.389***	0.073*	-								
BYHA	0.812***	0.034ns	-0.164***	-							
EGRV	-0.249***	-0.027ns	-0.281***	-0.106***	-						
DFFL	-0.312***	-0.023ns	-0.222***	-0.198***	0.401***	-					
DFPD	-0.330***	-0.026ns	-0.232***	-0.213***	0.414***	0.976***	-				
DFTFL	-0.261***	-0.043ns	-0.264***	-0.129***	0.432***	0.931***	0.939***	-			
DAP-75	-0.348***	-0.024ns	-0.265***	-0.214***	0.468***	0.923***	0.942***	0.945***	-		
DM	0.356***	-0.129***	-0.162***	0.435***	-0.035ns	0.039ns	-0.003ns	0.159***	-0.035ns	-	
PLHGT	-0.045ns	0.133***	0.271***	-0.153***	-0.125***	-0.019ns	-0.009ns	-0.119***	-0.039ns	-0.514***	-

6.5.2 Heritability estimates (broad-sense)

Heritability estimates for various yield traits across environments (irrigated + rainfed), irrigated and rainfed conditions are presented in Table 6.11. Heritability estimates across environments for SYHA (0.138), BYHA (0.07), and HI (0.04) were low. However, high heritability for SDWT (0.999) was observed. Phenological traits had low to moderate heritability estimates that ranged between 0.04 - 0.505. The heritability for EGRV could not be determined. This was because the genotype variance was negative.

Heritability estimates were low under irrigated conditions for yield and its components. The heritability for SYHA was 0.245, SDWT was 0.335, BYHA was 0.283 and HI was 0.171. However, phenological traits had moderate heritability ranging from 0.435 to 0.536 but very low heritability was obtained for EGRV (0.07). This trend was similar to observations across environments. However, very high heritability was obtained for SDWT (0.999) across environments compared to irrigated condition (0.335) (Table 6.11).

Under rainfed conditions, heritability for most traits was also low. However, there was a high heritability for SDWT (0.700) close to heritability obtained across environments (0.999) unlike irrigated (0.335) conditions. The heritability for EGRV, SYHA, BYHA and HI could not be calculated as a result of negative genotype variance.

Table 6.11: Heritability estimates (h^2_b) for yield components traits under irrigated condition, rainfed condition and across environments

Traits	Irrigated	Rainfed	Across environments
EGRV	0.073	-	-
DFFL	0.536	0.272	0.493
DFPD	0.524	0.295	0.505
DFTFL	0.494	0.227	0.453
DAP-75	0.455	0.216	0.412
DM	0.435	0.102	0.355
PLHGT (cm)	0.321	0.349	0.327
BYHA ($kgha^{-1}$)	0.283	-	0.077
SDWT(g)	0.335	0.700	0.999
SYHA ($kgha^{-1}$)	0.245	-	0.138
HI	0.171	-	0.040

EGRV= Plant growth vigor, DFFL= days to first flower emergence, DFPD= days to first pod emergence, DFTFL= days to 50% flowering, DAP-75= days to 75% podding, DM= days to maturity, PLHGT= plant height, BYHA= above ground biomass ($kgha^{-1}$), SDWT= 100-seed weight, SYHA= yield in $kgha^{-1}$ and HI= harvest index.

6.5.3 Identification of QTL for yield and related traits

6.5.3.1 General features of genetic linkage map

A total of 49 SSR markers were mapped into eight linkage groups (LG) that spanned a length of 335.04 cM of the chickpea genome at an average marker density of 7.21 cM (Table 6.12). Linkage group three (LG 3) was the smallest linkage group (8.73 cM) and had few markers (3) with average marker density of 2.9 cM. Linkage group eight (LG 8) was the second smallest and spanned 28.06 cM with marker density of 7.0 cM. Linkage group two (LG 2) spanned 90.63 cM which was also the longest with marker density of 15.1 cM. Linkage groups one and seven (LG 1 and LG 7) were almost of the same length spanning 35.99 cM and 36.27 cM each with marker density of 11.9 cM and 5.2 cM, respectively. Linkage groups four and five (LG 4 and LG 5) were also similar in length spanning 41.28 cM and 41.29 cM respectively. These two had marker densities of 5.2 cM each. Linkage group six (LG 6) spanned 51.82 cM which was the second longest with a marker density of 5.2 cM.

Markers in common between this map and the map produced by Winter *et al.* (2000), Tar'an *et al.* (2007), Rehman (2009), Nayak *et al.* (2010) and Hiremath *et al.* (2012) are represented by asterisk and detailed in appendix 9.0. A total of 32 markers out of 45 were common with one or more of these maps while 17 new markers were mapped (Figure 6.2a, b).

Table 6.12: General features of the genetic map of chickpea developed from 49 SSR markers for 188 $F_{3:5-6}$ population for ICCV 94954 x ICCV 01507

Linkage group	Length (cM)	Number of mapped markers	Average marker density (cM)
LG1	35.99	3	11.9
LG2	90.63	6	15.1
LG3	8.73	3	2.9
LG4	42.25	8	5.2
LG5	41.29	8	5.2
LG6	51.82	10	5.2
LG7	36.27	7	5.2
LG8	28.06	4	7.0
Total/Average	**335.04**	**49**	**7.21**

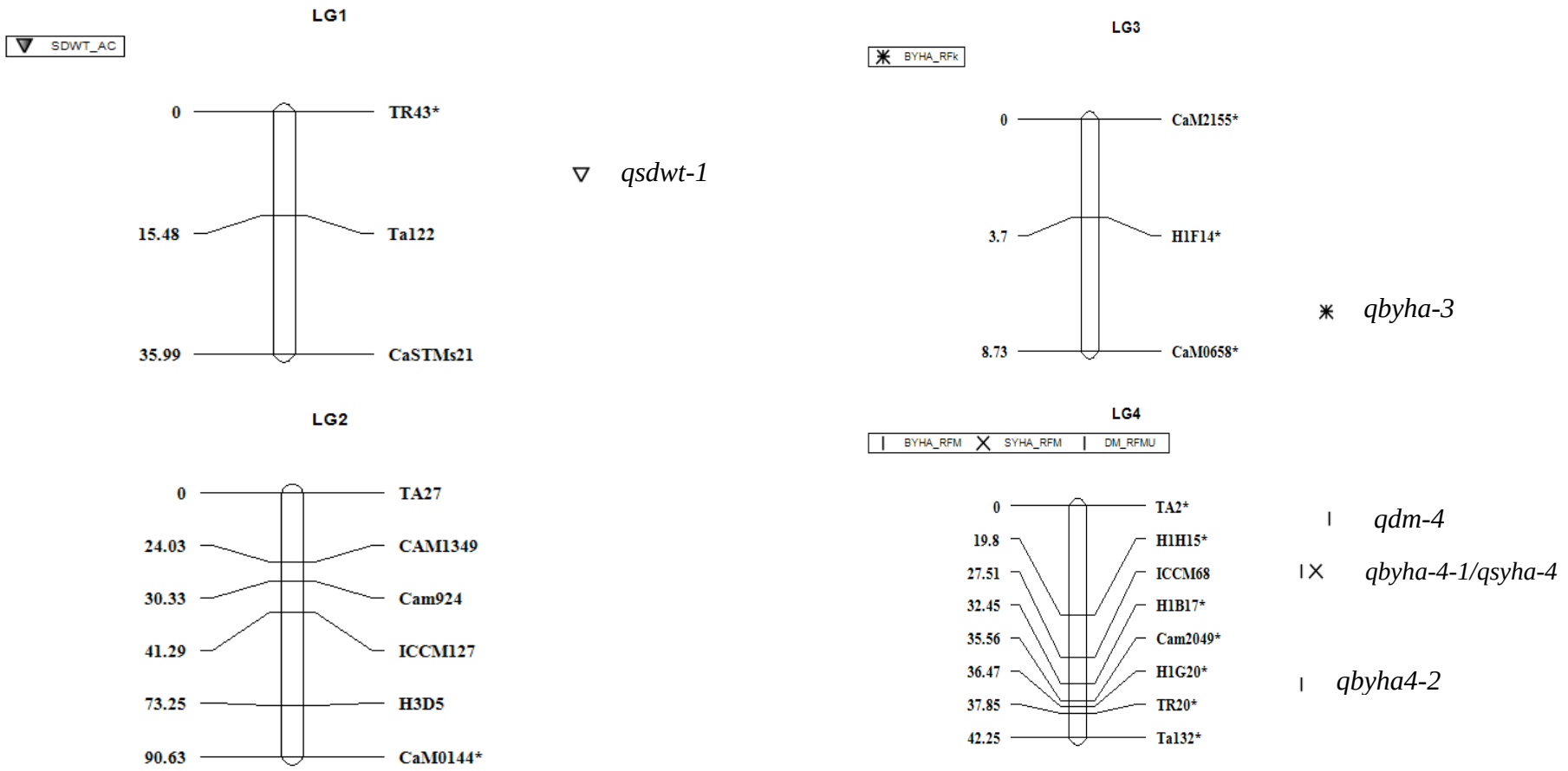


Figure 6.2a Linkage map showing QTL on LG 1, LG 3 and LG 4; *qsdwt-1* (QTL for 100-seed weight) *qbyha-3*, *qbyha-4-1* and *qbyha4-2* (QTL for above ground biomass) and *qdm-4* (QTL for days to maturity).

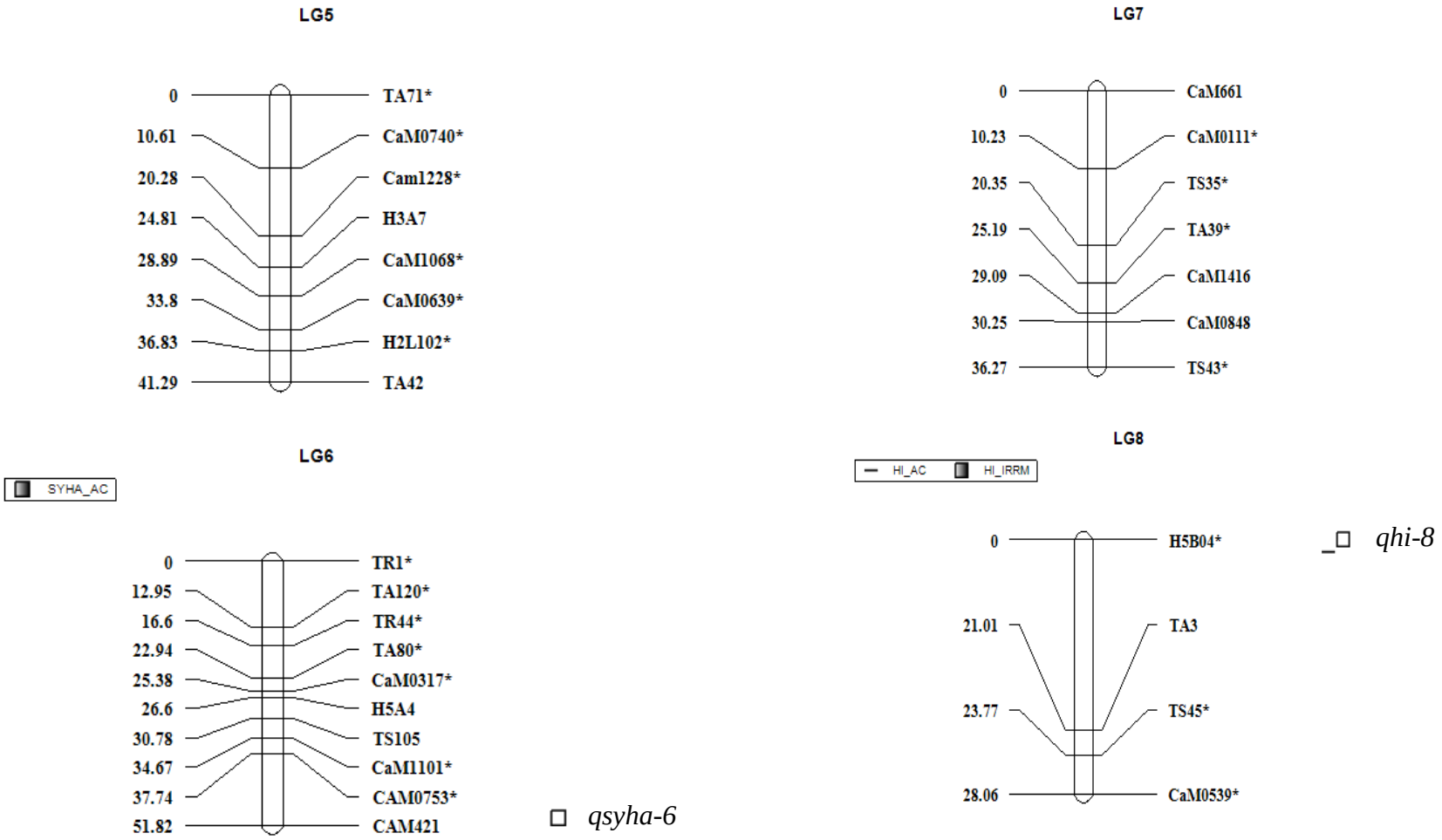


Figure 6.2b: Linkage map showing QTL on LG 6 and LG 8; *qsyha-6* (QTL for 100-seed weight), *qhi-8* (QTL for harvest index)
*; Markers mapped by Winter *et al.* (2000), Tar'an *et al.* (2007), Rehman (2009), Nayak *et al.* (2010) and Hiremath *et al.* (2012) (Appendix 9.0).

6.5.3.2 Mapping QTL for yield and yield related traits

A total of eight QTL were detected under different environments (Table 6.13). Three QTL were detected for BYHA under rainfed conditions at KATC and KALRO. One QTL, *qbyha-3,* was detected on LG 3 flanked by H1F14 - CaM0658 at interval of 5.03 cM with a logarithm of odds (LOD) of 3.3 and 8.7% phenotypic variation expressed (PVE). Two other QTL were detected on LG 4, *qbyha-4-1* and *qbyha-4-2*, between H1H15 - ICCM68 and H1G20 - TR20 respectively. The LOD values were 12.7 and 5.9 respectively. The QTL, *qbyha-4-1* had 32.5% PVE and was considered a major QTL while the other, *qbyha-4-2*, had 13.5% PVE and considered a minor QTL.

Two QTL for SYHA were identified, one under rainfed at KALRO and another across environments. Under rainfed conditions it was found on LG 4 (*qsyha-4*) between H1H15 - ICCM68 with LOD of 3.4 and PVE of 8.2%. This QTL was mapped on the same position with QTL (*qbyha4-1*) for BYHA under the same environment. A second QTL was found on LG 6, *qsyha-6,* was mapped between CAM0753 - CAM421 flanking markers, expressing 11.08% phenotypic variation with a LOD of 3.8.

One QTL for HI was mapped under irrigated conditions at KALRO on LG 8 (*qhi-8*) flanked by H5B04 - TA3 with a PVE of 9.9% and a LOD of 4.3. This was the same QTL identified on the same linkage group across environments. One QTL for SDWT was mapped on LG 1 (*qsdwt-1*) on position 8.0 between TR43 and Ta122 with a distance of 15.48 cM between the markers across enviroments. The LOD value was 3.2 and phenotypic variation expressed was 12.19%. One QTL for DM was mapped on LG 4 (*qdm-4*) under rainfed condition at Muserech which represented 13.3% phenotypic variation exppressed. The marker traits were TA2 - H1H15 with an average distance of 19.8 cM and a LOD of 4.3.

Table 6.13: Quantitative trait loci detected for above ground biomass (BYHA), grain yield (SYHA) days to maturity (DM), harvest index (HI) and 100-seed weight (SDWT), linkage group, position of mapped QTL, LOD, percentage variation expressed and contributing parent allele

Trait	QTL	Environment	Linkage	Interval markers	Interval	LOD*	Additive^	PVE (%)
BYHA	*qbyha-3*	KATC+Rainfed	LG3	H1F14 -CaM0658	5.03	3.2936	152.0293	8.6668
	qbyha-4-1	KALRO+Rainfed	LG4	H1H15 -ICCM68	7.71	12.7822	-51.9556	32.3926
	qbyha-4-2	KALRO+Rainfed	LG4	H1G20 - TR20	1.38	5.8727	-37.4196	13.4941
SYHA	*qsyha-4*	KALRO+Rainfed	LG4	H1H15 -ICCM68	7.71	3.3952	-13.0127	8.2405
	qsyha-6	Across[1]	LG6	CAM0753 - CAM421	14.08	3.8	-36.179	11.08
DM	*qdm-4*	MUS+Rainfed	LG4	TA2 - H1H15	19.8	4.2902	-0.5971	13.3181
HI[+]	*qhi-8*	KALRO+Irrigated	LG8	H5B04 - TA3	21.01	4.2585	0.0006	9.906
	qhi-8	Across[1]	LG8	H5B04 - TA3	18.25	3.4	-0.002	10.37
SDWT	*qsdwt-1*	Across[1]	LG1	TR43 -Ta122	15.48	3.2	0.038	12.19

Key: *= LOD score >3.0, ^= positive value denotes contribution from the female parent while a negative value is contribution from donor parent, SDWT= 100-seed weight, SYHA= grain yield and HI= harvest index. [1] =environment is defined by treatment (irrigation +rainfed) + location and HI[+]=the HI QTL was treated as one.

Contributions to the expressions of traits were mainly coming from the parent ICCV 05017 (donor) than ICCV 94954 (recurrent). The parent, ICCV 94954, contributed to high BYHA at KATC while the ICCV 05017 expressed more at KALRO. The contribution of ICCV 05107 was also similar to phenotypic observation across environments and under irrigated or rainfed conditions. However, contribution of ICCV 94954 to SDWT was mostly reductive as observed from phenotypic values.

6.6 Discussions

There were variations among genotypes under different environments but some were consistent. Plant vigour (EGRV) did not differ among genotypes indicating that all the genotypes had good growth at earlier stages. Leport *et al.,* (2006) found no differences among genotypes during the early growth stages. Good ground cover at an early stage also helps in reducing water loss through evaporation. The differences in number of days to maturity among genotypes were less than the differences in days to 50% flowering. This could be an indication that those plants that flowered late shortened the cycle in order to escape terminal drought. Reports indicate that one mechanism of drought adaptation in plants is maturing early (Gaur *et al.*, 2008). This had a penalty on yield as some genotypes yielded lower than the recurrent parent, which is in agreement with earlier findings (Caliskan *et al.*, 2008).

The overall performances of genotypes under rainfed conditions were lower than under irrigated conditions. Average yield for the best performing genotype, ICCX-060045-F3-P174-BP (1310.35 kg/ha) under rainfed conditions compared to the best genotype, ICCX-060045-F3-P188-BP (1502.11 kg/ha) under irrigated environments, translated to 14.63% yield loss. However, nine genotypes were 43% and above, higher yielding than the best parent (ICCV 05107). These lines could be recommended, after multi-location evaluations,

for short rainy seasons when rainfall is usually unpredictable. Six genotypes also showed stability across the environments (irrigated and rainfed conditions) and these were; ICCX-060045-F3-P188-BP, ICCX-060045-F3-P4-BP, ICCX-060045-F3-P159-BP, ICCX-060045-F3-P76-BP, ICCX-060045-F3-P179-BP and ICCX-060045-F3-P91-BP. Such genotypes can perform well under optimum and stress environments and could be recommended for both low dryland areas and dry highlands.

There was significant positive correlation between yield and SDWT, HI, BYHA and DM. However, in most cases the correlation was low except for BYHA that had 75% yield correlation. This is a clear indication that the four parameters determine chickpea yield and can be utilized for indirect selection of yield. Each of these traits has small effect contributing to yield increase. One hundred seed weight were reported to contribute positively to seed yield and that seed yield were correlated with number of seeds per plant and biomass (Talebi and Rokhzadi, 2013). Similarly other authors have reported similar observations (Sidramappa *et al.*, 2008; Kobraee *et al.*, 2010; Shamshi *et al.*, 2010; Biabani *et al.*, 2011). Phenological traits were all negatively correlated to yield indicating that these traits can adversely affect yield. Prolonged days to flowering may probably lead to more vegetative growth at the expense of flowers and pods and consequently lead to low yield especially under drought environment. This is because flowering and pod formation for such genotypes (late maturing) may coincide with terminal drought which could lead to substantial yield losses. Reported yield losses due to terminal drought ranged from 58-95% (Leport *et al.*, 2006). Further research by these authors indicated that time of pod set caused significant effect on yield where late podding resulted in smaller, fewer seeds per pod and consequently small seeds. Other findings indicated that phenological traits such as days to podding, days to maturity, and reproductive period were negatively correlated with seed yield (Sidramappa *et al.*, 2008).

Heritability of traits varied under rainfed and irrigated conditions and across the five environments. Heritability for SDWT was high under rainfed conditions (70%) and across environments (99.9%) compared to irrigated conditions (33.5%). This implies that the expression of high heritability of SDWT across environment was mainly from rainfed conditions. This indicates low environmental influence on SDWT due to stress conditions. In other research, a high heritability of 82% and 85.7% for SDWT was reported (Bakhsh *et al.*, 2007; Farhatullah and Khan, 2011) while in another study a heritability of 71% was reported (Abbo *et al.*, 2005). Malik *et al.*, (2009) reported a heritability of 99% while Thudi *et al.*, (2014) reported more than 90% heritability across all environments and locations and 67.1% under heat stress environment. This difference could be attributed to different genotypes used and environmental conditions under which the trials were conducted. In addition, heritability was reported to be influenced by several factors including type of genetic material, sample size, sampling method, conduct of research, calculation method and effect of linkage (Farshadfar *et al.*, 2008). High heritability of SDWT indicates that it could be considered for indirect selection for yield. The other components, SYHA, HI and BYHA had low heritability range of between 17% and 24.5%. This low heritability could be attributed to environmental influence and also interaction between genotype and environment. Yield is a complex trait controlled by many traits including HI and BYHA, SDWT and many others, each with additive effects on grain yield. It is also highly influenced by genotype x environment interactions (Kashiwagi *et al.*, 2008a) and thus selection for traits related to crop yield under drought was recommended than directly on yield (Krishnamurthy *et al.*, 2013). Heritability estimates for yield traits were not calculated under rainfed conditions due to negative genotypic variances. Similar results were reported in maize on days to anthesis and husk cover under low nitrogen (Ifie, 2013). Molecular approaches for traits that are difficult to

select have been in progress in the past few years. Good phenotypic data and polymorphic markers allow the identification of QTL and markers that are linked to the trait of interest.

A linkage map that spanned 335.04 cM in length was generated from 45 polymorphic markers out of 72 markers used to screen the parents; this was approximately 62.5% polymorphism. The low polymorphism could be attributed to the low genetic variability in chickpea as was reported earlier (Singh *et al.*, 2008; Chaturvedi and Nadarajan, 2010; Gaur *et al.*, 2012). In related findings 41% polymorphism between ICCV 96029 and CDC frontier was obtained while 78% polymorphism with sequence tagged microsatellite sites (STMS) between cultivated and wild chickpea was reported (Tekeoglu *et al.*, 2002). Rehman (2009) also found a similar polymorphism of 42%. Also, 307 SSR markers out of 2,409 (approx. 12.7%) were polymorphic between chickpea parents screened (Nayak *et al.*, 2010). The mapping of SSRs on the linkage groups was not different from what were mapped by others with similar markers (Winter *et al.*, 2000; Tar'an *et al.*, 2007; Rehman, 2009; Hiremath *et al.*, 2012). However, the orientation and distances differed. This could be attributed to the type and size of population used and number of markers. This therefore, resulted in less coverage of the map and consequently large intervals between markers of detected QTL. However, it was reported that with markers spaced about 10 cM to 15 cM apart, it is possible to identify few markers associated with the trait of interest if phenotypic data and QTL analysis was done well (Bernardo, 2008).

Eight QTL were detected on different regions of the chromosomes under different environmental conditions. Quantitative trait loci for above ground biomass (BYHA) were detected mainly under rainfed conditions, one on LG 3 while two others were detected on LG 4 by different markers. This could probably be a major and a minor QTL or it could probably be one QTL placed on a different location within the same LG due to marker recombination. One QTL for BYHA on LG 4 (32.4% PVE), was mapped on the same location with QTL for

yield (SYHA) (8.2% PVE) by the same markers H1H15 - ICCM68. The mapping of the two traits in the same region could indicate that this region might be a hotspot for yield traits and transferring this region will lead to varieties with multiple traits. Findings have shown that the LG 4 referred to as CaLG04 has been identified as '*QTL-hotspot'* region that harbors QTL for drought tolerant traits including several yield traits (Varshney *et al.*, 2014b). Thudi *et al.*, (2014b) reported 32 marker-trait associations (MTAs) for yield with a phenotypic variation of between 11.43 - 20.03%. QTL for yield traits were also identified on LG 1, LG 3 and LG 4 by Rehman, (2009). One additional QTL for SYHA was located in LG 6 (11.08% PVE) whose PVE was higher than QTL on LG 4 (8.9%).

A QTL for 100-seed weight was detected on LG 1 with a PVE of 12.19%. Seed weight was shown to be correlated with yield and HI hence is an important trait for selection. Other findings have mapped this QTL on other locations. Cobos *et al.* (2009) mapped two QTL for seed size on LG 2 and another on LG 4 associated with 32% PVE. QTL for seed weight was identified on LG 1 and LG 4 but considered less significant (Abbo *et al.*, 2005) while others were found on LG 4 and LG 8 that had 30.4% expression (Cobos *et al.*, 2007) and on LG 2 responsible for 14% PVE (Cobos *et al.*, 2009). Other findings indicated that a QTL for 100-seed weight was located on LG 1 and LG 4 explaining about 39% phenotypic variation (Hossain *et al.*, 2010) while previously 8 QTL for 100-seed weight were identified on LG 1, LG 3 and LG 4 each explaining between 6 - 13% phenotypic variation (Rehman, 2009). In a recent study, 70 multi-trait associations (MTAs) among 26 markers were identified for 100-seed weight that explained between 8.73 - 36.95% phenotypic variations and these MTAs were falling in '*QTL-hotspot'* region reported on LG 4 (CaLG04) (Thudi *et al.*, 2014b). In comparison with other findings there is a likelihood that QTL for seed weight are located in LG 1 with varying expressions among other LGs. There is need to pinpoint exact regions for future MAS applications.

A QTL for HI was detected on LG 8 with a PVE 9.9% and mapped on the same region across environments but the distances were slightly different and this could be due to recombination of the markers. This QTL was treated as one and there is the likelihood that the QTL for HI is in this region. Genotypes with high HI have better ability to partition the photosynthates into grain development during drought hence can result in better yield. Earlier findings reported that QTL for HI and drought tolerance score (DTS) were identified in LG 8 in the drier sites (Rehman, 2009). The author further noted that these linkage groups were also identified to have QTL for other traits such as stomatal conductance, canopy temperature and various phenological traits. The same LG 8 was also reported earlier to be associated with seed weight by Cobos *et al.* (2007) indicating that there is correlation between HI with seed weight and probably with drought tolerance traits. According to Thudi *et al.*, (2014b), 16 MTAs for HI were reported with phenotypic variation between 4.23 and 15.53%.

Days to maturity (DM) is also an important trait determining yield and drought tolerance mechanisms. Under drought stress plants shorten the cycle to escape terminal drought however, delayed maturity contribute to high yield under optimum conditions. From the results obtained, QTL for DM was detected on LG 4 (13.3%) sharing one marker with SYHA on the same linkage group. This could probably mean the two traits are linked. In addition, Rehman, (2009) mapped DM on LG 7 that was also associated with reproductive period. Five MTAs were identified for DM with one marker TA14 explaining 79.31% phenotypic variation (Thudi *et al.*, 2014b). Heritability for this trait was high indicating its importance for selection. Markers linked with DM and with negative effects are important for selecting genotypes with early maturity.

6.7 Conclusions

Several genotypes yielded higher than the parents across environments as well as under irrigated and rainfed conditions. Six genotypes, ICCX-060045-F3-P188-BP, ICCX-060045-F3-P4-BP, ICCX-060045-F3-P159-BP, ICCX-060045-F3-P76-BP, ICCX-060045-F3-P179-BP and ICCX-060045-F3-P91-BP performed significantly better than the best parent, ICCV 05017, across environments and under rainfed conditions. Under irrigated conditions, genotypes, ICCX-060045-F3-P174-BP, ICCX-060045-F3-P146-BP, ICCX-060045-F3-P23-BP, ICCX-060045-F3-P62-BP and ICCX-060045-F3-P46-BP, performed better than ICCV 05107. The genotypes listed above together with 20 high yielding genotypes should be evaluated in several locations alongside commercial checks for possible release as new varieties. There were positive significant correlations between yield and four other traits [100-seed weight (SDWT), harvest index (HI), above ground biomass (BYHA) and days to maturity (DM)]. These traits also exhibited high, moderate to low heritability estimates under different environments. A total of eight QTL were identified on linkage map spanning a total length of 335.04 cM, with marker density of 7.12 cM. Three QTL for BYHA were identified, one on LG 3 (8.67% PVE) and two on LG 4 (13.5 - 32.4% PVE), two for SYHA on LGs 4 & 6 (8.24 - 11.08% PVE) and one each for SDWT on LG 1 (12.19% PVE), HI on LG 8 (9.9% PVE) and DM on LG 4 (13.31%). However, more markers/genes need to be mapped in these regions. A highly saturated linkage map facilitates marker-assisted breeding as well as mapping of quantitative trait loci (QTL). Marker trait associations and genes associated with QTL for yield related traits will be useful for molecular breeding for yield in chickpea improvement. In addition selection of genotypes with high genetic value, based on identified QTL could be utilized for chickpea improvement through gene pyramiding by marker assisted recurrent selection (MARS).

CHAPTER SEVEN

7.0 General discussion, conclusions and recommendations

It has long been known that breeding drought tolerant chickpea with high yield is an economical, sustainable and viable option, yet not much progress has been made at developing varieties that meet farmers' needs. Drought remains a major challenge in chickpea production in Kenya. The main goal of this study was to generate information that would be useful to breeders for improvement of chickpea for drought tolerance. The objectives were to a) identify constraints to chickpea production and preferred farmer traits in varietal selection b) determine inheritance of drought tolerance traits and yield components in chickpea c) introgress drought tolerant root traits into Kenyan chickpea genotypes through marker assisted backcrossing and d) evaluate chickpea genotypes and identify quantitative trait loci (QTL) associated with yield under drought conditions.

A PRA study conducted in two regions in Kenya (Rift valley and Eastern) revealed that the major constraints affecting chickpea production were pest infestations, drought, birds' damage, lack of seeds and use of late maturing varieties in Bomet district (Rift valley). In Chepalungu, also in the Rift valley, diseases, pest damage and lack of training that tied in rank, drought and use of late maturing varieties were ranked as the most important constraints. In contrast, Mbeere South district (Eastern) had major constraints as; lack of market, drought, pest infestation and diseases. This is an indication that constraints are specific to regions since productions in the highlands (Bomet and Chepalungu) were constrained mainly by pest infestation, drought and varieties that were late maturing. This is because they plant chickpea as a relay crop during the time when rains are unreliable and hence increased pests' damage and terminal drought. They also needed varieties that were early maturing to avoid delaying operations for the main cropping season where they planted

maize and wheat. Farmers in Mbeere South planted chickpea for commercial purposes and therefore needed markets for their produce. In this district, the crop was planted during both seasons unlike in the highlands where chickpea was planted after the main commercial crop. The results from this study were in agreement with those from other crops which showed that needs and challenges of farmers were location specific (Ojwang, 2010; Were, 2011; Kiiza *et al.*, 2012). Other challenges identified in this study included the fact that farmers lacked seed and knowledge on chickpea production. Farmers relied on institutions for seed and from government organization such as Ministry of agriculture, livestock development and fisheries and non-governmental institutions and mainly mass media for training on chickpea production.

In addition, farmers in Bomet and Chepalungu districts preferred varieties that were high yielding, drought tolerant, early maturing, tolerant to pest [pod borers - *Helicoverpa armigera* (Hüb.)] and diseases, high germination percentage and good taste. On the other hand farmers in Mbeere South district preferred varieties that were high yielding, drought tolerant, resistant to field (pod borer) and storage pests (bruchids), early maturity and resistant to diseases. It was noted that the ranking of preferences in terms of importance differed among farmers in the districts. This was attributed to the diverse cropping systems, diverse needs, variety specific traits and constraints that are specific to a given agro-ecology. Bomet and Chepalungu are located in the dry highlands where chickpea is grown after main commercial crops unlike in Mbeere South which is located in the medium altitude semi-arid region and the crop is grown during both rainy seasons. The results from the study were in agreement with those from earlier studies which indicated that farmers preferred varieties that were disease resistant, early maturing, high plant vigour, good taste and high seed yield (Thagana *et al.*, 2009; Kaloki, 2010). Specific varieties and chickpea types (Desi and Kabuli)

were also ranked differently. Farmers generally preferred Desi types (e.g. ICCV 97105, ICCV 92944 and ICCV 00108) over Kabuli types (e.g. ICCV 00305 and ICCV 95423).

Breeding of chickpea varieties with farmer preferred traits (high yielding, drought tolerance, early maturity among others) requires knowledge of genes controlling these traits. During hybridization, recombination of alleles occurs and the aim is to have a variety with expressed desired genes. The second objective was to determine the gene effects controlling root traits and yield component(s) under rainfed conditions. As indicated earlier, chickpea is exposed to terminal drought which causes high yield losses. Root traits were identified to play a key role in chickpea adaptation to drought since root attributes enable the plant to mine water efficiently from deeper soil layers under dry environments. From this study, root traits from a cross between ICCV 00108 and ICC 8261 were controlled mainly by additive [a] gene effects. However, non-additive effects, dominance and epistasis (additive x additive, additive x dominance and dominance x dominance) also played a role in gene expression. Total root length (TRL) and RLD were controlled mainly by additive genes while root dry weight (RDW) was controlled by additive, dominance [d], additive x additive [aa] and dominance x dominance [dd] gene interactions. Root to shoot dry weight ratio (R/S) was controlled by additive genes and the three gene interactions ([aa], [ad] and [dd]). Shoot dry weight (SDW) was influenced by additive and additive x dominance [ad] gene effects. Previous work showed that [a] and [aa] gene effects were significant in RDW in ICC 283 x ICC 8261 and ICC 4958 x ICC 1882 and only [dd] was significant for ICC 283 x ICC 8261 (Kashiwagi *et al.*, 2008a). These results show that selection for root traits is recommended to be done at later generation in breeding programmes and large population size should also be maintained.

Yield is controlled by several component traits which are useful in indirect selection of the trait. Seed size (represented by 100-seed weight) was found to be influenced by additive effects. Non-additive gene effects, dominance and additive x additive, were also

significant. Seed size was reported to be influenced by additive and additive x additive gene effects by Kumhar, *et al.,* (2013) and Sharma, *et al.,* (2013) while dominant and additive gene effects were reported by Farshadfar, *et al.* (2008). Five genes controlling 100-seed weight were identified in this study and this was similar to the work reported earlier (Sharma *et al.*, 2013) while fewer genes were identified to control the expression of most root traits. Heritability was low for most of the traits and there was also presence of environmental variation.

Backcrossing methods have been recommended for introgression of desired traits. This is more advantageous for complex traits (root traits and yield) and with the aid of markers it is effective and takes shorter time to develop a variety. Two recurrent parents *Chania Desi II* (ICCV 92944) and *LDT 068* (ICCV 00108) were both crossed to a donor parent, ICC 4958. Although low polymorphic SSR markers were identified, those linked to a '*QTL – hotspot'* region were polymorphic among the F_1 backcrosses (BC_1F_1 and BC_2F_1). Yield components (seed weight per plant and 100-seed weight) for BC_2F_2 and root traits (RDW, SDW, PDW and R/S) for BC_2F_3 differed significantly in the two populations. The best 20 families from both crosses had higher means compared to the recurrent parents. Four families namely; EUC-03-BC_2F_3-P6-2-2-2-8, EUC-03-BC_2F_3-P22-1-2-7-8, EUC-03-BC_2F_3-22-1-2-7-13 and EUC-03-BC_2F_3-P6-1-3-9-2 expressed high TRL, RDp, RDW SDW and PDW in *Chania Desi II* x ICC 4958 compared to the recurrent parent. In terms of yield the families also had high seed weight per plant and 100-seed weight. In *LDT 068* x ICC 4958 cross, EUC-04-BC_2F_3-P52-1-4-7-20, EUC-04-BC_2F_3-P52-1-1-3 and EUC-04-BC_2F_3-P52-1-3-6-5 performed better than recurrent parent in terms of root traits but varied yield components.

Results also indicated that root traits and seed size (represented by 100-seed weight) were successfully inherited by the families as some recorded higher values than the donor

parent. The donor parent was reported to have large rooting system with large root biomass allocation (Kashiwagi *et al.*, 2005) and large seeds (Gowda *et al.*, 2011). Such successful introgression of root drought tolerance QTL in chickpea have been reported (Oyier, 2012; Varshney *et al.*, 2013a).

Heritability estimates for these traits were low and this could be attributed to environmental effects. High genetic correlations were also recorded among traits. Total root length (TRL) and RLD were positively correlated with RDW. Similarly SDW had high correlation with TRL, RLD and RDW of more than 50%. In addition, seed weight per plant had high positive correlation with number of seeds/plant. This means that SDW and number of seeds per plant are good indicators of root traits and yield, respectively, and could be used for indirect selection. These correlations are important in selection of traits that are difficult to breed/select for due to gene interactions and complex G x E interactions.

Drought was reported as a major constraint in chickpea production and high yielding genotypes under these conditions is highly desirable. The fourth objective was to evaluate chickpea genotypes and identify quantitative trait loci (QTL) associated with yield. Several genotypes (188 $F_{3:5-6}$) were evaluated under drought stress to identify high yielding potential lines. Six lines, ICCX-060045-F3-P188-BP, ICCX-060045-F3-P4-BP, ICCX-060045-F3-P159-BP, ICCX-060045-F3-P76-BP, ICCX-060045-F3-P179-BP and ICCX-060045-F3-P91-BP yielded more than 38% higher than the better parent, ICCV 05107, across the environments. This is an indication that they could do well under both irrigated and rainfed conditions. Under irrigated conditions genotypes, ICCX-060045-F3-P174-BP, ICCX-060045-F3-P146-BP, ICCX-060045-F3-P23-BP, ICCX-060045-F3-P62-BP and ICCX-060045-F3-P46-BP, performed twice more than the better parent. On the other hand ICCX-060045-F3-P188-BP, ICCX-060045-F3-P111-BP, ICCX-060045-F3-P4-BP and ICCX-

060045-F3-P118-BP had between 49% - 74% yield increase compared to better the parent under rainfed conditions.

Quantitatively inherited traits such as yield are highly influenced by G x E interactions (Kashiwagi *et al.*, 2008a) and selection can be based on correlated traits (Krishnamurthy *et al.*, 2013). Identification of quantitative trait loci using markers linked to complex traits such as drought tolerance and yield has been in progress (Varshney *et al.*, 2013b; Thudi *et al.*, 2014a). From the results obtained, there were positive correlations between yield and other traits (HI, BYHA, SDWT and DM). These traits were used alongside yield (SYHA) to identify QTL linked to them. A linkage map spanning total length of 335.04 cM was generated using 49 SSR markers. The average marker density was 7.12 cM and average intervals for the QTL between flanking markers ranged from 1.38 - 21.02 cM. Eight QTL where three for BYHA, one on LG 3 and two on LG 4 contributing from 8.67 - 32.4% phenotypic variation were mapped. In addition, two QTL for SYHA on LGs 4 & 6 that contributed from 8.24 - 11.08% phenotypic variation were mapped. One QTL each were mapped for SDWT on LG 1 that contributed 12.19% phenotypic variation, HI on LG 8 contributing 9.9% variation and DM on LG 4 contributing 13.31% of the variation. These QTL have been identified by other authors but some on different linkage groups. For example, 100-seed weight was identified on LG 2 with 12% PVE (Cobos *et al.*, 2009), LG 1 and LG 4 (Abbo *et al.*, 2005), LG 1 and LG 4 with 39% PVE (Hossain *et al.*, 2010). In the current study SDWT was identified on LG 1 (12.19% PVE) indicating that other than this LG other LGs also harbor SDWT trait. Yield was identified on LG 4, which also harbored QTL for biomass on the same location, and shared one marker with QTL for DM. Findings also reported this region to harbor several QTL for several traits including yield (Rehman, 2009; Thudi *et al.*, 2014b; Varshney *et al.*, 2014b). QTL for HI on LG 8 was also found to be linked with other traits including drought tolerance traits (Rehman, 2009). The identified QTL

however, had low PVE % showing that these traits are influenced by environment. Application of molecular markers linked to these traits and transfer of these regions into elite chickpea backgrounds will result in progress in chickpea improvement.

The major findings of the study were:

a) The major constraints affecting chickpea production were identified and found to be generally similar in the three regions but the ranking differed depending on cropping systems and agro-ecologies. These were; drought, pest infestation, late maturing varieties, diseases, lack of market and birds' damage. Farmers preferred chickpea that were high yielding, drought tolerant, early maturing, and resistant to pests and diseases.

b) Additive gene effects were highly significant for all root traits except total plant dry weight. Additive gene effects were also important in controlling 100-seed weight. Non - additive gene interactions for traits studied, except plant dry weight, total root length and root length density were also significant. Additive variances were higher than dominance variances indicating that these traits are fixable.

c) Through marker assisted backcrossing, root drought tolerant QTL was introgressed into two Kenyan chickpea varieties (*Chania Desi II* and *LDT 068*). Several developed lines had improved root traits compared to their parents. In addition, large seed size trait of the donor parent (ICC 4958) was also introgressed. Shoot dry weight was significantly positively associated with important root traits.

d) High yielding genotypes across environments (both irrigated and rainfed across locations) were identified. Also, genotypes specific to either irrigated or rainfed conditions, were identified. Eight QTL, three for biomass, two for yield, one each for harvest index, 100-seed weight and days to maturity, were identified under different environments.

Recommendations

There is a need to constantly involve farmers in the selection of varieties to take care of their preferences that are specific to their needs and localities which fit in their farming systems. Breeding for multiple traits will enhance adoption of developed varieties. Some socio-economic factors affecting chickpea production, such as lack of seed and market, need to be addressed for overall increased productivity of chickpea in Kenya. A survey covering wider localities is also necessary in order to involve more farmers due to wide and varied agro-ecological zones in Kenya.

The choice of breeding method largely depends on factors affecting inheritance of traits which is largely influenced by both additive and non-additive gene effects. It is recommended that a combination of methods such as recurrent selection and single seed descent (SSD) should be used when dealing with traits controlled by complex gene interactions. Large population should also be maintained and selection should be done at later generations.

The best 20 families identified with improved root traits from *Chania Desi II* x ICC 4958 and *LDT 068* x ICC 4958 through marker assisted backcrossing need to be advanced and evaluated alongside checks for possible identification and selection of superior lines. Genotypes identified through phenotypic evaluations need to be evaluated in multi-location trials for possible release as commercial varieties.

In future, drought tolerant lines developed through MABC and high yielding lines identified through phenotypic evaluation could be hybridized to pyramid these genes and develop multi-trait chickpea lines.

Markers/genes controlling drought and yield related traits need to be validated and deployed in breeding to aid conventional breeding methods hence reduce the time taken for breeding of complex traits. This will lead to improvement in chickpea yield production.

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APPENDICES

Appendix 1.0: Participatory Rural Appraisal Guiding questions: Focus Group Discussion

1. General information

a) County	
b) District	
c) Village	

2. What type of crops do you grow? List then rank

3. What acreage is under each crop? Indicate after ranking

4. Other than crops, do you keep livestock? List then rank.

5. What type of farming system do you practice? List then Rank

6. a) When do you plant? Which season (which is your main season)? General

b) When do you plant chickpea? Draw the calendar

c) Why do you plant during this season(s)? General reasons

d) What type of farming system do you practice in chickpea production? Rank

7. a) What type of varieties do you plant? List then rank

b) What are the preferred reasons for growing the varieties you mentioned? List then rank based on preference

c) What are the reasons for non preference of the other varieties? List then rank

b) Where do get the seed from? List then rank

8. a) How long have you planted chickpea?

b) How often do you change varieties?

i) If Yes, then suggest the scale below

ii) If yes, what are the reasons for changing varieties? List then rank based on importance

iii) If no, what are the reasons? List then rank (maybe opposite of above or other)

9 .a) i) What are the constraints in chickpea production? List them then ranks

ii) How have you been managing these constraints? List

10. a) Are there farmers in the group not planting chickpea? If yes would you accept if introduced?

b) If yes what qualities would you like them to have? List then rank

c) If not ready to accept, what are the reasons?

11. a) i) On average what is the yield. Suggest scale below (they can also give or number of bags)

Yld (kg/ha)	**Or no. of bags**
<100	<1
100-300	2-4
400-600	5-7
700-1000	8-10
>1000	>10

ii) How/what do you use chickpea for? List uses

b) On average what is the cost per kg? List If rate differ, indicate by no. of farmer

c) Are there factors determining cost? List and rank

d) Where do you sell? List and rank

e) When do you prefer selling and give reasons? List and rank each alongside.

f) What are your general comments about chickpea production in your area?

12. a) Do you receive any extension service? Who provides. List and rank

b) How do you get technical assistance from extension officers? e.g demonstration, field days etc List and rank.

13. What are the means in which you access/get information on farming activities/market? e.g mass media, electronic, face to face etc? List and rank

Appendix 2.0: Constraints to chickpea production by ranking method in Bomet and Chepalungu districts in the year 2012

	Bomet								Chepalungu					
	Kiplabotwa		Cheboror		Olbobo				Bing'wa		Chemeg'wa			
	M	F	M	F	M	F	MDS	Rank	M	F	M	F	MDS	Rank
Insect pests	4	1	4	2	1	1	3.8	1	4		2	2	2.5	2
Lack of training	-	-	6	-	-	-	0.2	10	-	-	1	1	2.5	2
Drought	-	2	3	3	2	3	2.8	2	3	-	3	-	1.5	3
Late maturity	3	5		4	-	2	1.7	5	1	2	5	-	2.5	2
Lack of seeds	6	-	1	1	-	-	1.8	4	-	6	-	-	0.3	7
Birds	2	-	2	5	3	4	2.3	3	-	4	6	4	1.3	4
Diseases	5	4	5	6	-	-	0.8	7	5	1	4	3	2.8	1
Market	1	3	6	-	-	-	1.5	6	6	6	-	5	0.8	5
Water logging	-	-	-	-	4	5	0.5	8	6	5	-	-	0.5	6
Threshability	-	-	6	-	5	-	0.3	9	-	-	6	6	0.5	6
Weeding	-	-	-	-	6	-	0.2	10	-	-	-	-	0.0	8

Key; M=Male, F=Female, MDS – Mean Derived Score 1=Highly Ranked; 6=Lower Rank, - = No response, A rank of 1 received a score of 5, 4 received 2, 3 received 3, 4 received 2 and 5 and above received a score of 1.

Appendix 3.0: Ranking of chickpea production constraints in Mbeere South district in the year 2012

	Ndia-Ndasa		Gategi		Maviani - Rurii		Maviani - Wavosyo		
	M	**F**	**M**	**F**	**M**	**F**	**F**	**MDS**	**Rank**
Lack of Markets	1	1	3	1	1	3	1	4.4	1
Drought	2	3	1	3	2	2	2	3.9	2
Pest infestation	2	4	1	3	3	1	3	3.6	3
Diseases (Blight)	3	2	-	2	4	4	4	2.4	4
Threshability	-	-	2	-	5	-	5	0.9	5
Lack of dehusking machine	-	-	4	-	-	-	-	0.3	7
Water logging	4	5	5	-	-	6	-	0.7	6
Poor timing at planting	-	-	-	-	-	5	-	0.1	8

Key; 1=Highest rank 6=Lowest rank, - = No response, MDS – Mean Derived Scores

A rank of 1 received a score of 5, 2 received 4, 3 received 3, 4 received 2 and 5 and above received a score of 1.

Appendix 4.0: General criteria for ranking of traits preferred by farmers in Bomet and Chepalungu districts in the year 2012

	Bomet District								Chepalungu District					
	Kiplabotwa		Cheboror		Olbobo				Bing'wa		Chemeg'wa			
Criteria	**M**	**F**	**M**	**F**	**M**	**F**	**MDS**	**Rank**	**M**	**F**	**M**	**F**	**MDS**	**Rank**
High yield	3	1	-	1	1	1	4.7	1	1	1	1	1	5.0	1
Drought tolerance	-	3	-	3	2	2	2.3	2		2	-	3	3.0	2
Earliness	4		4	2	4	3	2.2	3	4	4	2	2	3.0	2
Pest resistance	1	2	-	5	-	4	2.0	4	3	5	-	-	1.0	6
Disease resistance	2	-	-	4	-	5	1.2	6	2	3	-	-	1.8	3
Threshing ability	-	-	2	6	-	-	0.8	7	6		-	-	0.3	8
Good taste	-	4	1	-	3	-	1.7	5	5	6	4	6	1.3	5
Germination	-	-	3	-	7	-	0.7	8	7	-	3	4	1.5	4
Heavy seeds	-	-	-	-	8	3	0.7	8	-	-	5	5	0.5	7
Colour	-	-	-	-	9	6	0.3	8	-	-	6	-	0.3	8
Tolerant to waterlogging	-	-	-	-	6		0.2	10	-	-	-	-	0.0	9
Stability	-	-	-	-	5	7	0.3	9	-	-	-	-	0.0	9

Key; M=Male, F=Female, MDS – Mean Derived Score 1=Highly Ranked; 9=Lower Rank, - = No response, A rank of 1 received a score of 5, 2 received 4, 3 received 3, 4 received 2 and 5 and above received a score of 1.

Appendix 5.0: General criteria for ranking of traits preferred by farmers in Mbeere South district in the year 2012

	Ndia-Ndasa		Gategi		Maviani - Ririi		Maviani - Wavosyo		
Criteria	**M**	**F**	**M**	**F**	**M**	**F**	**F**	**MDS**	**Rank**
High yielding	1	1	1	1	1	1	1	5.0	1
Drought tolerance	2	2	2		2	2	2	3.4	2
Early maturity	3	4	2	1	-	-	-	2.0	4
Pest resistance to both field and storage	4	5	3	2	4	5	3	2.3	3
Tolerance to water logging	5	3	4	4	-	-	-	1.1	6
Easy to thresh and Winnow	-	-	-	-	3	-	5	0.6	8
Adaptability to intercropping	-	-	-	-	6	4	-	0.4	9
Resistance to diseases	7	7	5	3	5		4	1.3	5
Good taste	-	9	6	-	-	3	6	0.9	7
Colour	6	6	-	-	7	-	7	0.6	8
Soft testa	-	8	7	-	-	-	-	0.3	10

Key; M=Male, F=Female, MDS – Mean Derived Score 1=Highly Ranked; 9=Lower Rank, - = No response, A rank of 1 received a score of 5, 2 received 4, 3 received 3, 4 received 2 and 5 and above received a score of 1.

Appendix 6.0: List of forward and reverse primer sequences used in screening backcross progenies

Marker name	**Primer pairs (5' ⟶3')***	**Source**
NCPGR127	CATAATGCAAGGGCAATTAG/ CTCTTATCTTCATGTTGCCG	Gaur *et al.*, (2011)
NCPGR21	TCTACCTCGTTTTTCGTGCC/ TTGCTCCTTCAACAAAACCC	Sethy *et al.*, (2006)
*TA11	CATGCCATAAACTCAATACAATACAAC/ TTCATTGAGGACAATGTGTAATTTAAG	Winter *et al.*, (1990)
*TA113	TCTGCAAAAACTATTACGTTAATACCA/ TTGTGTGTAATGGATTGAGTATCTCTT	Winter *et al.*, (1990)
*TA118	ACAAGTCACATGTGTTCTCAATA/ GGAAAGGTTAAGAAATTTTACAATAC	Winter *et al.*, (1999)
TAA170	TATAGAGTGAGAAGAAGCAAAGAGGAG / ATTTGCATCAATGTTCTGTAGTGTTT	Winter *et al.*, (1990)
GA24	TTGCCAAAACCAATAACTCTG/ TCCCTTTTACACAAGGCCAG	Winter *et al.*, (1990)
ICCM0249	TTTCTTCGCATGGGCTTAAC/ GGAGATTTGTTGGGTAGGCTC	Nayak *et al.*, (2010)
*M13-CaM0204	GAAGACAAAGTAATTACACATCCTCA/ TGCACACATTCTTTCACGCT	journal.pone.oo27273.s001
CaM1903	TGTGATGCAACCTAACAGTCA/ CCATGTACACTTACACGGTAGAAGA	journal.pone.oo27273.s001

*Additional markers at BC_1F_1. Forward and reverse primers are separated by a slash respectively.

Appendix 7.0: DNA extraction procedure

1) 2 mg of dried leaf samples was placed into tubes containing 3 mm diameter steel bead (2 each), 560 µl of buffer (PL1) and 2 µl of RNAse was added and the tubes were capped
2) The grinding was done in a genogrinder (EL Lyzer from Genetix) 2-5 times at 300 rpm and vortexed then repeated the process twice for 2 minutes.
3) The tubes were then placed in water bath at 65 ºC for 45 minutess
4) The samples were centrifuged at 5000 rpm for 20 minutes
5) The contents were kept at -20 ºC for 20 minutes and centrifuged at 6200 rpm for 12 seconds
6) 450 µl of binding buffer (PC) was dispensed into MN square well block, added 400 µl cleared of lysate (supernatant) of each DNA sample and mixed by repeated pipetting
7) The lysate was transferred into binding plate and sealed with gas permeable foil (optional)
8) The contents were centrifuged at 5870 rps for 10 minutess
9) Washing with silica membrane: 400 µl of PW1 was added to each nucleospin plant II binding plate and centrifuged for 3 minutes at 5870 rpm, 700 µl of PW2 was added and centrifuged for 3 minutes at 5870 rpm repeated twice
10) Eluting DNA: Nucleospin plant II binding plate was placed on was placed on rack of tube strips and 50 µl of preheated buffer PE (70%) was dispensed to each well and centrifuged for three minutes at 5870 rpm and this process was repeated twice, DNA is collected in the new fresh tubes.

Appendix 8.0: List of 49 SSR polymorphic markers used in genotyping 188 F_3 families

1	TR43	18	H1B17	35	CaM0317
2	Ta122	19	H1H15	36	H5A4
3	CaSTMs21	20	TA2	37	TS105
4	ICCM127	21	TA71	38	TR1
5	Cam924	22	CaM0740	39	TS43
6	CAM1349	23	Cam1228	40	CaM0848
7	TA27	24	H3A7	41	CaM1416
8	H3D5	25	CaM1068	42	TS35
9	CaM0144	26	H2L102	43	TA39
10	CaM2155	27	CaM0639	44	CaM0111
11	H1F14	28	TA42	45	CaM661
12	CaM0658	29	CAM421	46	H5B04
13	ICCM68	30	CAM0753	47	TA3
14	TR20	31	TR44	48	CaM0539
15	H1G20	32	TA120	49	TS45
16	Cam2049	33	CaM1101		
17	Ta132	32	TA80		

Appendix 9.0: Markers mapped on generated genetic map by other authors

Marker	**LG**	**Authors**
CaM0111	LG 2	Nayak *et al.*, (2010)
CaM0144	LG 1	Nayak *et al.*, (2010)
CaM0317	LG 5*	Nayak *et al.*, (2010)
CaM0539	LG 8	Nayak *et al.*, (2010)
CaM0639	LG 8, LG 5	Nayak *et al.*, (2010), Hiremath *et al.*, (2012)
CaM0658	LG 3	Hiremath *et al.*, (2012)
CaM0740	LG 5	Nayak *et al.*, (2010)
CAM0753	LG 6	Hiremath *et al.*, (2012)
CaM1068	LG 5	Nayak *et al.*, (2010)
CaM1101	LG 6	Nayak *et al.*, (2010)
Cam1228	LG 5, LG 7	Nayak *et al.*, (2010)
Cam2049	LG 4	Nayak *et al.*, (2010)
CaM2155	LG 2, LG 5	Nayak *et al.*, (2010)
H1B17	LG 4	Hiremath *et al.*, (2012)
H1F14	LG 3	Hiremath *et al.*, (2012)
H1G20	LG 4	Hiremath *et al.*, (2012)
H1H15	LG 4	Hiremath *et al.*, (2012)
H2L102	LG 5	Hiremath *et al.*, (2012)
H5B04	LG 8	Tar'an *et al.*, (2007), Rehman, (2009), Hiremath *et al.*, (2012)
TA120	LG 6	Tar'an *et al.*, (2007)
Ta132	LG 4	Tar'an *et al.*, (2007), Rehman, (2009)
TA2	LG 4	Winter *et al.*, (2000), Rehman, (2009), Hiremath *et al.*, (2012)
TA27	LG 2	Hiremath *et al.*, (2012)
TA3	LG 8	Tar'an *et al.*, (2007), Hiremath *et al.*, (2012)
TA39	LG 5	Tar'an *et al.*, (2007), Winter *et al.*, (2000), Rehman, (2009), Hiremath *et al.*, (2012)
TA71	LG 5	Hiremath *et al.*, (2012)
TA80	LG 6	Tar'an *et al.*, (2007), Winter *et al.*, (2000), Rehman, (2009), Hiremath *et al.*, (2012)
TR1	LG 6	Tar'an *et al.*, (2007), Hiremath *et al.*, (2012)
TR20	LG 4	Hiremath *et al.*, (2012)
TR43	LG 1	Tar'an *et al.*, (2007), Hiremath *et al.*, (2012)
TR44	LG 6	Tar'an *et al.*, (2007), Winter *et al.*, (2000), Rehman, (2009), Hiremath *et al.*, (2012)
TS35	LG 5	Tar'an *et al.*, (2007), Winter *et al.*, (2000), Rehman, (2009), Hiremath *et al.*, (2012)
TS43	LG 5	Tar'an *et al.*, (2007), Hiremath *et al.*, (2012)
TS45	LG 8	Hiremath *et al.*, (2012)