PHENOTYPIC, PHYSIOLOGICAL CHARACTERS AND QUANTITATIVE TRAIT LOCI IN F2 GENERATION OF *Phaseolus vulgaris* UNDER DROUGHT STRESS IN MACHAKOS COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any other University or for any other award.

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Declaration by Supervisors:

We confirm that the work reported in this thesis was carried out by the student under our supervision.

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DEDICATION

This thesis is dedicated to the memory of two special people; my late mum - Lydia Kirinyet and Liz Cherono - my beloved late daughter.

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
CIAT	International Centre for Tropical Agriculture
CORR	Correlation
CTAB	Cetyl-Trimethyl Ammonium Bromide
DSI	Drought Susceptibility Index
DW	Dry Weight
EAR	Economic Review of Agriculture
EDTA	Ethylene diamine tetra acetic acid
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
GBS	Genotyping-by-Sequencing
GLM	General linear model
GWAS	Genome-Wide Association Study
KALRO	Kenya Agricultural and Livestock Research Organization
LSD	Least Significant Difference
MAB	Marker Assisted Breeding
MAS	Marker Assisted Selection

MOALF	Ministry of Agriculture Livestock and Fisheries
MT	Metric tons
PROC	Procedure
QTL	Quantitative Trait Loci
RIL	Recombinant Inbred Lines
SAS	Statistical Analysis System
SNP	Single Nucleotide Polymorphism
SPAD	Soil Plant Analysis Development
SPSS	Statistical Package for Social Science
SSD	Single Seed Descent
TES	Tris + EDTA + Sodium chloride
WUE	Water Use Efficiency

ABSTRACT

Currently, yield of common bean (*Phaseolus vulgaris* L.) in smallholder systems in Kenya is comparatively low; standing at 400 kg ha^{-1} against global average of 960 kg ha^{-1}. The low yield is attributed to drought which has become a major constraint affecting more than 60% of common bean production across the world. The most effective measure to mitigate effects of drought on common bean production is development of drought tolerant genotypes. Hence, there is need to select for drought tolerant genotypes from the local germplasm of common bean for hybridization. The current conventional method used in screening for drought tolerant bean genotypes involve long duration of field testing. Therefore, there is need to use other alternative method like, marker assisted selection (MAS) using DNA markers linked to quantitative trait loci (QTL) that could quicken the selection process and subsequent development of drought tolerant genotypes. The objectives were to; i) assess phenotypic and physiological characters associated with drought tolerance, ii) characterize F2 (KAT B1 × GLP2) population for drought tolerance using phenotypic and physiological characters, iii) determine QTLs associated with drought tolerance in an F2 (KAT B1 × GLP2) population of common bean. A series of experiments were carried out to elucidate the phenotypic and physiological characters and map quantitative trait loci (QTL) for drought tolerance in common bean. Six genotypes; KAT B1, GLP2, Nyota, Angaza, Faida and Metameta were assessed in a rain-out shelter under water stress for phenotypic

and physiological characters related to drought tolerance. Characterization of phenotypic and physiological characters was also carried out in F2 population consisting 116 individuals previously developed from KAT B1 × GLP2 cross. The F2 population comprising of 120 lines derived from KAT B1 and GLP2 were mapped for quantitative trait loci (QTL) linked with drought tolerance. The experiments were laid out in a randomized complete block design replicated three times. Stomatal conductance and stem biomass showed greatest variation among genotypes and had significant positive correlations with yield per plant under water stress. Estimates of expected genetic advance indicated that high gains and progress are attainable on genotypic selection for characters such as days to maturity, pod number plant^{-1}, stomatal conductance and leaf water potential at 34.97 %, 22.32 %, 22.87 % and 25.46 % respectively. High estimate values for heritability in broad sense were also obtained for these characters. A genetic linkage map with a total length spanning 634 cM of the common bean genome at an average distance of 2.02 cM between adjacent markers was generated using 229 single nucleotide polymorphism (SNP) markers. A total of twenty three QTLs were detected for the branch numbers, pod numbers, grain number per pod, stem biomass, leaf biomass, pod biomass, days to flowering and maturity, 100-seed weight, seed yield, stomatal conductance and leaf water potential. Two putative QTLs involved in drought adaptation mechanisms were detected; SCO4.1^{KG} associated with stomatal conductance on chromosome Pv04 and LWP1.1^{KG} associated with leaf water potential chromosome Pv01. Overall, this research provided insight into the genomic regions (QTLs) controlling traits related to drought tolerance in common bean. Given the long duration taken in developing improved common bean cultivars using conventional phenotypic selection, the identified QTLs should be incorporated in marker assisted selection in screening and selection of high yielding and drought tolerant genotypes hence, hastening common bean improvement efforts.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Common bean is grown over a wide range of environmental conditions across the world where it is exposed to great variations in soil moisture contents and seasonal droughts. Due to climate change, drought has become a major constraint affecting more than 60 % of common bean production across the world (White and Castillo, 1992; Beebe *et al*., 2009). In the main growing regions in Latin America where cultivation is done under rain-fed conditions, the yields are reported to often fall from 1.2 t ha^{-1} to below 0.53 t ha^{-1} as a result of intermittent drought (Fageria *et al*., 2014; de Faria *et al*., 2018). In Eastern Africa region, common beans are very vulnerable to diseases outbreak, weather fluctuations and soil fertility decline. This has led to a negative effect on the overall production (Katungi *et al*., 2010; FAO, 2018). In 2007, common bean were grown in approximately 25 million hectares across the globe producing 18.4 million metric tons, whereas in 2020, 33 million metric tons were produced worldwide in about 39 million hectares (FAO, 2020).

In Kenya, cultivation is primarily done by smallholder farmers under rain-fed conditions mainly for subsistence use. However, surpluses with market value of 850 million shillings are marketed accounting for 25 percent of total annual production (GoK, 2016). According to the MoALF (2019) area under bean cultivation was 1.036 million hectares

which produced 577,674 tons in 2011, whereas approximately 747,254 tons were produced in 1.162 million hectares in 2018 (Table 1.1). The steady increased in acreage under bean cultivation in Kenya over the last decade (Table 1.1) is directly linked to the recent unpredictable rainfall patterns as a result of climate change. Delayed rains increases the risk of cultivating the staples like maize and therefore most farmers prefer growing beans to lower the risk of total crop loss (Legesse *et al*., 2006).

Year	Area (Ha)	Production (90 kg bags)	Production (Tons)	Yields (Bags/Ha)	Yields (Tons/Ha)
2007	846,327	3,455,512	383,900	4.8	0.4
2008	610,428	2,901,237	261,111	4.8	0.4
2009	960,705	5,170,696	465,363	5.4	0.5
2010	689,377	4,339,980	390,598	6.3	0.6
2011	1,036,738	6,418,596	577,674	6.2	0.6
2012	1,056,046	6,919,544	622,759	6.6	0.6
2013	1,083,604	7,938,805	714,492	7.3	0.7
2014	1,052,408	6,844,357	615,992	6.5	0.6
2015	1,006,106	8,658,156	779,234	8.8	0.8
2016	1,171,710	8,090,667	728,160	7.8	0.7
2017	1,153,846	8,033,215	703,661	7.4	0.6
2018	1,162,015	8,221,018	747,254	8.7	0.8

Table 1.1: Common bean production trends in Kenya 2007-2018

Source: **MoALF,** Ministry of Agriculture, Livestock and Fisheries (2019).

Most of the varieties grown in Kenya exhibit high variability in adaptation, growth habits, maturity, plant size, seed shape and colour (Kimani *et al*., 2005). The common types in terms of acreage (accounts for 75 % of the total area) and market preferences are the red mottled beans (Korir *et al*., 2005). Agro-ecological conditions and market forces, are the main factors which attribute to spatial distribution of seed types. Rose coco is the

dominant variety grown in the major producing counties of Kakamega, Busia, Siaya, Nakuru, Bomet, Elgeyo, Muranga and Kirinyaga because of the market preference and as a major component in the local diet as well as a relatively low input production costs compared to other bean types (Korir *et al*., 2006, Katungi *et al*., 2010). In lower Eastern Kenya; Machakos and Makueni, farmers prefer Mwitemania because it is an early maturing variety and gives a significant yield during the October-December season when the rainfall is adequate. Although Mwitemania and Canadian wonder are high yielders, the area under these varieties has been diminishing because of the challenges associated with fertility of the soil, poor rainfall distribution and the emergence of serious diseases (Nyabundi *et al*., 1995; Allen *et al*., 1996). The newly released bean varieties in market such as Cianku and Tasha (KEPHIS, 2011) are early maturing, richer in iron and zinc, low in acidity, broad adaptability, tolerant to diseases and pest (Korir *et al*., 2006). However, they are low to moderately tolerant to drought which has been identified as the biggest challenge to production and yield stability of common bean and has been reported to be endemic in most of the agro ecological zones in Kenya. As a result, the yields are low and rarely exceed 400 kg/ha. Under favourable production conditions in Kenya, most of the high yielding varieties produce an average of 860 kg/ha (Kimani *et al*., 2007; Katungi *et al*., 2010; FAO, 2018).

More than 60 % of the total area under common beans lies in agricultural land prone to water deficits causing severe yield reduction that may total up to 90 % (Katungi *et al*., 2010). Reports show that only 34 % of the area in the growing zones in Kenya get sufficient precipitation (Ngetich *et al*., 2014) implying that there is significant exposure

of the crop to the risk of drought. The effects of drought stress are often aggravated by inadequate and unpredictable precipitation, high temperatures, incidences of high levels of solar radiation, as well as soil with low fertility (Tiwari *et al*., 2017). Thus, in Kenya irrigation cannot be a practical alternative to ameliorate the effects of drought in such areas given the ever growing scarcity of water resources and competition for them. As such, common bean production in Kenya is done solely under rain-fed environments (Katungi *et al*., 2010). Therefore, drought mitigation measures focusing on improving ways by which the common bean crop absorb limited moisture in the soil and to use it efficiently in crop development, accumulation of the biomass and pod filling are needed to ensure sustained crop productivity in marginal areas (Beebe *et al.*, 2013).

Screening common bean cultivars for important economic characters, such as tolerance to water stress has been one of the key objective in common bean genetic improvement program by CIAT since 1993 (Broughton *et al*., 2003; Beebe *et al*., 2008). In Kenya, KALRO-Katumani and KALRO-Thika have been at the forefront in developing varieties that are drought tolerant since the 1970s. In 1984, three lines; GLP×1127, GLP×92 and GLP×1004 with tolerance to water stress were identified at KALRO-Thika and they were recommended for production in areas prone to drought such as lower Eastern Kenya (Nyabundi *et al*., 1995). However, among the released varieties none received market preference, hence, subsequent breeding efforts at KALRO-Katumani focused on large seeded red mottled types that are palatable and highly preferred in the market (Kimani *et al*., 2007). Between mid-1990s and early 2000s, among the new genotypes released were four large seeded beans namely; KAT X56, KAT X69, KAT B1 and KAT B9 (Kimani *et*

al., 2007). However, due to the ongoing changes in global climate, accompanied by unprecedented environmental phenomena these varieties no longer guarantee good performance in marginal areas as before due to their susceptibility to severe water deficit stress, hence, the need for further research particularly focusing on the development of genotypes with better drought tolerance traits that will ensure stable production. It is currently held that, an efficient and effective selection procedure will enhance the development of improved genotypes since the conventional breeding method that rely on phenotypic selection has not been successful for selecting superior genotypes (Asfaw *et al*., 2012; Padilla-Chacón *et al.*, 2017). Knowledge about the inheritance of important agronomic and physiological characters as well as the integration of genomic method in common bean breeding programs would make it easier and faster to select superior genotypes through locating QTLs that contribute to water stress tolerance (Alvares *et al*., 2016).

In this study, genotypes with contrasting drought tolerance were used in the comparative analysis. Comparing the genetic expression pattern of important traits associated with the drought stress is certainly a strategy to identify the traits that may be responsible for contributing towards tolerance ability. The current study aimed at providing valuable information that might be used to screen and select drought tolerant common bean varieties for agricultural zones with frequent soil water deficit.

1.2 Statement of the problem

In Kenya, the current on farm yields is comparatively low; standing at 400 kg of grain per hectare against the global average of 960 kg per hectare (FAO, 2018). Low yield is generally contributed by a number of factors, for instance the declining fertility of the soil, intensification of drought due to climate change and the outbreak of diseases in major common bean producing regions of the country and the low yield potential genotypes which are also drought susceptible. Hence, there is a great need to identify elite genotypes through screening the local germplasm of common bean in order to select high yielding, drought tolerant varieties for cultivation in water stress areas as well as using them in hybridization programme as parents. However, one of the main challenges hindering this process is the lack of an efficient selection technique.

The current conventional method used in the identification and selection of common beans is normally a highly protracted procedure involving several years and long duration of field testing. It normally take an average of 11 years to screen and select a variety that is superior (White and Singh, 1991; Blair *et al*., 2007). Therefore, selection for drought tolerance using conventional selection methods take a substantial period of time to come up with a well-adapted cultivar and also requires huge investment in terms of resources. Furthermore, problems associated with environmental sensitivity combined with complex physiological interactions often reduces the potential of associating traits to agronomic benefits. In addition, the phenotypic measurements often do not reveal the genetic potential of the plant due to influence by the environment. Thus, any tool that will help in

shortening the procedure for screening and selection as well as reveal the true plant genotype is necessary.

1.3 Justification

Owing to the long duration expended to identify common bean genotypes with superior characters, the genomic tools particularly the molecular marker technology has the potential of making screening and selection activities in crop breeding programs quite efficient and effective. The identification of markers that can facilitate the selection of genotypes with drought tolerant traits at an early stage of crop development program would ensure rapid release of a new variety (Mukeshimana *et al*., 2014). Besides offering an opportunity to select for traits of interest, markers linked to complex traits can also be useful in a negative selection programme to select against negative characteristics. The utilization of marker technology especially QLT mapping in the study of drought tolerant traits of common bean genotypes is, however very limited in East Africa, particularly Kenya. As such, the application of marker-assisted selection (MAS) in common bean crop breeding programmes has not been realized (Mukeshimana *et al*., 2014; Lobaton *et al*., 2018).

Therefore, identification of QTLs controlling tolerance to water stress in common beans is prerequisite to implement marker-assisted selection (MAS). Evaluation of germplasm under water stress would enhance the process of identifying major traits associated with tolerance to water stress. Hence, facilitating screening and selection of drought tolerant

genotypes at early generations (viz., F2 generation), thereby cutting cost of running field trials, space as well as saving on time.

1.4 Hypotheses

i. Phenotypic and physiological characters are associated with drought tolerance in selected common bean genotypes.

ii. Characterization of F2 population (KAT B1 × GLP2) for drought tolerance can be achieved using phenotypic and physiological characters.

iii. Quantitative trait loci (QTL) are associated with drought tolerance in an F2 (KAT B1 × GLP2) population of common bean.

1.5 Objectives

1.5.1 General objective

To evaluate the phenotypic, physiological and quantitative trait loci (QTL) for efficient and rapid screening and selection of common bean genotypes to improve production.

1.5.2 Specific objectives

i. To assess phenotypic and physiological characters associated with drought tolerance in selected common bean genotypes.

ii. To characterize F2 (KAT B1 × GLP2) population for drought tolerance using phenotypic and physiological characters.

iii. To determine QTLs associated with drought tolerance in an F2 (KAT B1 × GLP2) population of common bean.

1.6 Significance of the study

The findings obtained may be used by the plant breeders as a basis for screening and selecting drought-tolerant common bean genotypes. The basic genetic information established has a broad relevance in related studies in other crops. The QTLs controlling tolerance to drought in common beans detected in this study will offer a good insight of the genetics of drought adaptation traits to the plant breeders and therefore, will accelerate the process of selection and development of tolerant varieties of crops using marker assisted selection (MAS). Thus, the farmers will benefit from adoption of improved varieties much faster and quickly resulting in increased production.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of common bean

The wild relatives of the modern common beans originated from Central and South America; in the region stretching from Northern Mexico to Northwestern Argentina where it was cultivated as early as 6000 BC (Gepts, 1998). The Mesoamerica and the Andes gene pools are two distinct isolated evolutionary lines of common beans that exist at the agro-ecological centers of origin. Studies have indicated a higher diversity which exist among Mesoamerica form compared to the Andean form (Gepts, 1998). Evidence from comparative morphological and molecular relationship (Gepts, 1998; Cortés *et al*., 2013) are useful in distinguishing the gene pools. The two diverged eleven thousand years ago (Velasquez and Gepts, 1994). In general, the seeds of the Mesoamerican form are small with the weight of 100 seeds averaging at 25 g and known to have a phaseolin type I, a special type of storage protein (Beebe *et al*., 2001; Wortmann, 2006). The Andean lineage are characterized by phaseolin type II and comparatively larger seeds weighing more than 40 g/100 seeds (Cortés *et al.,* 2013).

2.2 Biology of common bean

Common bean variably termed as dry or kidney bean, belongs to the family leguminosae. Generally, a warm season herbaceous crop falling under the species, *vulgaris*. They are mostly twining, climbing vines extending up to 3-6 m high while some forms are sub-erect herbaceous bush growing up to 22-55 cm in height (Gentry, 1969; Webster *et al*.,

1977). They bear alternate, green, pinnately compound trifoliate leaves. The leaflets are smooth-edge 7 cm wide and 11 cm long. Flowers are either axillary or terminal arranged in pairs or solitary in 15-35 cm racemes. The plant is largely self-pollinated, however, if an insect coated with pollen come in contact with the stigma, cross-pollination is possible (Wortmann, 2006). Each flower gives rise to one pod, once they are fertilized. The pod may contain 5-13 seeds that vary widely in size and the seed colour are either red, black ,white, brown or mottled depending on the variety (van Schoonhoven and Voysest, 1991). Bush bean is by far the predominant type cultivated across Africa (Beebe *et al*., 2001; Buruchara, 2007). The life cycle duration for the determinate types of common beans ranges from 60-90 days, while that of the indeterminate climbing types ranges from 250-300 days. Harvesting of green pods takes place 15-20 days after flowering and the yields ranges between 5 and 7.5 tons/ha (Wortmann, 2006). If they are grown for dry beans production, it requires another 25-30 days for grain filling. The potential yield for dried beans is up to 3.8 tons per hectare however, average for small holder farms is 0.5-1.5 t/ha (Wortmann, 2006). Padilla-Chacón *et al.* (2017) reported that the green biomass of common beans is 1.6 kg/m^2 translating to 16 tons of green biomass per hectare.

2.3 Production of common bean

2.3.1 Agronomic requirements

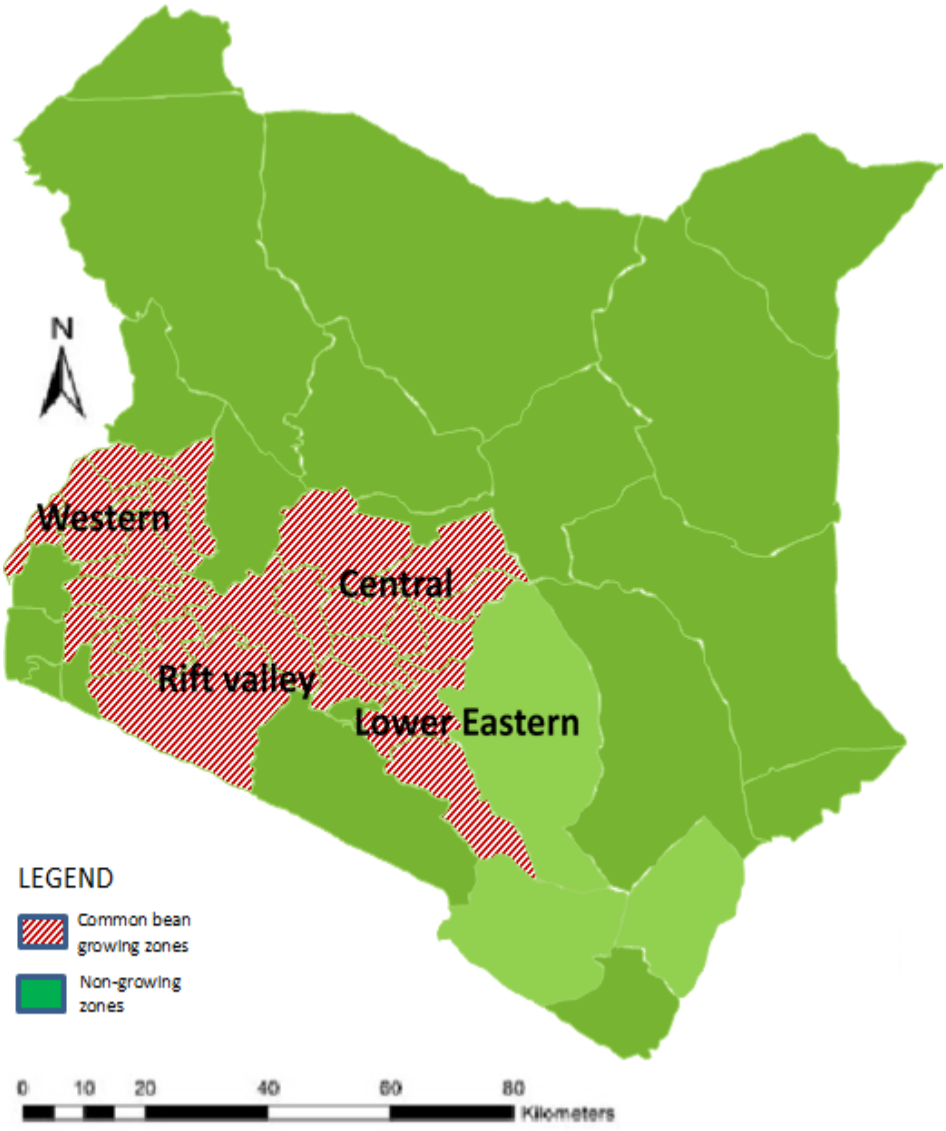
Beans are affected by exposure to long periods of low temperatures at any stage of growth, hence cold places are unsuitable for cultivation of the crop (Wagara and Kimani, 2007; Asfaw *et al*., 2013). Usually with adequate soil water, the crop is not affected by high temperatures, although pollination is inhibited by high nocturnal temperatures.

Moderate precipitation (550 mm) is required throughout a growing season but in the periods during and after flowering adequate amounts are essential. Most common bean varieties take 45 to 100 days from emergence to physiological maturity therefore considered a short-season crop (Buruchara, 2007). Climbing varieties are suitable for cooler high altitude areas as their growth cycle could extend for more than 180 days from establishment (Graham and Ranalli, 1997; Gomez *et al*. 2004). Common bean does well on a wide variety of soil characterized by good drainage, high content of humus and pH ranging from 6.5-7.5 (Wortmann *et al*., 1998). Most cultivars do not withstand water logging. In Kenya, the crop cultivation is done in areas rising to elevation of 1000-2200 m above sea level in the warmer mid-elevation such as lower Eastern, East and West of Rift Valley and Nyanza regions (Figure 2.1).

2.3.2 Common bean growing zones in Kenya

The main bean growing zones of Central, Western and Rift Valley (Figure 2.1) normally experience two rainfall patterns in a year coinciding with cropping seasons for common beans. The main sowing season run between May and August whereas short rain season between October and December. However, in the lower Eastern parts of Kenya (Figure 2.1) the rains between October to January are considered too unreliable to get a good yield, therefore their main sowing season is from March to May because it is reliable and adequate.

Figure 2.1: Common bean production zones in Kenya. Source: Google earth map pro. (2019).



2.4 Economic importance of common bean

Common bean is one of the most widely consumed legumes in Kenya, accounting 40 % of protein in the local diet (Kavoi *et al*., 2016). It contain essential amino acids like isoleucine and threonine in relatively low concentrations whereas moderate concentration of leucine, lysine, methionine and valine are found. However, tryptophan, isoleucine and phenylalanine are found in high concentration (Bressani and Elías, 1984). Common bean is also excellent source of fibre, contain phenolic anti-oxidants and composed of slow-release carbohydrates (Spilsbury *et al*., 2004; Shenkut and Brick, 2003; McDougall, 2017). Common beans appear to significantly reduce chances of developing diseases like cancer, heart diseases and diabetes because it is cholesterol free and low in fat content. Most health organizations recommend regular consumption of common bean (Leterme and Munoz, 2002). The slow digestion of common beans causes gradual increase in blood sugar making it an excellent appetite suppressant. Research has also found it to be effective in managing weight loss due to its ability to delay the reappearance of hunger (Bailey *et al*., 2015; Díaz-Gómez, 2017). It has the potential of alleviating malnutrition because of its high nutritional value, making it an important complement in carbohydrate-rich foods such as maize, rice and cassava (Anjali, 2015). In Kenya, common bean is appreciated and consumed by several rural and urban households because it is comparatively cheap protein source than animal protein hence it is recognized as the “meat of the poor” (Pachico, 1993).

Common bean is eaten either as green immature pods, boiled dry grains and/or green leaves, hence useful in staggering food supply. The green immature pod is mostly liked

because of its good taste and relatively short time for preparation (less than 40 minutes) (Pachico, 1993). In several regions around the world the pre-cooked bean seeds are canned either with tomato sauce or alone. It is generally eaten with a wide range of recipes like *Githeri* (mixture of beans and maize) or as cooked dry grains. It may also be made into soup or mashed and served with stew. Consumers' acceptance of a particular bean variety is influenced by the pigmentation and size. The red colour imparted on the food after cooking makes the large reddish coloured bean as the most preferred by the consumers (Katungi *et al*., 2010). In some specific locations where there is a variety of bean seeds, trade-off between some superior traits and the seed colour is quite likely. For instance, varieties with good flavour are preferred by the consumers (Okwiri *et al*., 2005). In other areas, leaves are eaten as vegetable (Hillocks *et al*., 2006).

2.5 Constraints to common bean production and mitigating strategies

Abiotic stresses particularly unfavorable temperatures, drought, soil acidity and low soil fertility continue to pose threat to production of beans in several farming systems across the globe (Acosta-Gallegos and White, 1995; Lizana, *et al*., 2006; Zupin *et al*., 2017). The expansion of bean production into more marginal environments in Africa and Latin America exposes the crop to more threats from abiotic stresses (Ariani and Gepts, 2015; Briñez *et al*., 2017). Furthermore, the recent global climatic changes has created extreme environmental conditions for bean production that may require serious investments in the development of bean cultivars better adapted to high temperatures and water stress (Porch, 2008; Battisti and Naylor 2008). Greater adaptation to abiotic stresses may help bean producers to obtain good bean yields in regions with less ideal conditions (Miklas *et*

al., 2006). Though common beans require moderate fertility [N (0) P (40 kg ha^{-1}) and K (20 kg ha^{-1})], constraints associated with soil factors such as low fertility and acidity often limit production in many places in the world. Despite common bean being susceptible to drought stress, the production of this crop in different regions across the world is carried out under water stress conditions. This is due to insufficient moisture supply. Common bean require an average of 500 mm/growing season by rainfall or irrigation (Wortmann and Allen, 2003; Hillocks *et al*., 2006). This high variability may pose huge challenges in field screening for these traits. In addition to the difficulties associated with the evaluation of beans for abiotic stress tolerance, is the overall effects of the genotype × environment interaction in bean performance (Brick *et al*., 2008; Beaver *et al*., 2008; Binagwa *et al*., 2018).

To identify breeding lines with superior performance for traits such as tolerance to abiotic stress, breeders have to work with replicated field trials and larger populations. Further, several cycles of selection and the use of multiple parents as sources of favourable alleles may be needed if any significant progress is to be realized (Muñoz-Perea *et al*., 2006; Brick *et al*., 2008; Beaver *et al*., 2008). The development of genomic tools like molecular marker technology has opened opportunities leading to the identification of traits associated with abiotic stress tolerance by the bean breeders (Ishitani *et al*., 2004). It should be noted, however, that the ability to detect the link between the markers and the different traits will greatly determine the effectiveness of marker-assisted breeding. Ideally, the genetic variability associated with the abiotic stress tolerance to a large

extend should be explained by the markers and that the genotype × environment interaction should not greatly affect the expression of the trait.

2.6 Drought response

2.6.1 The physiology of drought response

Plants exposed to drought stress show several physiological changes; stomatal conductance, leaf and water potential that are important to the plant in mitigating stress (Anjum *et al*., 2017). Typically, drought occurs mainly due to low and less-frequent precipitation, leading to soil water deficit that is difficult to be extracted by plant roots (da Silva *et al*., 2013). Severe stress can cause significant reduction in plant water status due to insufficient water absorption caused by low soil moisture and the increase in loss of water during transpiration process. Drought stress responses of flowering plants are physiologically complex regulatory process involving a number of quantitative traits, which has made breeding for drought tolerance in the common bean very problematic (da Silva *et al*., 2013). Normal physiological processes are affected by reduced plant water status hindering their growth and development, causing senescence and wilting, finally leading to death of the plant (Anjum *et al*., 2017).

The effect of drought is exacerbated by high temperature stress, which promotes plant water loss through increased evaporation (Polania *et al*., 2016). Drought responsive traits in common bean have been the subject of study in various field experiments and in more controlled greenhouse environments (da Silva *et al*., 2013). The association between drought stress and traits related to yield has continued to receive much attention, because

of their importance as yield output indicators and their measurability in a large germplasm. The drought response among bean genotypes has been characterized on basis of physiological processes like photosynthesis, photosynthate acquisition and partitioning indices (Costa-França *et al*., 2000). Basic physiological processes like cell growth and photosynthesis are indirectly affected by drought through decreased stomatal conductance during early stages of stress development leading to limited evaporation and less diffusion of CO_2 in the leaf mesophyll. During long periods of drought stress, certain biochemical changes at the cell level enables better drought tolerance and yields. Under drought stress tolerant genotypes produce higher yield compared to the susceptible genotype due to fine regulation of stomatal movement and the ability to assimilate CO_2 with stomatal closure. This ensures limited loss of water in the daytime and maintenance of relatively high water content in the night. Increased efficiency in water use as well as limited accumulation of the reactive oxygen species by the tolerant genotype also explain its superior performance under water stress (Rosales *et al*., 2013; Ruiz-Nieto *et al*., 2015). Adaptation studies has enabled the identification of genotypes with drought tolerant traits, making it the basis for further studies on the mechanisms of tolerance to water stress (Hieng *et al*., 2004; Zupin *et al*., 2017).

The ability of plants to tolerate drought stress is related to its pattern of water consumption (Costa-França *et al*., 2000). Certain genotypes are known to display water spending behaviour such as maximization of transpiration, enables the plant to thrive under adequate water supply and produce high yields (Zupin *et al*., 2017). Genotypes with high adaptation to harsh environmental conditions have developed mechanisms for

conservative water use during drought ensuring their yield are less affected. The ability to save water by drought tolerant genotypes is linked to plasticity at cellular and biochemical levels, high photosynthetic rate, high stomatal conductance and photo-inhibition (Lizana *et al*., 2006). Genotypes with high partitioning indices under drought stress indicate better ability to distribute photosynthates to major sinks such as the developing pods and seeds hence, better yield (Beebe *et al*., 2008, 2013).

2.6.2 Response to drought at the molecular level

It is very important to understand the various reactions occurring in the plants' metabolic pathways due to drought stress and the processes regulating stress adaptation in order to identify key markers that might assist in distinguishing genotypes with different levels of tolerance (Montalvo-Hernández *et al*., 2008). Blair *et al.* (2011) analyzed the transcriptomic changes and phenotypic responses in two common bean cultivars, one susceptible and one tolerant, exposed to well watered and water stressed conditions. The up-regulated genes with higher expression in in the leaf during the flowering and grain-filling developmental stages of the drought tolerant bean cultivars was the cysteine-rich secretory, antigen 5 and Pr-1 (CAP) superfamily protein, both potential targets for multiple stress breeding. Drought stress affects plants at the molecular level through different ways: by altering the gene expression, modifying post translational regulation that leads to activation of proteins and by directly acting on the protein itself (Hieng *et al*., 2004; Ariani and Gepts, 2015). These actions are interdependent and only key proteins that are active enable a response that is beneficial for the plant.

Modern transcriptomic and proteomic tools have greatly facilitated the screening of genes with varied expression and proteins that changes in abundance or activity in plants subjected to drought stress. Recent advances in sequencing and the publication of full genome sequences have greatly contributed to the identification of genes and proteins of model legume, barrelclover (*Medicago* truncatula) and of crop legumes, such as, common bean (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum*) (Young *et al*., 2011; Jain *et al*., 2013; Wu *et al*., 2014). Classification of genes and proteins obtained from screening studies according to their known ontologies is important in order to investigate their connections and interactions in metabolic pathways using systems biology and bioinformatics methods. A combination of these approaches has led to the identification of several genes known to have differential expression and proteins with changed abundance in common bean under drought stress (Zadražnik *et al*., 2013; Schmutz *et al*., 2014; Zadražnik *et al*., 2017).

2.6.3 Mechanisms of adaptation to drought in common bean

Common bean grow in a wide range of habitats thus, is exposed to seasonal droughts and fluctuating soil moisture availability. Therefore, several adaptation strategies encompassing diverse mechanisms have been developed by the crop that allows maintenance of plant water status within reasonable range to ensure normal metabolic functions under drought stress (Beebe, 2012; Beebe *et al.,* 2013). As such, an individual

trait may not be a good indicator of drought tolerance since the yield under drought stress is jointly influenced by a number of traits (Kao *et al.,* 1994).

An increase in the synthesis of abscisic acid, is one of the immediate responses triggered by water deficit stress, leading to stomatal closure, thus, causing reduction in photosynthetic activity. Moreover, as a defense mechanism, a set of genes such as *EREBP/AP2* and bZIP that are involved in a series of regulation processes are released by the plant to overcome water deficit stress (Hoyos-Villegas *et al*., 2017). Therefore, several mechanisms must integrated in a breeding programme to ascertain drought tolerance in breeding cultivars. The drought tolerance mechanisms have been categorized into three groups, namely, drought escape, drought tolerance, and drought avoidance (Levitt, 1980).

2.6.3.1 Drought escape

Drought escape is the ability of the plant to complete its developmental stages before the setting in of serious soil and crop water deficits **(**Bray, 1997**)**. This mechanism is characterized by high rate of development of phenological events such as early flowering and early maturity and photosynthates remobilization into the grain. According to Ouyang *et al*. (2017), remobilization of photosynthate to the grain at the onset of drought could be particularly important to permit grain filling as stress increasingly become acute at the end of the season.

Phenotypic plasticity is also another mechanism that contribute to increased performance under drought (Acosta-Gallegos and White, 1995). This particular trait, enables genotypes to dramatically shorten their growing cycle thus escaping drought conditions later in the growing season. Early maturity may offer a bean crop a chance to escape drought. However, pattern of water availability determine their relative usefulness (Amede *et al*., 2004). According to Pastenes *et al*. (2004), earliness is only beneficial for crops grown where soil moisture was adequate early in the season. Flowering time is the most critical stage for optimizing adaptation, hence, yield in environments with varying water availability and distribution in a growing season (Beebe *et al.*, 2013). The most important criterion for improving adaptation to drought in common bean is through matching crop phenology with the environmental factors and rainfall patterns (Acosta-Gallegos and White, 1995). According to White *et al*. (1994), the shoot biomass based on strong general combining ability effects as well as high heritability estimates, leaf nitrogen concentration and a strong positive correlations with seed yield appear to be the most promising indicators of adaptation to drought. Limited studies have reported the correlation involving a number of traits that confer short field duration (earliness), high grain yield and drought tolerance in common bean genotypes. Weller *et al*. (2019) attempted to carry out a study in this regard using a few number of scores to characterize the grain yield and phenological data to determine shorter field duration.

Agronomic characters like the grain number per pod, pod number per plant and 100 seed-weight are some components of yield used in quantifying drought tolerance in common bean (Beebe *et al.*, 2013). According to Board and Maricherla (2008), remobilization of

photosynthate (stem/leaf reserve to grain filling) is measured using trait indicators like pod harvest index (PHI), pod partitioning index (PPI) and harvest index (HI) and grain filling index (GFI). Harvest index (HI) is a key trait to identify genotypes with superior adaption to drought stress through better mobilization of photosynthates (Beebe *et al.*, 2013).

The components of HI can be distinguished as pod partitioning index (PPI), stem biomass reduction (SBR) and pod harvest index (PHI) (Beebe *et al.*, 2008). PHI is measured as the ratio of pod biomass at harvest in relation to that of canopy biomass (CB) value at mid-pod filling growth stage when the vigor of the genotype is believed to be at its maximum. SBR indicates the extent of mobilization of stem reserve to grain filling. It is the value of stem biomass at harvest in relation to that of stem biomass at mid-pod filling. According to Klaedtke *et al.* (2012), a genotype that is able to combine all the above three plant traits with greater canopy biomass or shoot vigor values should yield higher compared to the other genotypes under drought stress conditions.

2.6.3.2 Drought avoidance

Drought avoidance refers to the plant's ability to uphold high potential of water in their tissues, despite a reduced soil moisture the availability **(**Bray, 1997**)**. This is realized through a deep and extensive rooting system, decreased evaporation surface, reduced water loss through decreased leaf conductance, leaf movement/rolling that enables reduced absorption of radiation as well as increased hydraulic conductance (Beebe *et al.*, 2013).

The most important and natural mechanism that enables plants to reduce water loss is a reduced total leaf area. This is connected with inhibited expansion of developing foliage, small-size young leaves, reduced number of leaves, and /or loss of leaves due to senescence, all these are counterproductive in terms of grain yield as a result of a reduced photosynthetic area (Acosta-Gallegos and Kohashi-Shibata, 1989). However, an increase in leaf thickness and the ratio of surface area of mesophyll to total leaf area may compensate for the reduced area (Broughton *et al*., 2003; Muñoz-Perea *et al*., 2006). Adaptive leaf mechanism including greater amounts of leaf pubescence, lower leaf temperature, leaf reflectance and leaf movements are important in creating tolerance along the path of moisture movement leading to reduced loss of water from leaf cells to air. Studies have confirmed importance of extensive root growth in ensuring less stress of the plant due to drought effects through permitting exploration of a greater soil volume to extract more moisture (Ramirez-Vallejo and Kelly, 1998; Traub *et al.,* 2017). Rooting systems among common bean species show considerable variation in architecture (Lizana *et al*., 2006). However, specific genotypes exhibited great variation in response to a certain environment (Acosta-Gallegos and White, 1995).

Stomatal conductance which is a measure of water vapor flux from the surface of the leaf to the atmosphere is usually measured with a portable leaf porometer. It plays a crucial role in the regulation of plant water balance and therefore determines efficiency of water use (Builes *et al.,* 2011). However, it is difficult to get accurate measurements of stomatal conductance in a relatively large population of plants due to the fluctuation of the main environmental factors like wind, solar radiation and humidity that are known to affect

stomatal conductance. Water use efficiency (WUE) is another important attribute that determines the performance a crop under terminal drought conditions (Passioura, 2007). It explains the plant's rate of production of assimilate relative to the rate at which water is lost to the atmosphere.

2.6.3.3 Drought tolerance

Drought tolerance is the ability of the plant to cope with low tissue water potential despite soil water deficit (Assefa *et al.*, 2017). Inherent drought tolerance is due in part to the ability to maintain turgor through increased cell elasticity, osmotic adjustment, desiccation tolerance by protoplasmic tolerance and decrease in size of the cell. This strategy involves coordinating the physiological and biochemical changes that occur at the cellular and molecular levels and the separation of ions in the plant (Blair *et al*., 2012). Reducing the number of stomata play a great role in common bean plant tolerance to drought by reducing water loss through transpiration hence, helping in the conservation of soil moisture. The sunken stomata help to minimize water loss by reducing movement of air over the stomata, thereby creating a humid microclimate, resulting in reduced rate of evaporation and the water potential gradient. On the other hand, CAM mode of photosynthesis help the plant to conserve water because the stomata stay closed during the day minimizing water loss and light energy converted to chemical energy (Fowler, 1984; Schmid *et al*., 2008; Töpfer *et al*., 2020). Differences in osmotic adjustment have been reported among the common bean varieties (Miklas *et al.*, 2006). However, tissue elasticity is considered to be more important than osmotic adjustment as the reduction in tissue elasticity leads to better extraction of soil moisture (Muñoz-Perea

et al., 2006). Under water stress environment, bean plant cells accumulate three kinds of osmotica: salts, small organic solutes and hydrophilic, to help in osmotic adjustment.

Complexities related to measuring and interpreting effects of osmotic adjustment and changes in tissue elasticity have prompted researchers to attempt correlating the effects of turgor maintenance to rates of tissue elongation. A positive and highly significant correlation was found between drought tolerance index and seedling elongation in 25 bean cultivars (White and Singh, 1991). Desiccation tolerance is also another mechanism contributing to drought tolerance. Mitochondrial and chloroplast membranes appear to be sensitive to desiccation and water loss that causes formation of lesions in the membranes (Omae *et al*., 2012). Estimation of the overall cell membrane stability under desiccation have been attempted through use of simple conductivity measurements. However, none of these approaches has been reported in common bean experiments (Muñoz-Perea *et al.,* 2006).

2.7 Sources of traits for adaptation to drought

Common bean is a relatively sensitive legume to abiotic stress. However, remarkable progress has been achieved towards breeding for tolerance to various abiotic stresses. The intra-specific crosses that take advantage of the naturally occurring variability within *Phaseolus vulgaris* has led to improvement in drought tolerance (Beebe *et al*., 2008). However, further progress in drought tolerance could be realized through making inter-specific crosses with other species of the genus *Phaseolus*. Several species of the genus *Phaseolus* that are cross compatible with common bean are found in different ecological

regions ranging from tropical rainforest to arid desert (Allen *et al*., 1998). Some of the secondary gene pool which can be readily crossed with common bean include wild species; tepary bean (*Phaseolus acutifolius*) and its wild relative *Phaseolus parvifolius* which are a good source of traits for hot and dry climates as it evolved in semi-arid or arid environments (Beebe *et al*., 2008).

2.7.1 Genetic sources for improving drought tolerance

Different roots and shoots traits are responsible for improved drought adaptation in common bean. Deep and extensive root system ensures maximum uptake of water while on the other hand decreased leaf conductance enables optimal use of absorbed water to produce grain during drought stress (Beebe *et al.,* 2013). White and Castillo (1992) evaluated the yield of common bean genotype whose shoot had been grafted onto selected root of diverse origin and grown under drought. The effect of the union on the general growth and yield of common bean under drought stress condition was found to be quite small. Haghighi and Ascher (1988) examined the mechanisms of the root system's effect on leaf water status in a graft between *Phaseolus vulgaris* as the scion and *Phaseolus acutifolius* as the stock, and found that genotype of the root determines the water potential of a plant. Modifying the root systems by selecting and breeding for beneficial root traits offers an inexpensive way to improve the productivity of drought stress common bean (Thung *et al*., 1999).

Field screening has led to identification of common bean lines that are relatively drought-tolerant such as BAT 477, A 195 and BAT 1289 (Sponchiado *et al.*, 1989; White and Singh, 1991). Preliminary studies have also identified a number of drought QTLs for

BAT 477 under drought stress conditions (Blair *et al.,* 2006). Breeding efforts aiming to improve drought tolerance has also recently resulted in small seeded Mesoamerican lines SER and SEN with up to 36 % yield greater than BAT477 under unfavourable environments (Briñez *et al*., 2017; Gonçalves *et al*., 2019). According to Traub *et al.* (2017) genotypic variability in drought tolerance between the common and tepary beans was not related to differences in mesophyll tolerance of dehydration.

A study conducted over two seasons under field conditions in CIAT, Colombia showed two outstanding accessions; G 40159 and G 40068 of *Phaseolus acutifolius* and inbred lines; RAB 650 and SEA 23 were highly adapted to water stress conditions (Rao *et al.,* 2004). Rao *et al.* (2006) also identified three lines namely; SER 16, SEA 5 and SER 5 with higher pod partitioning index, pod harvest index (PHI), lower value of seed phosphorus content, a lower proportion of pod wall biomass and leaf area index (LAI) and associated it with their superior adaptation to drought conditions. Klaedtke *et al*. (2012) demonstrated using an interspecific populations that had high capacity to mobilize photosynthate in common bean lines adapted to drought resulted in improved yield. Assefa *et al*. (2013), showed in a separate study that the capacity to remobilize photosynthate to grain under stress for better harvest index is an important drought tolerance trait. Nevertheless, under severe drought conditions, nitrogen mobilization, harvest index (HI) and water-use efficiency (WUE) are severely affected (Lizana *et al.,* 2006). Water-use efficiency (based on carbon isotope discrimination, CID) is not a promising indicator of adaptation to drought based on the results measured under field

conditions (White and Singh, 1991). However, these findings require verification as the study was performed over one season with limited number of parental genotypes.

Combining genes from race Durango to race Mesoamerica provided drought tolerant lines like L88-63, a superior black seeded line (Frahm *et al.*, 2004). A double cross of race Durango (Guanajuato 31) and race Mesoamerica (BAT 477) also yielded SEA 5 and Pinto Villa mainly grown in the highland environments of Mexico (Acosta-Gallegos and White, 1995; Teran and Singh, 2002). More recently, an introgression of Andean lines and Mesoamerican sources registered significant gains in yield (Briñez *et al*., 2017; Traub *et al.*, 2017). Some of the drought tolerant Andean genotypes such as; KAT B1, SEQ 102, SEQ 1003 selected in other studies are now widely used in many breeding programs in Kenya, Rwanda and Uganda respectively (Kimani *et al*., 2007; Nabateregga *et al*., 2019). According to Singh (1995), CIAT has been screening bean germplasm since 1993 with an aim of identifying high yielding cultivars that may increase production in diverse socioeconomic and agro-ecological conditions within East Africa. Most work focused on large seeded Andean bean genotypes with farmer preferred traits. The study resulted to the identification and release of Andean bean genotypes; GLP2, GLP×92G and GLP×1127, which were recommended for production in wet high potential highland regions of Kenya (Nyabundi *et al*., 1995). In late 1990's, four lines; GLP×1004 and KAT X69 with tolerance to water stress were identified at KALRO-Thika and they were recommended for production in areas prone to drought such as lower Eastern Kenya (Nyabundi *et al*., 1995). However, among the identified varieties none was preferred in the market, hence, subsequent breeding efforts at KALRO-Katumani focused on large

seeded red mottled types that are highly preferred in the market. Between early and mid-2000s, among the new genotypes released were two large seeded namely; KAT B1 and KAT B9 (Kimani *et al*., 2007).

2.8 Genetic diversity of common bean

Genetic variation in common bean occurs as a result of the processes pertaining to evolution (selections, mutations, random genetic drift and migration) and also due to the man's influence through selection and domestication (Mitranov, 1983; Ramirez-Vallejo and Kelly, 1998; Alvares *et al*., 2016). An important factor to be considered in crop breeding is genetic variability which is an essential prerequisite to obtain progenies that are high yielding (White and Singh, 1991; Polania *et al*., 2016). In this regard, variability is the difference that is present among individuals as a result of differences in their genetic make-up and/or due to the influence by the surrounding environment. The variation within the populations could be phenotypic, genotypic or these two factors interacting together (Radkov and Mitranov, 1983). According to Warner (1952) and Mitranov (1983), the core of plant breeding is the existence of variability among individuals within a population. Therefore, the basis for improving a crop is genetic variation (Langat *et al*., 2019). Plant breeders utilize the variations in directing and controlling evolutionary process to develop new varieties. Thus in selection and breeding, this is of immense importance.

In plant breeding, heritability is important primarily as a measure of the value for selecting a particular character in different progenies and as a transmission index (Broughton *et al*., 2003). Dimova and Svetleva (1992) defined heritability in terms of the amount of phenotypic variance that is heritable and is the best measure of transmissibility of a character passed down to the offspring by the parents . It is, not only, a property of a character under a study but also of a population to be sampled, of the environmental conditions to which the individuals are exposed to, and the way in which measurements for the phenotype were taken (Menuccini *et al*., 2000; Costa Franca *et al*., 2000).

Broad sense heritability estimate is the ratio of the total genotypic variance, including dominance, additive and epistatic variance to the phenotypic variance whereas narrow sense heritability estimate is only the additive portion of the total phenotypic variance and it shows to which extent the phenotypes are influenced by the genes inherited from parents (Rao, 2001; Singh, 2001; Torres *et al*., 2006). Both the narrow and broad sense heritability are important in plant breeding since the effectiveness of selection relies only on the additive portion of genetic variance in reference to whole component of total variance (Broughton *et al*., 2003; Wentworth *et al.,* 2006; Zadražnik *et al.,* 2017). Mitranov (1983) indicated that the low heritability estimates for quantitative characters could be as a result of their sensitiveness to environmental factors. According to Johnson *et al.* (1955), the narrow-sense heritability, although usually the major factor, is not always the best measure when considering the heritability of a trait in populations because it does not give an adequate assessment of the influence of genetics on a trait, unlike the broad sense heritability, which captures the relative effects of both genes and

environment on phenotype. Genetic advance is termed as differences between genotypic values of a generation that is obtained from a selected population over the base population mean value. Low values of genetic advance is an indication of non-additive gene action whereas high values signifies additive gene action (Lizana *et al*., 2006).

The estimates of heritability will be useful if accompanied by high values of genetic advance (Langat *et al*., 2019). Therefore, the importance of heritability estimates increases when heritability and genetic advance are used alongside the selection differential; the amount by which the mean of the entire group is exceeded by the selected lines (Johnson *et al*., 1955). The estimates for heritability should always be examined alongside that of genetic advance, because high heritability alone does not necessarily translate to high genetic progress (Lizana *et al*., 2006). Grain yield and other economic characters that are polygenic in nature, usually have low heritability since they are often influenced by the environment (Checa and Blair, 2012). If differences between environmental variability and genotypic variability are small then, selection will be efficient and the selected trait will be transmitted from the parent to its progeny (Omae *et al*., 2012). Furthermore, the genetic association between breeding materials needs to be understood in order to be able to maintain the genetic diversity as well as sustain selection gain in the long term.

2.9 Molecular techniques used in plant accessions

Molecular markers have revolutionized the crop breeding procedures. The genetic differences inherent between individual organisms can be elucidated by the molecular markers. Generally they are used as chromosome landmarks for important traits that

facilitate the introgression of QTLs into the desired chromosome regions (Collard *et al*., 2005). The genes controlling the target traits and the phenotype of the traits of interest are useful as genetic markers (O'Boyle *et al*., 2007). The polymerase chain reaction (PCR) technologies are the most efficient way to study the DNA polymorphism (Collard *et al*., 2005; Xu and Crouch, 2008; Lee *et al*., 2015).

The molecular markers help to reveal the different traits and are useful in showing the diversity among the crop species (Collard *et al*., 2008; Xu and Crouch, 2008; Lee *et al*., 2015). Some of the most important attributes that make molecular markers suitable for marker assisted selection (MAS) include ability to show variation among different individuals (detectable DNA polymorphism), detection of the different forms of the individual that can be inherited simultaneously, well distributed and a good coverage of genome. Others attributes may include, the ability to show clear difference between closely related individuals, comparatively cheap, fast in yielding information as well as not complex to use (Collard *et al*., 2008; Xu and Crouch 2008; Lee *et al*., 2015; Trapp *et al*., 2016; Langat *et al*., 2020).

2.9.1 Single nucleotide polymorphisms (SNPs)

The Single nucleotide polymorphisms markers have ability to clearly distinguish between resistant and susceptible traits hence making it very suitable for marker assisted selections. In genomic DNA of some individuals of a population there are single paired bases sequences occurring in different positions referred to as single nucleotide polymorphisms (Jehan and Lakhanpaul, 2006). At the moment the most reliable markers

used in genomics are the SNPs. They are now widely used in plants, animals and human genomic studies (Schneider *et al*., 2001; Jehan and Lakhanpaul, 2006; Linares *et al*., 2012). Currently, SNPs are applied in several areas such as effect of certain drug in the body; study the genetic diversity in organisms and evolution among plant species (Jehan and Lakhanpaul, 2006). The PCR-CTPP technology amplifies the DNA bands to different lengths making easier to identify the various marker locations, therefore ideal for single nucleotide polymorphisms analysis. This technique is considered to be most suitable to study molecular markers because it is simple, quick and relatively inexpensive (Linares *et al*., 2012).

2.10 Genotyping-by-Sequencing (GBS)

Genotyping-by-Sequencing (GBS), is a simple multiplexed genomic approach used to discover single nucleotide polymorphisms (SNP) for use in performing genetic analyses and genotyping studies. The GBS method greatly reduces genome complexity by combining restriction enzyme with the high-throughput sequencing capacity of Illumina platforms to cleave the DNA ready for next generation sequencing (NGS) (Davey *et al*., 2011; Beissinger *et al*., 2013; Sonah *et al*., 2013). As a technically low cost, quick, specific, highly reproducible technology, GBS is suitable tool for plant breeding and plant genetics studies, including genomic diversity studies (Gore *et al*., 2007; Fu, 2012; Lu *et al*., 2012), genome-wide association studies (Elshire *et al*., 2011) and genomic selection (Baird *et al*., 2008; Poland *et al*., 2012).

2.10.1 Application of genotyping-by-sequencing (GBS) in plant breeding

Plant breeding is one of the field that has greatly benefited from the applications of GBS. The approach offers a low-cost tool for genotyping breeding populations, providing plant breeders with the opportunity to implement GWAS, diversity studies, and linkage analysis (Poland and Rife, 2012). Preliminary work on genomic selection demonstrate great opportunity to hasten the speed of developing new improved varieties in plant breeding programs (Baird *et al*., 2008). Since GBS approach has been shown to be robust across a range of species, prior knowledge of the species genomes is not necessary as a prerequisite to implement GBS, as both SNP discovery and genotyping are achieved simultaneously (Poland and Rife, 2012; Narum *et al*., 2013).

In genomics-assisted breeding programs, GBS through the next generation sequencing (NGS) technologies has been used to re-sequence some recombinant inbred lines (RILs) to be used during the analysis and mapping of traits of interest (Deschamps *et al*., 2012). The development of RAD technique through construction of a GBS linkage map using the next generation sequence technologies (NGS) (Baird *et al*., 2008), has led to QTL analysis in barley (Chutimanitsakun *et al*., 2011). In the absence of reference genome sequences in plant breeding programs, GBS is an excellent platform for integrating molecular markers and genotyping of large populations (Narum *et al*., 2013).

2.11 QTL mapping for drought tolerance

The breeding programs have been hastened through the discovery of several traits associated with drought tolerance (Bernier *et al*., 2008). Initially the process was slow, tedious and expensive. Plant tolerance to abiotic stress is the work of several genes referred to as quantitative traits. The quantitative trait loci (QTLs) are the regions in the genome that contains genes associated with quantitative traits (Collard *et al*., 2005; Trapp *et al*., 2016). There are enormous advantages offered by applying genomics over the conventional methods in the study of drought related traits. The gene technology analyses the quantitative traits into single genetic identifiers called QTLs. The QTLs can be manipulated through biotechnology and widely used in marker assisted selection (MAS) (Tuberosa and Salvi, 2006).

Research activities involving genetic analysis of drought tolerance in crop plants have been made easier by the advent of molecular markers. There is no need for extensive measuring of phenotype in the field. Measuring the phenotypic characters requires extensive field testing however the molecular markers have greatly reduced testing over time and space through monitoring the genetic loci conferring drought tolerance traits (Nguyen *et al*., 1997). The markers linked with the trait are excellent indicators for selection for drought tolerance using MAS in breeding programs. Several studies involving QTL mapping have revealed that drought tolerance is a complex quantitative trait controlled by a large number of genes (Schneider *et al*., 1997; Blair *et al*., 2012; Trapp *et al*., 2016).

QTLs coding for drought tolerance in common beans have been found to exist in the same chromosome as those controlling different plant and yield components like weight of 100 seeds, plant yield, plant biomass, harvest index, partioning index for pod, harvest index, chlorophyll content, pods per plant, flowering duration, number of seeds per pod, maturity period and days to filling of grain (Blair *et al*., 2012; Asfaw *et al*., 2012). Drought related QTLs/genes in common bean were identified in the recombinant inbred lines (RILs) population derived from a cross between two cultivars; drought tolerant line SEA5 and drought susceptible line CAL96 (both cultivars from CIAT, Cali, Colombia) (Mukeshimana *et al*., 2014).

A total of 14 QTLs that are drought related were mapped after evaluating various parameters of common beans in different environments in populations that were grown under drought stress and non-stress conditions (Mukeshimana *et al*., 2014). In a different study Asfaw *et al*. (2012) crossed a drought susceptible DOR364 with drought-tolerant BAT477 to develop a population of recombinant inbred lines (RILs) that were grown under eight environments differing in drought stress (tolerance). Two different techniques were used to identify QTLs related to drought tolerance traits; multi-environment mixed model was able to identify 9 QTLs while composite interval mapping was able to identify 64 QTLs. The composite interval mapping generated 37 % of the mapped phenotypic characters such as chlorophyll content and pod partitioning traits.

2.11.1 Applications of QTL and molecular markers in breeding for drought tolerance

Mapping the quantitative trait locus (QTL) is an approach for detecting loci related to complex quantitative traits, such as, tolerance to drought stress. Multiple populations of common bean developed from crosses of tolerant and susceptible parental genotypes belonging to either intragene pool or intergene-pools of both Andean and Mesoamerican derivative, have been genotyped and their linkage maps generated (Traub *et al*., 2017). Novel sequencing technology and genetic markers, such as, SNPs have greatly improved the precision and resolution of genetic linkage maps, enabling the identification of several QTLs involved in tolerance to multigenic traits such as tolerance to drought, biomass production, yield partitioning, as well as micronutrient accumulation (Polania *et al*., 2016). QTLs associated with drought response in common bean have been reported to co-segregate with QTLs for yield, phenology, canopy biomass and biomass partitioning.

High variability present within the common bean germplasm and the strong influence of the environmental conditions on the presence of minor QTLs has greatly limited the practical use of the reported QTLs. Effort to establish controlled and uniform growth conditions to evaluate a large recombinant inbred line (RIL) population of over hundred genotypes, has however, proven not very practical. Hence, there is need for a compromise approach that consist testing a sub-sample of the most diverse RILs for a selected segregation trait in multiple trials sites to validate major QTL (Wang *et al*., 2014). Common bean of Mesoamerican origin, especially those belonging to the race

Durango has always been used as sources of drought tolerant traits used for much of the work in QTL mapping and development of drought-tolerant cultivars (Wang *et al*., 2014).

2.11.2 Marker-assisted selection (MAS)

Though phenotypic measurements are still commonly used in breeding programs in most field nurseries or greenhouse facilities to select for secondary traits, they are expensive and there is a danger of ambiguity due to variation in environmental conditions (Nguyen *et al*., 1997). Mapping QTLs provide the best alternative for selection for traits associated with tolerance to water deficit stress and higher productivity in harsh agro-ecological zones. According to Trapp *et al*. (2016), a number of reports indicate that different QTLs co-locate such as those related with components of yield and those linked to tolerance to drought stress. The traits for drought tolerance and grain yield under stress is revealed by QTLs for root traits, yield and its component and phenology found in the same loci (O'Toole, 2004). The same pair of genes found in different regions under different drought scenarios might be a likely candidate for breeding programs using MAS.

Before QTLs are applied practically in breeding programs they go through several QTL application research steps. The first step is the confirmation of QTL, followed by wide scale testing and finally validation of the markers. In era of genomic research, precise phenotypic measurements need to be carried out in order for QTLs to be successful (Cichy *et al*., 2015; Polania *et al*., 2016). Improving bean cultivars for drought tolerance using MAS based approaches is likely to be achieved through introgression of QTLs with

substantial, multiple influence on yield and its components under water deficit stress (O'Toole, 2004; Lee *et al*., 2015). Markers linked to drought tolerance QTLs have been reported in several studies, however they have not been adopted for use in MAS programs.

2.11.3 Potential for improving common bean for drought tolerance

Although the role of some traits like water use efficiency, stomatal conductance and plant water relations in adaptation to water stress have been revealed through physiological studies, the precise mechanisms underlying these traits have not been characterized. Additionally, not so well understood are the comparative significances of other traits such as quantitative traits.

In a diverse crop species like common bean with a huge potential for introgressing with closely related species, important genetic diversity are yet to be unrivalled in other mechanisms and character traits which could have significant roles in tolerance to water deficit stress. Hence, there is a need for continuous scrutiny of those physiological processes and traits. Moreover, the controlled environments for screening that can enhance the manifestation of traits need to be properly adjusted and the association between physiological characters and related QTLs be further investigated.

Though encouraging results have been reported on several QTLs related studies, most have been on a limited number of RIL populations mainly obtained from crosses of the Mesoamerican derivatives (Blair *et al*., 2007; Suárez *et al*., 2008; Lee *et al*., 2015).

Further studies on populations that were developed from Andean gene pool are necessary in order to disclose the existing variability in QTL alleles for water stress tolerance and for further analysis of the influence of heritability on the already identified QTL alleles. It is imperative to have larger number of highly polymorphic microsatellite to evaluate crosses acquired from the same genetic heritage. Furthermore, marker technology that is highly saturated like DArT (diversity arrays technology) could be appropriate for high resolution QTL mapping.

For effective genetic analysis, large populations with more than 100 RIL are needed, however, there are inherent difficulties associated with creating a RIL populations for more than 300 lines especially in common bean, given that it is a low multiplication species compared with other crops (Schneider *et al*., 1997). This also has an effect in the preservation of RILs population. Besides, RILs the advanced backcross population could provide better prospects in the determination of QTLs that would operate with no blurring influence by unrelated genes emanating from undesired origin.

Backcrossing has been found to fix important alleles in the genetic make-up of a new population. To successfully carry out marker assisted selection (MAS) for drought tolerance, knowledge of the interaction between the QTLs and the multiple heritability is critical, as crop improvement programs involves wide range of crop varieties representing different gene pools, races as well as genetic backgrounds. Another challenge encountered in trying to understand the genetics of drought tolerance is associating the underlying genetic and mechanistic factors with the QTLs. For instance, genes regulating

the expression of transcription factors, or those involved in hormonal production, carbon and nitrogen metabolism such as structural genes could be candidate gene for QTLs controlling drought tolerance. Structural genes such as proline and trehalose that are involved in metabolite biosynthesis in common beans, would be of interest because of their influence on tolerance to water deficit stress (Schneider *et al*., 1997; Lee *et al*., 2015). Hormones in the signaling pathway of the abscisic acid (ABA) could also be a source of candidate genes underlining a number of the QTLs that have been verified to date, or that remains to be identified (Condon *et al*., 2002; Amede and Schubert, 2003; Nadeem *et al*., 2019). From a breeding perspective, the significant levels of tolerance to water stress exhibited by *Phaseolus acutifolius* species could make interspecific crosses with common bean *Phaseolus vulgaris* to be very attractive.

Characterizing *Phaseolus acutifolius* accessions for drought tolerance has elucidated response mechanisms that could be useful in complementing the already identified common bean traits and lay emphasize on selection of the most crucial traits. However, so far, interspecific crosses have not yielded much results in regards to what can really be transmitted to common beans (Blair *et al*., 2007; Lee *et al*., 2015). Reports from physiological studies indicate that extensive rooting system typical of accessions of *Phaseolus acutifolius* could probably contribute to drought tolerance. The root structure of *Phaseolus vulgaris* can also be modified in useful ways through inter-specific crosses with *Phaseolus coccineus*. The rooting structure for *Phaseolus coccineus* is comparatively thicker compared to *Phaseolus vulgaris* hence better penetrating capacity

in soil with higher density- a trait that is useful for soils with compact structure that can limit root development (Schneider *et al*., 1997; Suárez *et al*., 2008).

The medium to long term goal of breeding is matching of mechanisms and character traits to a particular environments in regards to drought patterns. Thus, it is an iterative process to identify genetic diversity, to define general classes of traits and test it in range of environmental conditions (Schneider *et al*., 1997; Amede and Schubert, 2003). There is existing great potential to improve drought tolerance in common beans. To exploit this potential it requires a systematic application of physiological and genomic tools and a continuous analysis of genetic and mechanistic factors in a diverse germplasm either from intra species and interspecies. At the moment, it appears that the most important traits are those associated with root depth and remobilization of photosynthate, though there could be an emergence of other traits in future (Condon *et al*., 2002; Amede and Schubert, 2003; Nadeem *et al*., 2019).

The effectiveness of genomic tools could be improved through better understanding of the physiology of drought response and the mechanisms of drought tolerance. The sensitivity of beans to different aspects of soil, including soil infertility and compaction has an effect on the expression of desirable root characters (Blair *et al*., 2012; Polania *et al*., 2016; Traub *et al*., 2017). This fact shows the complexities related to the study of tolerance to water stress in common bean and has wide implications in the ultimate expression of water stress tolerance in the field. Beans may also be influenced by the environmental conditions which affect photosynthates remobilization to grain. A cocktail

of strategies involving efficient schemes of breeding, managed stress conditions, scaling-up of the use of phenotyping tools, alongside the genomics and MAS, would probably lead to efficiency in genetic improvement of drought tolerance in common beans (Cichy *et al*., 2015; Polania *et al*., 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out at the Dryland Research Centre, KALRO-Katumani, lower Eastern Kenya (01^{o} 34' S and 37^{o} 14' E). The region experiences frequent drought that causes bean yield reduction of up to 70 % due to the prevailing arid and semi-arid conditions (Sennhenn, *et al*., 2017). It is located at an elevation of 1600 m above sea level and receives an average annual rainfall of 711 mm. The average temperature is 26 °C throughout the year with a mean annual minimum and maximum temperature of 17 °C and 31 °C, respectively (KMD, 2020).

3.2 Identification of phenotypic and physiological characters associated with drought tolerance in selected common bean genotypes

3.2.1 Study materials for identifying drought tolerance traits

Materials for the study comprised six common bean genotypes; KAT BI, GLP2, Nyota, Faida, Metameta and Angaza. All are known to be phenotypically diverse. They possess contrasting drought tolerance characteristics (Table 3.1). Previously five genotypes were used in identifying drought tolerance traits (Ramirez-Vallejo and Kelley, 1998) and in another similar study three genotypes were used (Acosta-Gallegos and Kohashi-Shibata, 1989). This indicate that the six genotypes that were used in this study were higher than those reported.

Table 3.1: Origins and characteristics of genotypes used in the study

Genotype	Origin	Location	Characteristics
Nyota	Andean	Local collection, Kenya	Tolerant to water stress
Faida	Andean	Local collection, Kenya	Susceptible to water stress
Angaza	Andean	Local collection, Kenya	Moderately tolerant to water stress
Metameta	Andean	Local collection, Kenya	Susceptible to water stress
KAT B1	Andean	KALRO, Katumani	Tolerant to water stress
GLP2	Andean	CIAT, Uganda	Susceptible to water stress

EAGC (2019). East Africa Grain Council, East Africa Cross Border Trade Bulletin.

The genotypes; KAT B1 and GLP2 (experimental checks) are improved genotypes developed by the Kenya Agricultural Research and Livestock Organization (KALRO) and Centre for International Tropical Agriculture (CIAT) respectively. They harbor high genetic diversity (Kimani *et al*., 2005; Katungi *et al*., 2011; KEPHIS, 2011) and served as parents of QTL mapping population for water stress tolerance. The two varieties have high preference in the market and also widely grown across the country. In addition, both varieties are of Andean type, similar to most of those found in Kenya (Kimani *et al*., 2007; KEPHIS, 2011). KAT B1 is a medium yellow average yielding cultivar known for its drought tolerance traits and it served as the drought tolerant control while GLP2 a large red mottled high yielding cultivar was used as the susceptible control. It was developed for high altitude areas. Nyota, Faida, Metameta and Angaza are new common bean varieties recently released by KALRO. They were chosen through field screening of the local germplasm collections in KALRO. Nyota and Angaza are known to possess drought tolerant properties whereas Faida and Metameta are susceptible to water stress.

Though, some of the recently released varieties such as Tasha and Cianku have almost similar traits with KAT BI and GLP2, they were not selected as parents to develop mapping population for the study because they have few contrasting characteristic between them.

3.2.2 Water stress experiment

The water stress experiment was carried in a rain-out shelter between January 2017 and May 2018. Plants were grown in plastic buckets/pots measuring (20 cm length x 20 cm diameter) considered to have adequate space for root growth for three plants. The base of each bucket was perforated to allow drainage of excess water. Each bucket was filled with 4.1 kg of sandy-clay-loam soil (ratio 4:1:3) with known characteristics mixed with 25 kg farmyard manure. The experiment consisted of six genotypes, with two blocks and one treatments (water-stress) in a randomized complete block design and was replicated three times (with water stress treatment as the main treatment and genotypes as the sub treatment). Buckets were watered to the upper limit of field capacity as determined using time domain reflectometer (model TDR 100) before sowing. A total of 540 seeds were sown in 108 plastic buckets. Five seeds were planted per plastic pot. Two weeks after germination, seedlings with undesirable attributes were discarded to maintain three vigorous seedlings per bucket. Thinning was not done to one plant because the pot had adequate space for three plants and there was no completion for resources. After emergence, the seedlings were irrigated every two days until they were fully established. Control treatments (block 1) received regular water supply throughout the experimental period and maintained at 75 % of field capacity as determined by time domain

reflectometer. Seventeen days after sowing, the water stress plants (block 2) were subjected to severe drought by withholding water supply for ten days until the soil water content decreased to 30 % of field capacity as determined by time domain reflectometer. The plants were re-irrigated after stress treatment, when some leaves started showing signs of wilting.

3.2.3 Data collection

Data collection started at the tenth day after withholding water to induce drought stress. Data were recorded for both well watered and stressed plants to determine the degree of water stress. Different parameters were recorded on thirty six randomly sampled plants in each replicate. Measurements were normally recorded from 10.30 am to 11.30 am.

3.2.3.1 Measurement of days to flowering and maturity

Data was recorded every two days for number of days to flower and to reach physiological maturity. Days to flower (DF) were calculated by getting the difference between date of sowing and date when 50 % of the plants in the replicate had fully opened their flowers. Days to physiological maturity (DPM) were obtained by getting the differences between dates of sowing and dates when 50 % of the pods had lost their (green pigmentation) colour and would burst open easily when pressed (Polania *et al.,* 2012).

3.2.3.2 Measurement of 100-seed weight and seed yield

Harvesting was carried out at physiological maturity and the seeds for individual plants were kept separately in paper bags and left to dry naturally in air under room temperature (25 °C). Seed moisture content was at 14 % (determined using seed moisture meter) when the weight of 100 seeds for each genotype was measured. The grain yield for each genotype was obtained by weighing all the seeds.

3.2.3.3 Measurement of above ground biomass

The above ground biomass was determined at two stages: mid-pod filling and harvest. At the mid-pod filling stage, randomly picked plants were cut at the soil surface. Then each sample was separated into stems (SB), leaves (LB) and pods (PB) respectively. Different components were placed separately into paper bags and sealed, weighed and oven dried at 80 °C to constant weight and recorded. The above process was repeated for each genotype at harvest time. The data obtained were used in calculating the drought indices.

3.2.3.4 Measurement of number of plant branches

At mid-growing stage (31-36 days after sowing) the average number of branches per plant for each genotype was computed from data obtained from each of the randomly selected plants per replicate.

3.2.3.5 Measurement of number of plant pods

The mean number of pods from each randomly selected plants was obtained, recorded and then used in the determination of the average number of pods for each genotype.

3.2.3.6 Measurement of stomatal conductance

Leaf stomatal conductance measurements were carried out on three different dates at an interval of five days starting a week from the onset of flowering. The randomly selected plants per replicate were evaluated for both stress and well watered treatments. Measurements were carried out on the third fully expanded leaves of plants between 10.30 and 11.30 am using a portable photosynthesis system (LI-6400, LICOR Inc., Lincoln USA). The leaf was put between two conductance elements in the device that compares the flow rate and water vapor mole fraction of air that enters and leaves the chamber. The total conductance to water vapour was computed as a function of transpiration and vapor pressures in the leaf and cuvette.

3.2.3.7 Measurement of leaf water potential

Leaf water potential was measured on stressed and non-stressed plants every three days between 10.30 am and 1.30 pm at intervals of one hour by observing the presence of sap on the cut surface of the leaf stalk using a portable plant water status console (Soil Moisture Equipment Corp., Santa Barbara, California). Two fully expanded leaves (1^{st} and 2^{nd}) were randomly selected from each replicate for leaf water potential

measurements. Prior to detaching, the leaf was enclosed in a reflective plastic bag to suppress transpiration, allowing the stem water potential to equate with leaf water potential (Turner, 1981). The leaf petiole was cut (in slanting manner) and enclosed in the pressure chamber ensuring the cut end extend through a rubber stopper in the pressure chamber cover. The cover around the petiole was tightly sealed to prevent any gas from escaping during the pressurization. The pressure in the chamber was slowly increased by opening the compressed nitrogen valve until the sap is pushed out at the end of the petiole. The valve was closed to stop any further increase in pressure as soon as a sap started oozing out from the xylem vessels. After the leaf water potential (measured in MPa) was recorded from the pressure gauge, the pressure was released completely through an outlet valve and the sample removed (Turner, 1981; Phene *et al*., 1990).

3.2.3.8 Measurement of water use efficiency

The water use efficiency was calculated as total biomass above ground produced (after mid-pod filling) over a week expressed over transpiration measurements obtained for the same period. Water use efficiency for biomass (WUEB) yield was estimated from biomass yield according to Stanhill (1987) as follows;

WUEB= Biomass yield (ton) /Total water use (4.5 litres)

3.2.3.9 Measurement of drought intensity index

Estimation of the drought intensity index (DII) was carried out by following the formulae proposed by Fischer and Maurer (1978). The DII is used to calculate the severity of

drought stress between two growing conditions based on the mean yield of all genotypes. The formula for drought intensity index used was;

$$\mathrm{DII} = 1 - \left(\frac{\times_{\mathrm{WS}}}{\times_{\mathrm{WW}}}\right)$$

Where;

$\times_{ws}$ – is the overall mean yield of all the genotypes under water stress conditions

$\times_{ww}$ – is the overall mean yield of all the genotypes under well watered conditions

(DII)− is the drought intensity index - values are between 0 and 1, where values less than 0.3 signifies minor water stress and above 0.7 signifies severe water stress (Ramirez-Vallejo and Kelly, 1998), whereas moderate water stress is represented by values ranging between 0.3 and 0.6 (Urrea *et al*., 2009).

Drought susceptibility index (DSI) of a specific genotype is calculated as the improved yield performance under both stress and well watered condition. It is given by;

$$\mathrm{DSI} = \left(1 - \frac{\mathrm{Y_{ws}}}{\mathrm{Y_{ww}}}\right) / \mathrm{DII}$$

Where;

Y_{ws} − is the mean grain yield of a specific genotype under drought stress condition.

Y_{ww} − is the mean grain yield of the same genotype under well watered condition.

DSI − is the drought susceptibility index (Fischer and Maurer, 1978).

The smaller the DSI value of a genotype the greater its drought tolerance ability.

Percent yield reduction is the difference in performance of a genotype under both environments expressed in percentage. The percentage of yield reduction is calculated based on the following formula;

$$\text{PYR} = \left[\frac{\text{mean yield of a genotype under } \boldsymbol{ns} \text{ condition} - \text{mean grain yield of a genotype under } \boldsymbol{ds} \text{ condition}}{\text{mean grain yield of a genotype under } \boldsymbol{ds} \text{ conditions}}\right] \times 100$$

Where;

ns − is non stress

ds − is drought stress

PYR − is percent yield reduction.

The lower the values of percent of yield reduction the better stability of the genotype across different treatments and the vice versa.

Geometric mean (GM) is an estimation of the average grain yield of a genotypes between well watered and drought stress condition;

$$\text{GM} = \sqrt{\text{y}_{\text{ws}} \times \text{y}_{\text{ww}}}$$

Where;

Y_{ws} − is the average grain yield under the stressed treatment and

Y_{ww} − is the average grain yield under the well watered treatment.

Harvest Index (HI) was calculated as the percentage weight of total biomass above ground at mid-pod filing that is partitioned to the grain. The HI (%) for each genotype is expressed as;

$$\text{HI} = \left[\frac{\text{Grain biomass dry weight at harvest}}{\text{Total biomass above ground dry weight at midpod filling}}\right] \times 100$$

Where,

HI − harvest index

3.3 Characterization of F2 population (KAT B1 × GLP2) for drought tolerance using phenotypic and physiological characters

3.3.1 Study materials for characterizing phenotypic and physiological characters in F2 lines

An intraspecific cross between two common bean genotypes GLP2 and KAT B1 grown in a greenhouse was carried out to develop F2 progenies. The two genotypes have contrasting drought tolerance characteristics; GLP2 is drought susceptible while KAT B1 is tolerant to drought based on their productivity under water stress conditions.

3.3.1.1 Procedure for developing F2 population

Field trial was conducted in early 2017 to raise a breeding population from the two parents. GLP2 and KAT B1 plants were grown in two adjacent plots (4 m x 4m each). The rows were 75 cm apart to maintain plant population density of 200,000/hectare.

Since the flowering period of GLP2 is longer (36 days) compared with KAT B1 (31 days), it was sown five days earlier to ensure both parents flowered at the same time. The immature flower buds of GLP2 were opened using tweezers and its immature anthers were removed. Anthers from KAT B1 were then rubbed on the stigma of GLP2 in order to transfer pollen. The opened immature buds were carefully closed. Each bud was then tagged. Twenty eight F1 seeds obtained from the crosses were grown and allowed to self-pollinate to obtain the F2 seed that formed the next generation.

3.3.2 Evaluation of experimental materials

A total of 116 F2 seeds were planted along with parental genotypes during 2017/2018 in a rain-out shelter and evaluated for phenological, physiological, yield and yield related components traits and drought tolerance.

3.3.3 Estimation of genetic variability

Estimation of genetic parameters and correlation was carried out as per the procedures described by Robinson *et al*. (1949), Burton and Devane (1953), Hanson *et al*. (1956), and Falconer and Mackay (1996). The genotypic and phenotypic components of variance for the traits were calculated according to a formula by Burton and Devane (1953), as follows:

Genetic variance$\left(\sigma_g^2\right)$ = (Genotype mean square (GMS) - Error mean square (EMS))/ (Number of replications (r))

$$\text{Environmental variance}(\sigma_e^2) = \text{Error Mean Square}$$

$$\text{Phenotypic variance}(\sigma_p^2) = \sigma^2{}_g + \sigma^2{}_e$$

The genotypic, phenotypic and environmental variances were used for the estimation of phenotypic and genotypic coefficient of variation as suggested by Burton and Devane (1953), as follows:

$$\text{PCV \%} = \frac{\sqrt{\sigma^2 p}}{\times} \times 100$$

$$\text{ECV \%} = \frac{\sqrt{\sigma^2 e}}{\times} \times 100$$

$$\text{GCV \%} = \frac{\sqrt{\sigma^2 g}}{\times} \times 100$$

Where:

PCV % = Phenotypic coefficient of variability

GCV % = Genotypic coefficient of variability

ECV % = Environmental coefficient of variability

$\sigma^2{}_p$ = Phenotypic variance

$\sigma^2{}_g$ = Genetic variance

$\sigma^2{}_e$ = Environmental variance

$\times$ = Grand mean

The GCV and PCV values were classified as low, moderate and high according to Robinson *et al.* (1949) as follows: Low = <10 %, Moderate = 10 – 20 % and High = > 20 %

Broad-sense heritability was calculated according to the method by (Hanson *et al*., 1956).

$$h^2_B = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where;

h^2_B = heritability in broad sense

σ^2_g = Genetic variance

σ^2_p = phenotypic variance

The heritability values were ranked according to Robinson *et al.* (1949) as follows: Low = < 30 %, Moderate = 30 – 60 % and High = > 60 %.

The expected genetic advance or gain (GA) of selection for the trait was computed as:

$$GA = K\sqrt{\sigma^2_P \, h^2_B}$$

Where;

K = 1.40 intensity of selection at 5 %

$\sigma^2 p$ = phenotypic variance

h^2_B = Heritability in broad sense

The genetic advance as percentage of the mean:

$$\text{GAM} = \frac{\text{GA}}{\times} \times 100$$

Where:

GA = Genetic advance

× = Grand Mean

The GAM were classified as low, moderate and high according to Robinson *et al.* (1949) as follows: Low = < 10 %, Moderate = 10 – 20 % and High = > 20 %.

3.4 Identification of quantitative trait loci (QTL) associated with drought tolerance in an F2 (KAT B1 × GLP2) population of common bean.

3.4.1 Study materials for QTL mapping

Mapping population consisting of 120 F2s was raised from selfing 28 F1 seeds that were obtained from a single cross between KAT B1 and GLP2. A typical QTL population consists of 100 to 300 lines, each of which is evaluated both for phenotypic traits and

molecular markers (Beavis, 1994, 1998). The two parents differ greatly in terms of grain yield, physiology and phenology characters under drought stress conditions.

3.4.2 Phenotypic evaluation

The hundred and twenty F2 lines were evaluated along with their parents for physiological, phenological, yield and yield related traits following a randomized block design in a rain-out shelter.

3.4.3 Molecular analysis

3.4.3.1 DNA extraction

DNA was extracted from young fresh leaves (300 g) following the protocol described by Mahuku (2004). The emerging trifoliate leaves of the individual parents and the F2 progenies were harvested and lyophilized. Mortar and pestle were used to macerate the leaves (1 g) mixed with acid-washed sterilized sand to produce a fine powder. The content then transferred into a 1.5-mL Eppendorf tube followed by the addition of 500 µL of TES extraction buffer (pH 8) containing 0.2 M Tris-HC1 (pH 8), 10 mM EDTA (pH 8), 0.5M NaC1, 1 % SDS and proteinase K (final concentration of 50 µg/mL). The mixture was thoroughly agitated then incubated in water bath at 65 °C for 30 minutes. Afterward 500 µL of 7.5 M ammonium acetate solution was added. The preparation was gently shaken and incubated at -5 °C for 10 minutes and the sample were centrifuged for 15 minutes at 15,000 rpm. The supernatant was transferred to a new Eppendorf tube and 500 µL of ice-cold isopropanol solution was added and the mixture incubated at -20 °C

for 2 hours. Afterwards the mixture was centrifuged for 10 min at 15,000 rpm to pellet the DNA. After decanting the supernatant, DNA pellet was washed with 800 µL of 70 % ethanol. The DNA pellet was then air dried for 15 min and dissolved in 250 µL of Tris buffer (10 mM Tris-HC1 1 mM EDTA [pH 8]). The DNA solution was transferred to a 1.5 mL tube and 5 µl of RNaseA (20 mg/mL) was added and incubated at 37 °C for 1 hour to remove RNA.

3.4.3.2 PCR amplification

PCR amplification was carried out using 10 µl total reaction mixture following modified procedure by Mahuku (2004). A volume of 1 µl 50 ηg/µl DNA template was added to a PCR premix (modified) containing 10 nM forward and reverse primers (reverse 5'-CAAA CCTCATCATCATATCCCAC-3' and forward primer; 3'-GAGAGGAGTGCAGCTTTC TGG-5'), 1 µl 2 mM $MgCl_2$, PCR buffer, 0.5 µl of 0.1 mM dNTPs mix, 2 µl of 0.4 U/µl of DNA Taq Polymerase (BR Biochem Life Science Ltd) and the mixture was brought to a final volume of 10 µl by adding deionized water. The PCR amplification was carried in a programmed thermocycler. The amplification conditions were; initial denaturation at 94 °C for 3 minutes followed by 35 cycles (denaturation at 94 °C for 20 seconds, annealing at 55 °C for 40 seconds, extension at 72 °C for 3 minute) and a final extension at 72 °C for 5 minute.

3.4.3.3 Gel electrophoresis

Agarose powder weighing 2 g was put in a 200 ml flask. 100 ml of TAE (40 mM Tris-acetate, 1 mM EDTA) buffer was measured and added into it and swirled to achieve a

uniform mix. The mixture was heated in the microwave at 95 ºC until the agarose was completely dissolved in the buffer. Ethidium bromide (EtBr) with a concentration of 0.5 µg/ml was then added and shaken well to mix it properly. The preparation was incubated in a 65 °C water bath. With the help of the clamps, gel tray was tightly attached to a casting apparatus. An appropriate gel comb was then put into the gel mold to create the wells. The molten agarose was poured into the gel mold and placed under cool temperatures until it solidified. The comb was removed and carefully placed in electrophoresis chamber filled with the gel electrophoresis buffer. Loading dye comprising 0.25 % bromphenol blue, 0.25 % xylene cyanol and 30 % glycerol was added into to the DNA samples that was to be separate. The electrodes were then connected to the power pack and the gel left to run at 100 v for 45 minutes until the DNA migrated to 75 % of the distance towards the positive node. The gel was carefully removed, once the run was completed. The power supply then turned off and the gel was carefully removed from the electrophoresis chamber, and the buffer drained off. The gel tray was placed on paper towels to absorb any extra running buffer. The gel was then placed in the UV transilluminator and switched on. DNA bands showed up as orange fluorescent bands.

3.4.3.4 DNA sequencing

The PCR product were sent for sequencing where the BARCBean6K_3 SNP array (Song *et al*., 2016) with 1578 SNPs was used to genotype the 120 F2 population. The SNP genotyping was conducted on the BeadXpress Illumina platform following the Infinium® HD Assay Protocol (Song *et al*., 2013). The SNPs positions along the chromosomes were used to anchor the scaffolds of the v0.9 assembly and to position SNPs on the *Phaseolus*

vulgaris 1.0 assembly. The program GenomeStudio Genotyping Module v1.8.4 was used to complete SNP allele calling (Illumina, Inc., San Diego, US).

3.4.3.5 Linkage map construction and QTL analyses

A genetic linkage map of the common bean F2 population was constructed using the software JoinMap v.4.0 at minimum logarithm of odd (LOD) scores of 5.1 between markers (Van Ooijen, 2006). The order of markers in each linkage group was established by maximum likelihood method. Kosambi mapping function (Kosambi, 1994) was used to convert the recombination frequencies to marker distances in centiMorgans (cM).

The whole genome of *Phaseolus vulgaris* map version 1.0 was the reference in locating the physical position of the SNP markers (Goodstein *et al*., 2012; Schmutz *et al*., 2014). QTL analysis for each trait was conducted through the composite interval mapping (CIM) of Win QTL cartographer 2.5 software (Wang *et al*., 2014). Initially, the genomic position of markers associated with drought tolerance was identified through single marker analysis (SMA). Markers and the target trait showing significant association at a $p \leq 0.05$ were considered to identify the same QTL. Then, CIM was performed to locate QTL regions more accurately using a model with a window size of 10 cM, 1 cM walk speed, 5 significant background markers and a reverse forward linear regressions for each chromosomal position (Mukeshimana *et al*., 2014).

LOD thresholds score ($p \leq 0.01$) for defining the position of significant QTL was determined using 1000-permutation test runs for each trait (Churchill and Doerge, 1994). Peak LOD threshold score of 3.0 was set for identifying QTLs based on the permutation results. Designation of the identified QTL was carried out by following QTL nomenclature guidelines for common bean as described by Miklas *et al*. (2003) and Ender *et al*. (2008). According to the guidelines the QTL name consist of three parts; abbreviation for the trait name, linkage groups designation number and the serial number of the QTL controlling a trait. For example, $SY2.3^{KG}$, whereby SY which is the abbreviation for the trait; seed yield, the first number shows the location and for this case is chromosome 2 (Pv02), while the second number (3) indicates the order of discovery of this QTL and the superscript 'KG' is the abbreviation for the population name i.e. KAT B1/GLP2.

3.5 Data analysis

Prior to analysis, data for each trait was tested for homogeneity of variance using Bartlett's test (Bartlett, 1937). Analysis of variance was performed using the SAS V9.2 (SAS, 2011) and the general linear model (PROC GLM) procedure was used to perform combined analysis of variance to test for genotypic differences, treatment effects and genotypic-treatment interactions. Means were separated using Least Significant Differences at $p \leq 0.05$. Simple correlation analysis between grain yield and different traits in drought stress and well watered conditions were determined following the Pearson's correlation method and those traits which had relatively high correlation with the yield in both conditions were considered as the best predictor. Regression analysis was performed by applying MSTAT-C. Excel software (2010) was used to calculate the

drought stress indices as described by Fischer and Maurer (1978) to determine the drought tolerance of the different genotypes.

CHAPTER FOUR

RESULTS

4.1 Identification of phenotypic and physiological characters associated with drought tolerance in selected common bean genotypes

4.1.1 Variation of characters

Results presented in Table 4.1 indicate that variability was found in all the eleven characters except for the leaf biomass as shown by the presence of significant differences ($p \leq 0.05$) among the genotypes. On the other hand, water stress treatment had no significant effect ($p \leq 0.05$) on the leaf biomass. For all the characters except leaf biomass and days to maturity, the interaction of water stress and genotype was significant ($p \leq 0.05$). Genotypes showed varied response to water stress treatment; drought susceptible genotypes were affected greatly by water stress treatment than drought tolerant genotypes (Plate 4.1).

The highest variation among the genotypes was explained by stem biomass (23.51 %), followed by stomatal conductance (19.08 %). Number of grains per plant and pod biomass explained 15.17 % and 14.99 % of genotypic variation respectively. The rest of the characters were responsible for between 4.16 % and 12.48 % of variation. The mean's performance of the characters revealed that water stress caused a reduction of between 2.44 and 59.28 %. Stem biomass (SB) was the most affected (59.28 % reduction) and days to maturity (DM) being the most stable (1.87 % reduction).

Table 4.1: Effect of water stress on various characters of common bean genotypes grown under well watered (ww) and water stress (ws) treatments during 2017-18

	Susceptible		**Tolerant**		**Average**		**Reduction/ Increase (%)**	**Treatment(T) (df=1)**	**Genotype(G) (df=5)**	**G×T (df=5)**	**CV %**
	ws	ww	ws	ww	ws	ww					
NBP	6.00	9.15	11.33	15.84	8.66	12.49	30.50	*	*	*	17.07
NPP	5.33	6.00	9.33	11.33	7.05	8.16	13.60	**	*	**	8.48
NGP	3.00	3.33	4.00	4.66	3.55	4.05	12.34	**	*	*	15.17
SB	2.17	5.44	3.05	7.20	2.63	6.46	59.28	*	**	**	23.51
PB	0.20	0.60	0.54	0.92	0.35	0.69	49.27	**	*	*	14.99
LB	1.35	1.68	1.95	2.34	1.57	1.95	3.48	ns	ns	*	11.25
WUE	2.51	1.61	9.44	7.34	5.52	4.10	34.63	*	**	**	7.08
SCO	21.56	15.78	33.78	28.20	21.94	27.46	25.15	**	**	**	19.08
LWP	-0.74	-0.48	-0.51	-0.48	-0.62	-0.48	29.16	*	**	*	4.16
DF	30	31	45	46	37.85	40.33	13.71	*	*	**	5.11
DM	31	65	46	85	60.50	79.00	1.87	ns	*	ns	9.54

*, **, significance levels at $p \leq 0.05$, and $p \leq 0.01$, respectively; CV, coefficient of variation; NBP, number of branches per plant; NPP, number of pods per plant; NGP, number of grains per pod; SB, stem biomass; PB, pod biomass; LB, leaf biomass; WUE, water use efficiency; SCO, stomatal conductance; LWP, leaf water potential; DF, days to flowering; DM, days to maturity.

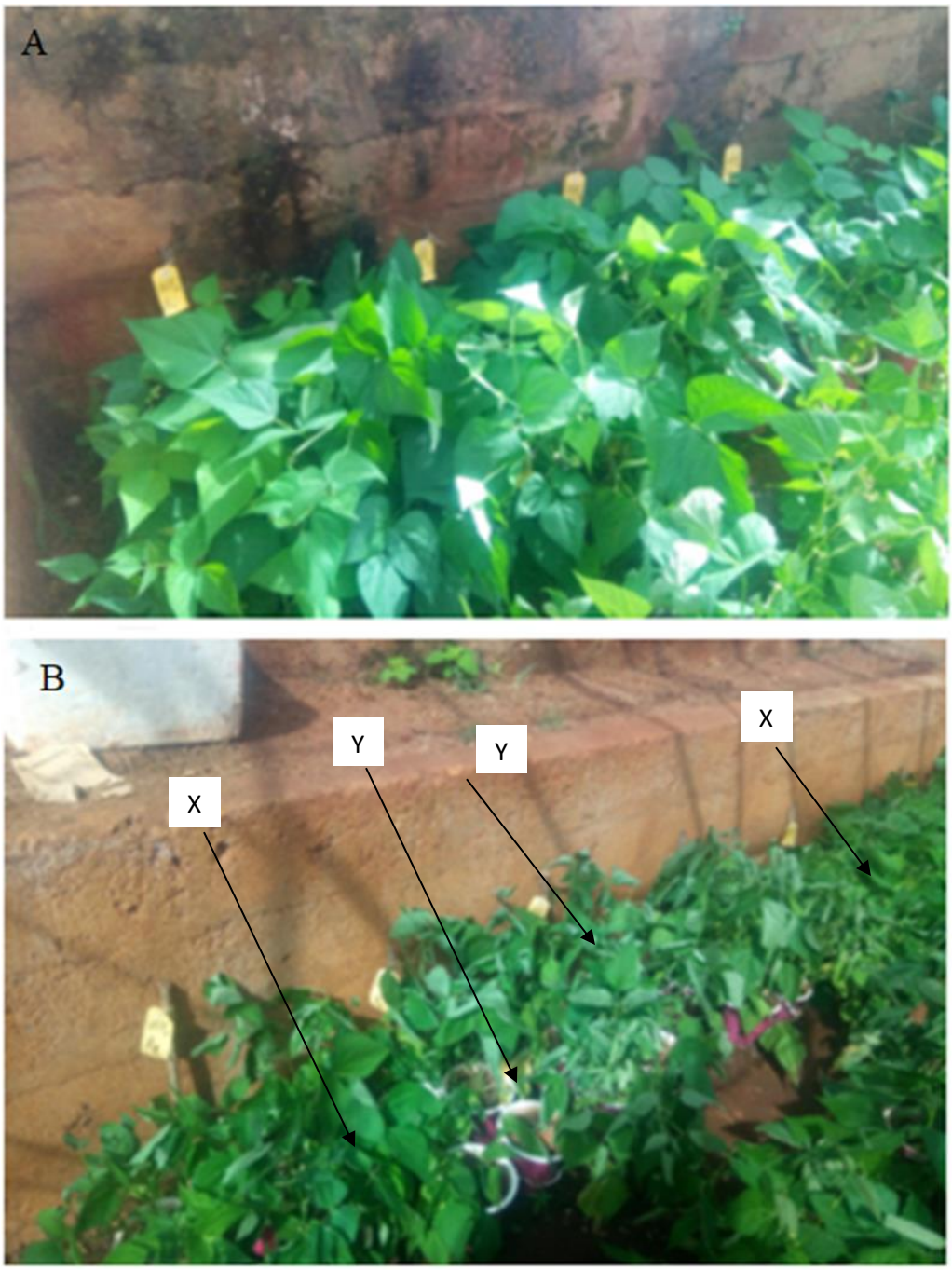


Plate 4.1: Effect of water stress on common bean genotypes: (A) before inducing water stress (B) after inducing water stress. Y, drought susceptible varieties; X, drought tolerant varieties.

An average of 3.55 and 4.05 in the number of grains per pod (NGP) were observed under well- watered and water stress treatment respectively demonstrating 12.34 % reduction in yield when the beans were under water stress. The decrease observed in number of pods per plant (NPP) in response to the induced water stress ranged from 5.33 to 9.33 with a mean of 7.05 when compared to the respective adequately watered treatments which ranged from 6.0 to 11.33 with mean of 8.16. Under well watered condition, all the genotypes showed similar behavior (-0.48 ΨMPa) in leaf water potential, whereas significant differences were noticed under water stress treatment. Nonetheless, leaf water potential (LWP) values for plants growing in adequately watered environment were considerably lower in comparison with that for plants subjected to water stress treatment.

Under water stress condition there was an overall reduction of 25.15 % in stomatal conductance (SCO) among all the genotypes. It was noted that there was increase in water use efficiency under water stress treatment causing an increase of 34.63 % from well watered treatment. Days to flowering (DF) ranged from 31 to 46 days with a mean of 40.3 days in well water treatment and from 30 to 45 days with a mean of 37.5 days under water stress treatment. On average flowering time took 1.5 days early (13.71 % reduction) under water stress compared with well watered treatment. Exposure to water stress caused genotypes to mature after 60.5 days (1.87 % reduction) earlier compared to that under normal supply of water. Water stress caused a reduction in stem biomass by 59.28 % from the well watered treatment. For leaf biomass, exposure to water caused a decrease of 3.48 % from the well watered treatment (Table 4.1).

4.1.2 Variability among traits, genotypes and water treatments

Tests for homogeneity of variance revealed homogenous error variance for each trait and thus, allowed for the analysis of variance. Four factors; replication, genotype, treatment and interaction were involved in the analysis of variances (Table 4.2). All the traits except days to flowering and maturity were significantly affected by replication effect.

Water treatment effect showed significant differences for all the traits assessed except for leaf biomass and days to maturity. Similarly, the interaction between the genotype and water treatment (G×T) had a significant effect on all the traits except days to maturity. Variances due to genotypes were statistically significant for all the characters except leaf biomass. Water use efficiency and days to flowering were not significantly affected by G×R interaction (Table 4.2).

Table 4.2: Combined analysis of variance of main effects on characters of common beans

Source of variation	Df	Mean value and level of significance										
		NBP	NPP	NGP	SB	PB	LB	WUE	LWP	SCO	DF	DM
R	2	9.02^{**}	10.51^{**}	0.78^{**}	2.00^{**}	0.06^{**}	0.02^{**}	0.01^{*}	0.01^{*}	0.01^{*}	0.02^{ns}	0.08^{ns}
G	5	26.28^{*}	16.04^{**}	1.03^{**}	1.19^{*}	0.14^{*}	0.26^{ns}	32.55^{**}	15.01^{**}	145.62^{**}	186.25^{**}	301.65^{**}
G*R	10	1.86^{**}	1.31^{*}	0.44^{*}	0.79^{**}	0.01^{*}	0.11^{*}	0.17^{ns}	0.21^{*}	7.14^{**}	0.01^{ns}	0.07^{*}
T	1	12.25^{*}	11.11^{**}	2.25^{**}	13.59^{*}	1.02^{*}	1.27^{ns}	18.01^{*}	0.18^{*}	273.57^{**}	20.25^{**}	1334.25^{ns}
G*T	5	1.71^{**}	1.17^{*}	0.45^{*}	0.64^{**}	0.01^{*}	0.03^{*}	1.02^{*}	0.01^{*}	0.67^{**}	1.05^{**}	22.05^{ns}
Model	23	8.33	5.71	0.68	6.68	0.08	0.12	8.16	0.02	46.80	41.59	650.00
Error	12	2.55	0.41	0.33	1.14	0.01	0.03	0.11	0.00	22.24	0.00	0.00
Corrected total	35											

*, **, significance levels at $p \leq 0.05$, $p \leq 0.01$ respectively; R, Replication; G, genotype; T, treatment; NBP, number of branches per plant; NPP, number of pods per plant; NGP, number of grains per pod; SB, stem biomass; PB, pod biomass; LB, leaf biomass; SCO, stomatal conductance; WUE, water use efficiency; LWP, leaf water potential; DF, days to flowering; DM, days to maturity.

4.1.3 Drought susceptibility index

The calculated estimates for drought tolerance indices for individual genotypes are shown in (Table 4.3). The geometric mean productivity for Faida and GLP2 under the two watering regimes was 142.5 and 131.9 respectively which were lower compared with that for Nyota, Metameta, Angaza and KAT B1 which were; 235.7, 237.3, 229 and 258.2 respectively. Variation of drought susceptibility index ranged from 0.26 to 0.83. Nyota registered the lowest drought susceptibility index values (0.26) and yield reduction rate (42.8 %) and therefore, considered the most drought tolerant genotype followed by KAT B1 with yield reduction rate and drought susceptibility index values (47.5 % and 0.37) respectively. GLP2, Faida, Metameta and Angaza were considered the most drought susceptible genotypes because of their relatively high values of drought susceptibility index and yield reduction rate.

Table 4.3: Geometric means for grain yield, percentage yield reduction and drought susceptibility index of six common bean genotypes

	Drought tolerance indices		
Genotype	GM	PYR (%)	DSI
Nyota	235.7	42.8	0.26
Faida	142.5	78.5	0.74
Metameta	237.3	63.1	0.57
GLP2	131.9	87.1	0.83
Angaza	229	61.3	0.6
KAT B1	258.2	47.5	0.37
Average	205.77	63.38	0.56

GM, Geometric mean; PYR, percentage reduction in grain yield; DSI, Drought susceptibility index.

4.1.4 Correlations between characters evaluated under well watered and water stress treatment

Significant correlation coefficients between various characters were found (Table 4.4 and 4.5). Highly positive and significant relationships involving key characters were established; number of grains per pod and number of pods per plant (r = 0.61), number of grains per pod and number of branches (r = 0.57), number of grains per pod and pod biomass (r = 0.55), number of grains per pod and stomatal conductance (r = 0.68), water use efficiency and leaf biomass (r = 0.65), days to flowering and leaf biomass (r = 0.51) (Table 4.4). Water use efficiency exhibited positive and significant correlation with days to flowering (r = 0.48) and days to maturity (r = 0.42) under water stress treatment (Table 4.4) while no correlation was realized between water use efficiency and days to flowering and maturity under well watered treatment (Table 4.5). Under both treatments, a positive association was observed for days to flowering with leaf water potential and stomatal conductance whereas the association of days to maturity was significant but negative with leaf water potential and stomatal conductance. Correlations between number of grains with stomatal conductance, leaf water potential and water use efficiency was negative and non-significant under well watered treatments (r = -0.11, r = -0.07 and r = -0.03) respectively (Table 4.5). However, under water stress treatment, there was a significant positive correlation (r = 0.68, r = 0.26 and r = 0.32) between number of grains with stomatal conductance, leaf water potential and water use efficiency (Table 4.4). Among the twelve studied characters, only WUE did not display a strong association with any other trait under well watered treatment. (Table 4.5).

Table 4.4: Correlation coefficients between various characters of six common bean genotypes under water stress treatment in a

Characters	DF	DM	SCO	LWP	WUE	HSW	SB	LB	PB	NBP	NPP	NGP
DF	1	-0.09	0.60**	0.32*	0.48**	0.03	0.41**	0.51**	0.31*	0.04	- 0.45**	-0.15
DM		1	-0.53**	-0.41**	0.42**	0.17	0.22*	0.29*	0.27*	0.34**	-0.08	-0.34**
SCO			1	-0.48**	-0.31*	0.25*	0.36*	0.23*	0.21*	0.28*	0.31*	0.68**
LWP				1	0.22*	0.31*	0.29*	0.37*	0.41*	0.35*	0.42**	0.26*
WUE					1	0.22*	0.35*	0.65**	0.23*	0.43**	0.17	0.32*
HSW						1	0.28*	0.48**	0.13	0.29*	0.24*	0.18
SB							1	0.54**	-0.18	-0.31*	-0.26*	0.12
LB								1	-0.06	-0.47**	-0.17	0.09
PB									1	-0.15	-0.46**	0.55**
NBP										1	0.65**	0.57**
NPP											1	0.61**
NGP												1

rain out shelter during 2017-2018

*significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; DF, days to flowering; DM, days to maturity; SCO, stomatal conductance; LWP, leaf water potential; WUE, water use efficiency; HSW, hundred seed weight; SB, stem biomass; LB, leaf biomass; PB, pod biomass; NBP, number of branches per plant; NPP, number of pods per plant; NGP, number of grains per plant.

Table 4.5: Correlation coefficients between various characters of six common bean genotypes under well watered treatment in a rain out shelter during 2017-2018

Characters	DF	DM	SCO	LWP	WUE	HSW	SB	LB	PB	NBP	NPP	NGP
DF	1	-0.25*	0.41**	0.18	-0.06	0.11	-0.19	0.27*	0.21	0.24	0.14	0.38*
DM		1	-0.22	-0.06	-0.07	0.39*	0.28*	0.04	0.08	0.19	0.07	0.43**
SCO			1	0.10**	0.36*	-0.12	-0.08	-0.23	-0.03	-0.12	-0.04	-0.11
LWP				1	-0.53**	-0.07	-0.20	-0.16	-0.13	-0.01	-0.48*	-0.07
WUE					1	0.15	-0.02	-0.09	-0.01	-0.04	-0.02	-0.03
HSW						1	0.17	-0.08	-025*	0.48**	0.32*	0.33*
SB							1	-0.35*	0.65**	0.63**	0.43**	0.53**
LB								1	-0.29*	-0.41**	-0.51**	0.42**
PB									1	0.22	0.46**	0.51**
NBP										1	0.44**	-0.38*
NPP											1	0.36*
NGP												1

*significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; DF, days to flowering; DM, days to maturity; SCO, stomatal conductance; LWP, leaf water potential; WUE, water use efficiency; HSW, hundred seed weight; SB, stem biomass; LB, leaf biomass; PB, pod biomass; NBP, number of branches per plant; NPP, number of pods per plant; NGP, number of grains per plant.

4.1.5 Contribution of individual characters to grain yield

Stomatal conductance as the predictor variable showed the highest values of adjusted R^2 (0.220) followed by number of pods per plant with adjusted R^2 value of 0.1807 (Table 4.6). Thus, of the total variability in yield the two characters; stomatal conductance and number of pods per plant could be able predict 22.00 % and 18.07 % respectively, whereas 17.65 % of the total variability in the grain yield could be predicted by number of branches per plant (R^2 adjusted = 0.1765). The rest of the yield attributes, individually could only contribute between 1.07 and 5.30 % of the variation in yield.

Results also showed that 10.27 % of total variation in yield could be explained by total biomass above ground (i.e. stem, leaf and pod biomass). Physiological characters (water use efficiency, leaf water potential and stomatal conductance) were able to account for a total of 29.54 % of the total yield variation respectively. However, days to flowering (R^2 adj= 0.0107) showed the least ability to predict grain yield. Thus, it is evident that stomatal conductance is the most important predictor variable among all other characters.

Table 4.6: Stepwise regression analysis of grain yield and individual characters under well watered and water stress treatment

Yield attribute	Regression equations	R^2	R^2-adjusted	Contribution to total variation in grain yield (%)
Number of branches/plant	y= 0.1212 +1.5865NGP	0.2280	0.1765**	17.65
Number of pods/plant	y= 2.864 -12.896NGP	0.2319	0.1807**	18.07
Stem biomass	y= 8.3629 - 24.752NGP	0.0235	0.0184**	1.84
Leaf biomass	y= 12.102 - 12.596NGP	0.0356	0.0313*	3.13
Pod biomass	y= -0.6517 +5.3557NGP	0.0127	0.0530**	5.30
Days to flowering	y= -0.0309 +6.5219NGP	0.0716	0.0107*	1.07
Days to maturity	y= -0.1028 +9.4473NGP	0.0853	0.0243*	2.43
Stomatal conductance	y= -0.227 +22.496NGP	0.238	0.220**	22.00
Leaf water potential	y= 14.243 +14.181NGP	0.0194	0.0459*	4.59
Water use efficiency	y= -0.609 +9.1098NGP	0.0347	0.0295*	2.95

*, ** Significant at $p \leq 0.05$, $p \leq 0.01$ respectively, ns, not significant; R^2, coefficient of determination; NGP, Number of grains /plant.

4.1.6 Effects of water stress treatment on various characters of common bean

There was varied genotypic performance within and across the water treatments among all the characters assessed (Figures 4.1 - 4.11). The number of branches per plant showed significant differences ($p \leq 0.05$) both among genotypes and water treatments (Figure 4.1). Although, Nyota registered the highest number of branches, it was not significantly different ($p \leq 0.05$) from KAT B1 and GLP2 under well watered treatment. However, it had significantly higher number of branches than that of Faida, Metameta and Angaza under well watered treatment. Under water stress, Nyota produced the highest number of branches per plant followed by KAT B1 and Angaza. GLP2, Metameta and Faida produced significantly lower number of branches under water stress treatment. The number of branches produced under well watered treatment by each genotype was significantly different from that produced under water stress treatment (Appendix 1).

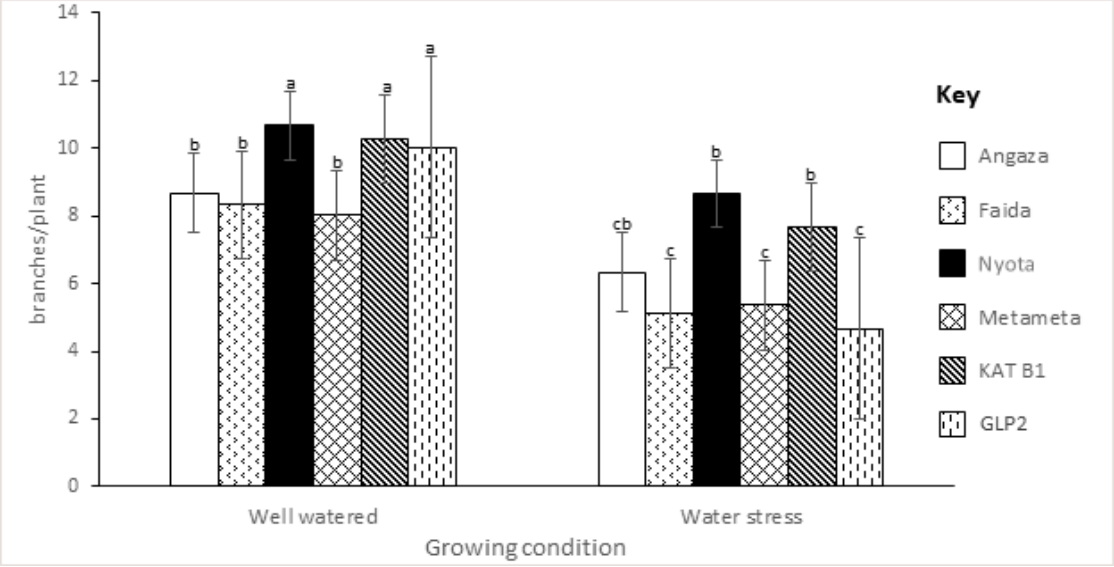


Figure 4.1: The effects of genotype and water treatment on number of branches per plant of common bean genotypes. Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

When grown under well watered treatment, Nyota, KAT B1 and GLP2 produced relatively high number of pods per plant; 10.33, 9.67 and 9.33 respectively in contrast with significantly ($p \leq 0.05$) lower 8.57, 5.33 and 3.37 values observed under water stress treatment (Figure 4.2). Metameta, Faida and Angaza grown under well watered treatment presented significantly lower ($p \leq 0.05$) number of pods per plant than that produced by Nyota, KAT B1 and GLP2. Under water stress treatment, GLP2 showed the highest drop in the number of pods per plant whereas Nyota yielded significantly higher ($p \leq 0.05$) number of pods per plant compared to all the other genotypes. Significant differences ($p \leq 0.05$) were observed in the genotypes for number of pods per plant in water stress treatment (Appendix 2)

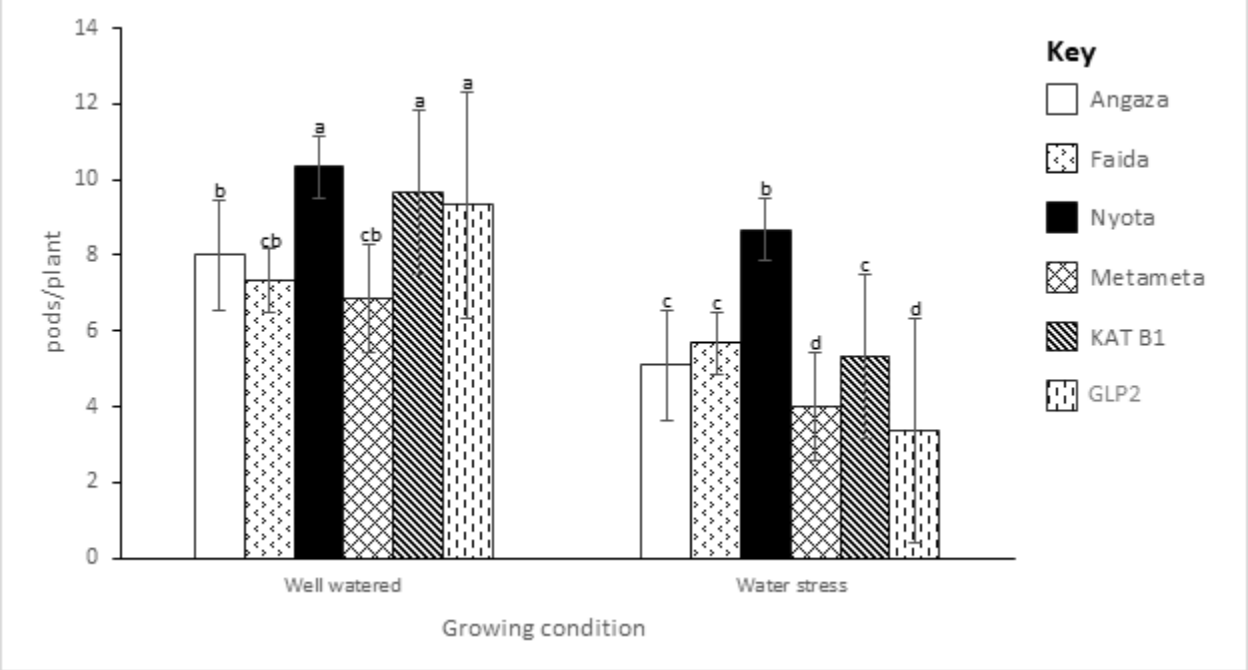


Figure 4.2: The effects of genotype and water treatment number of pods per plant of common bean genotypes. Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

The number of grains per pod were markedly affected in GLP2, Faida, Metameta and Angaza when subjected to water stress treatment. In contrast, Nyota and KAT B1 are not significantly different in number of grains per pod under water stress treatment as those observed under well watered treatment (Figure 4.3). Interestingly, the number of grains per pod yielded by Faida and Metameta in either treatment were almost similar. The water stress treatment was significant ($p \leq 0.05$) in affecting the number of grains per pod of all the genotypes (Appendix 3)

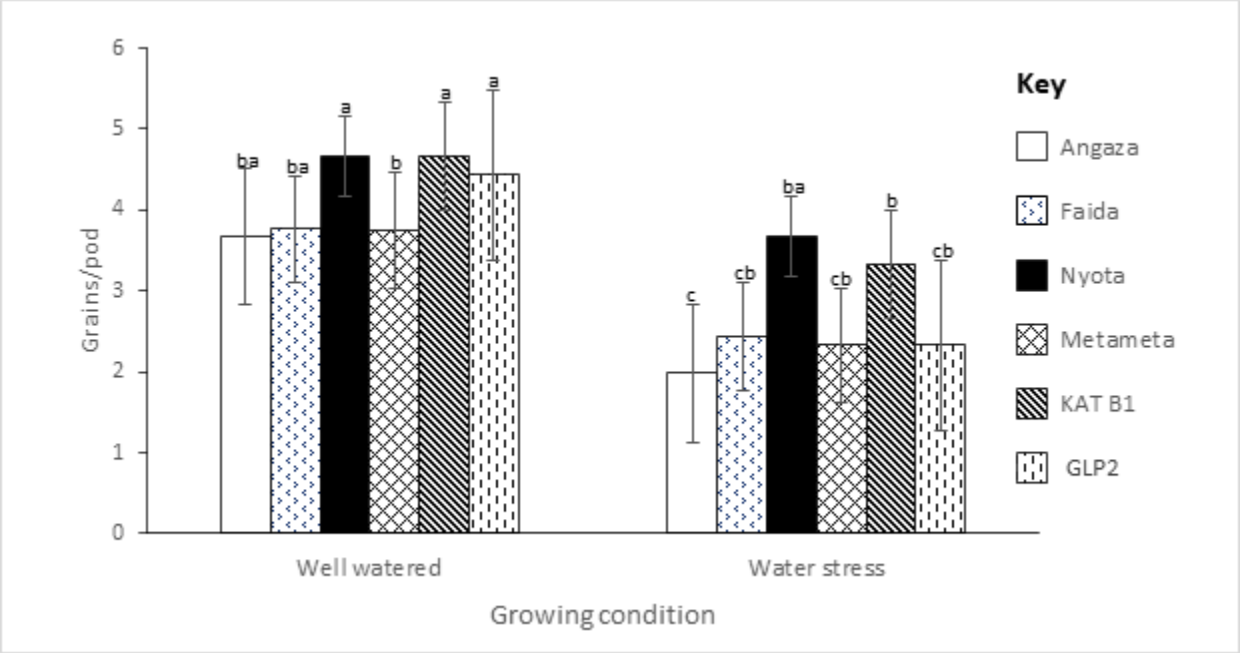


Figure 4.3: The effects of genotype and water treatment number of grains per pod of common bean genotypes. Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

Statistical analysis showed there was no significant differences ($p \leq 0.05$) in the leaf biomass within the genotypes (Figure 4.4). The water stress treatment significantly ($p \leq 0.05$) affected the leaf biomass of all the genotypes. Angaza displayed the highest leaf

biomass, followed by Nyota and KAT B1 when they were growing under well water conditions. Faida produced the least amount of leaf biomass under well watered condition. When subjected to water stress treatment, the relative performance of the different genotypes in terms of leaf biomass accumulation showed almost similar trend seen under well watered treatment (Appendix 4).

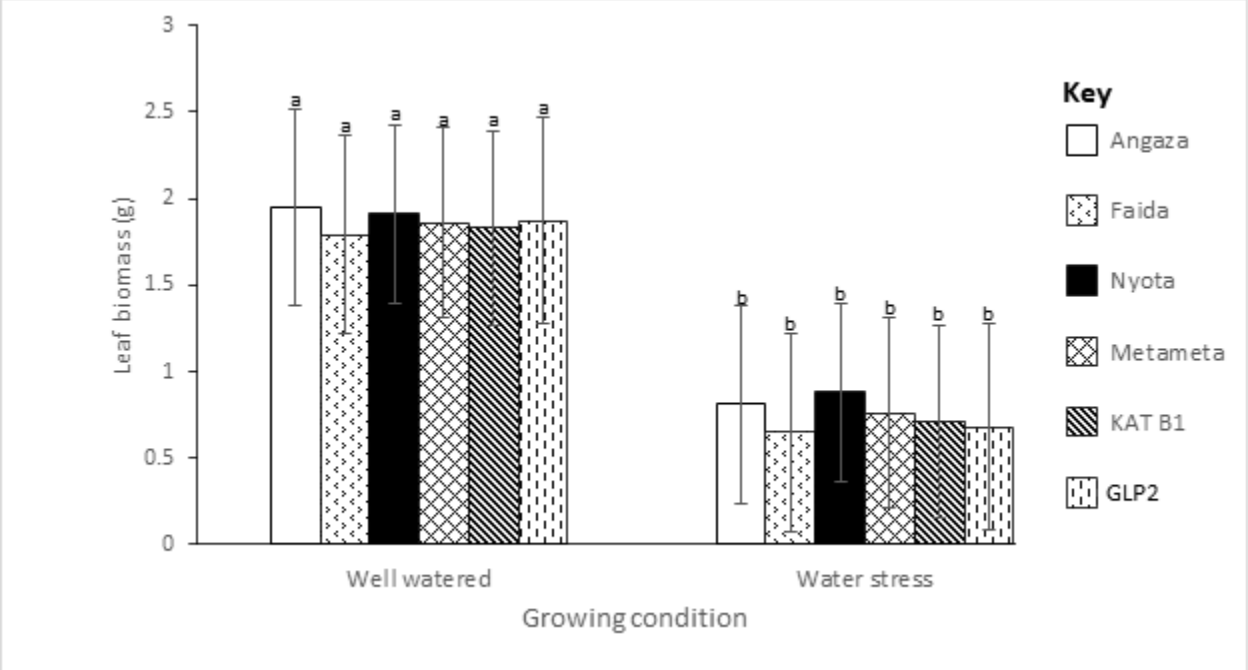


Figure 4.4: The effects of genotype and water treatment on leaf biomass of the six common bean genotypes. Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

Pod biomass values showed significant differences ($p \leq 0.05$) both among the genotypes and between water treatments (Figure 4.5). All the genotypes showed significantly lower pod biomass under water stress treatment compared to well-watered conditions. Under well watered conditions, Angaza, Nyota and KAT B1 produced significantly ($p \leq 0.05$) higher amount of pod biomass compared to other genotypes. The amount of pod biomass

produced by Metameta and Faida under water stress, was not statistically different ($p \leq 0.05$) from that produced under well watered treatment (Appendix 5).

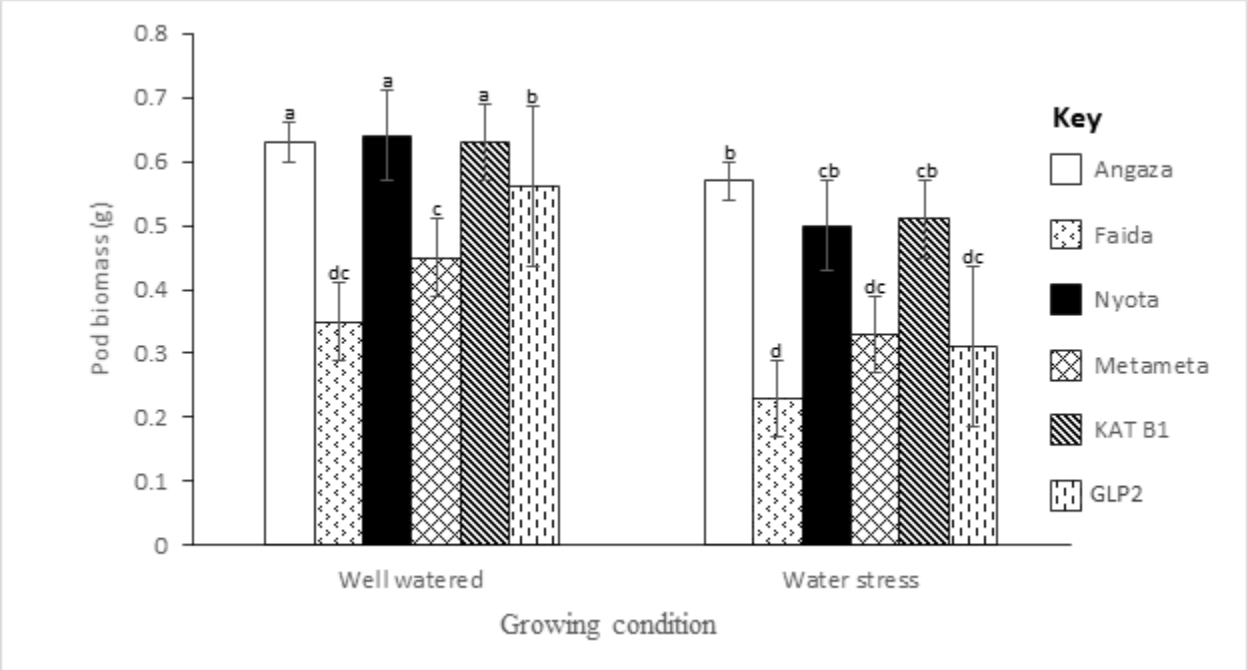


Figure 4.5: The effects of genotype and water treatment on pod biomass of common bean genotypes. Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

Results revealed that water stress reduced significantly ($p \leq 0.05$) the amount of accumulated biomass in the stem of all genotypes (Figure 4.6). In general, stem biomass differed significantly between the genotypes with Nyota accumulating the highest stem biomass compared to other genotypes when subjected to water stress treatment. Metameta and GLP2 yielded significantly lower amount of stem biomass under water stress conditions (Appendix 6).

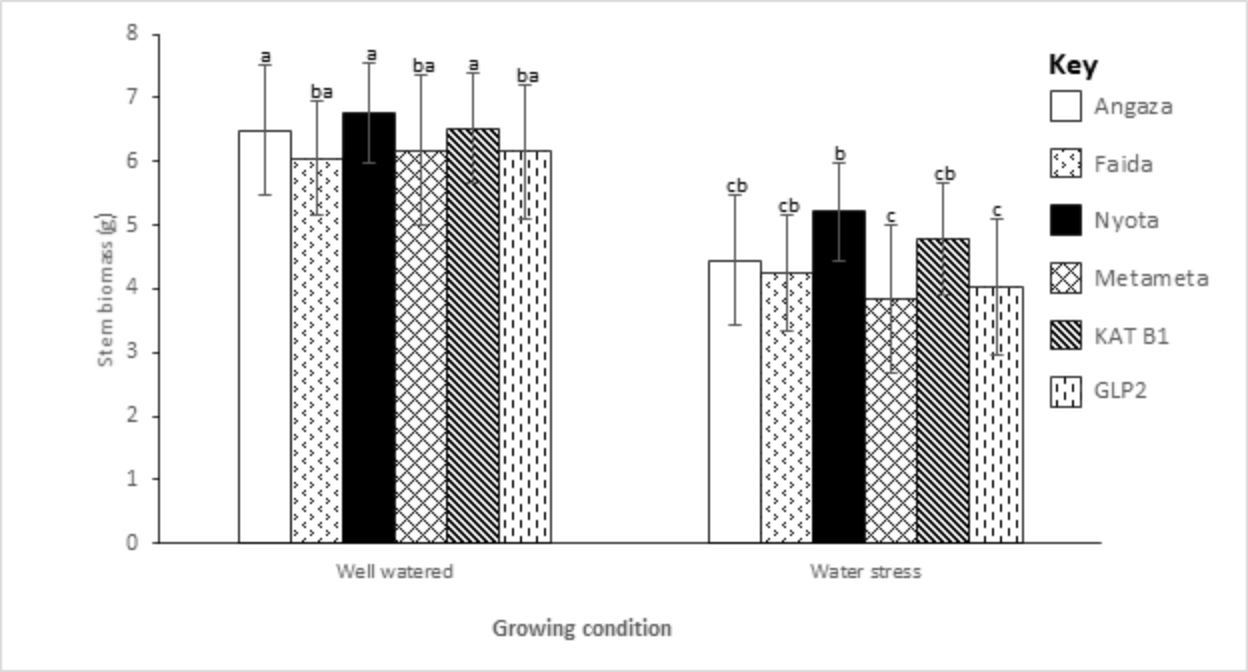


Figure 4.6: The effects of genotype and water treatment on stem biomass of common bean genotypes. Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

For water use efficiency measurements (Figure 4.7), under well watered treatments, the genotype Nyota showed the highest mean value of 4.74. However, it was not significantly different ($p \leq 0.05$) from the other genotypes. Under water stress treatment, genotypes showed high variation in water use efficiency allowing to establish statistical significance among the different genotypes. Nyota and KAT B1 were was the most efficient, whereas GLP2 was the least water use efficient genotype. In general, water use efficiency increased significantly ($p \leq 0.05$) in all genotypes when the plants were subjected to water stress (Appendix 7).

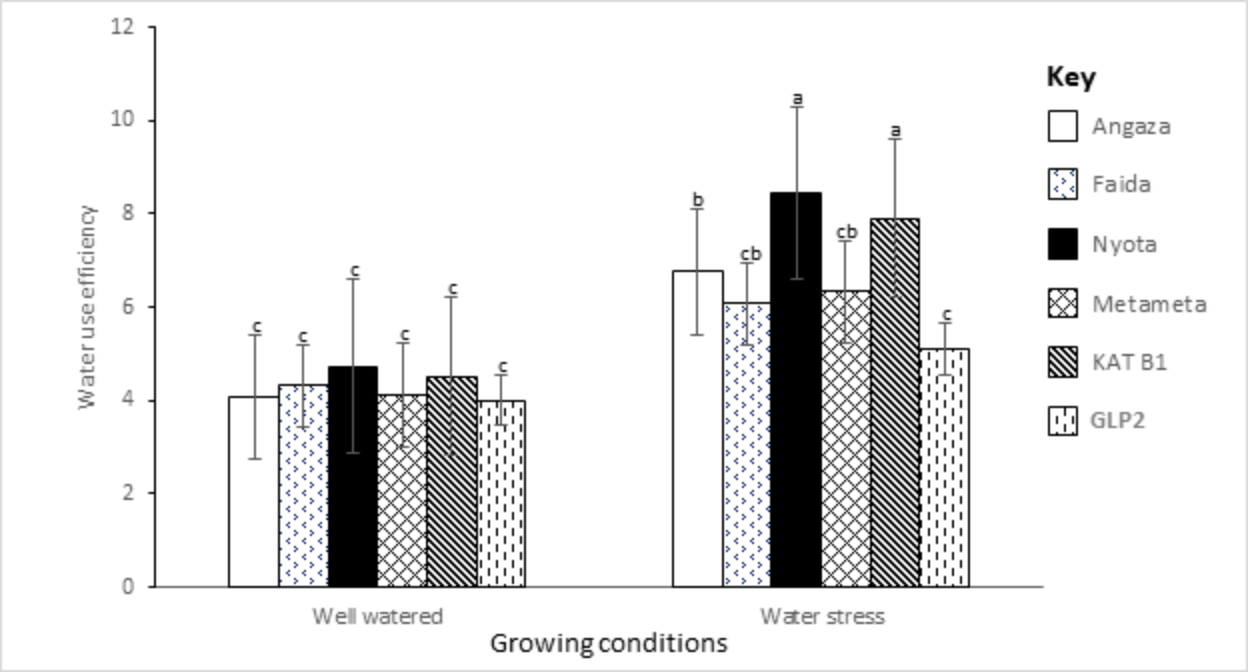


Figure 4.7: The effects of genotype and water treatment on water use efficiency (WUE) of common bean genotypes. Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

Genotypic variation for stomatal conductance was evident under both well watered and water stress treatments (Figure 4.8). Under well watered treatment, the value of stomatal conductance for Angaza was the highest (33.79 mol m^{-2} s^{-1}) while for Metameta this value was the lowest (25.15 mol m^{-2} s^{-1}). Under water stress treatment, the observed values for Nyota, Angaza and KAT B1 were significantly ($p \leq 0.05$) higher than for the other genotypes. Generally, GLP2, Metameta and Faida were the most affected by water stress and showed values of 12.17, 10.64 and 11.32 mol m^{-2} s^{-1} (Appendix 8).

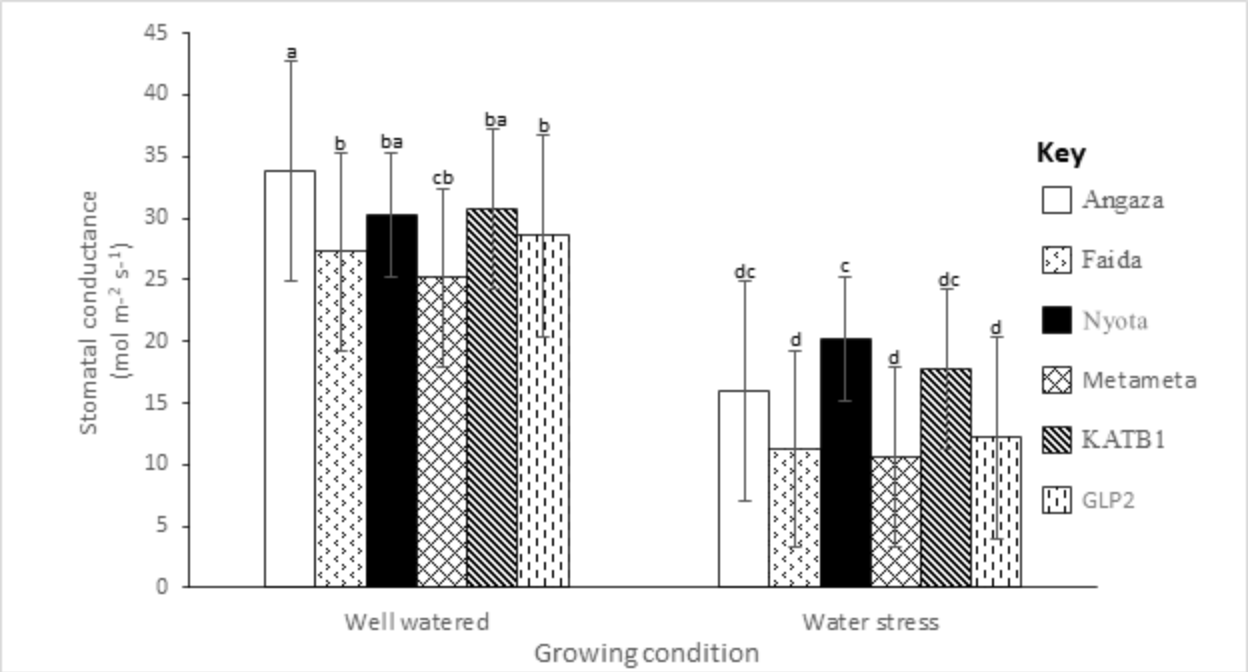


Figure 4.8: The effects of genotype and water treatment on stomatal conductance of common bean genotypes Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

The genotypic effects was not significant ($p \leq 0.05$) for leaf water potential under well watered treatment. However, significant differences were observed when compared with those grown under water stress conditions (Figure 4.9). Important differences ($p \leq 0.05$) was observed between the genotypes under water stress treatment. The tolerant genotypes (Nyota and KAT B1) exhibited significantly higher (less negative MPa) leaf water potential under water stress treatment than the susceptible genotypes (Faida, GLP2 and Metameta). The genotype GLP2 showed the lowest leaf water potential under water stress condition among the tested genotypes -0.74 MPa (Appendix 9)

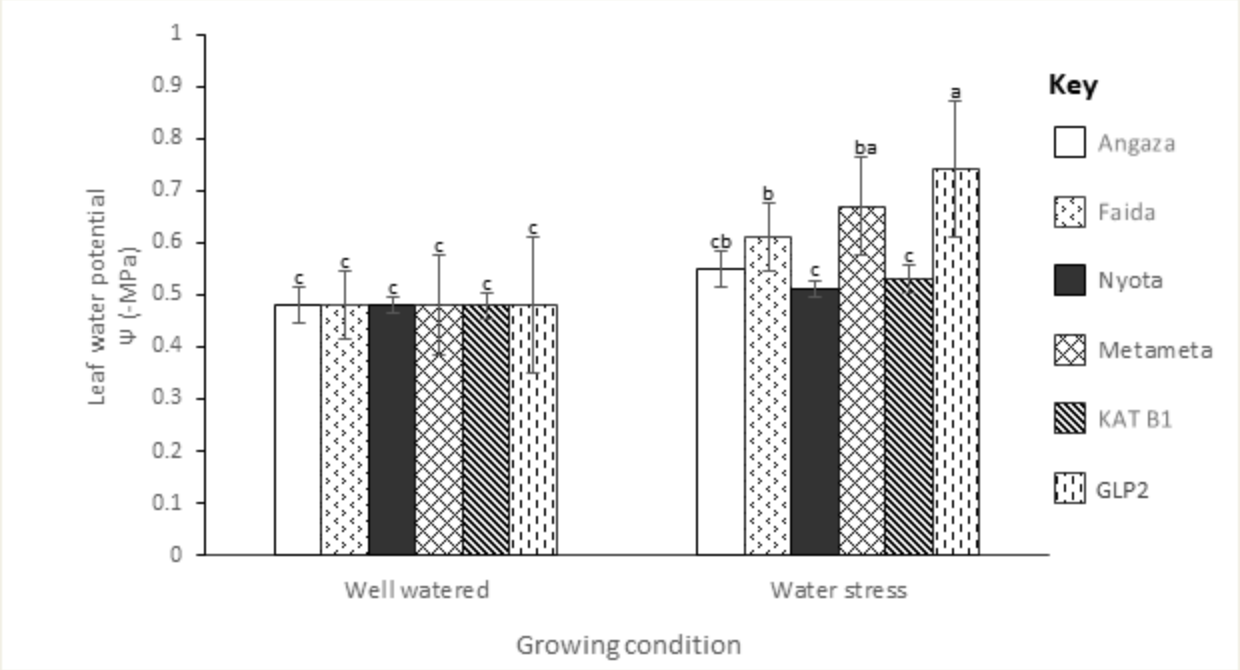


Figure 4.9: The effects of genotype and water treatment on leaf water potential of common bean genotypes. Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

Significant differences ($p \leq 0.05$) were observed in the genotype and the water treatment for days to flowering (Figure 4.10). The genotypes that were growing under water stress treatment flowered earlier than their counterparts under well watered treatment. Under well watered treatment, KAT B1 was early to flowering at 32.5 days. This was significantly ($p \leq 0.05$) shorter period to flowering compared to both Faida and Metameta that flowered after 45.5 days. Though Angaza, Nyota and GLP2 took an average of 4 days less to flowering compared to both Faida and Metameta, the difference was not significant ($p \leq 0.05$). Under water stress treatment, both Angaza and Metameta took relatively longer time to flowering compared to other genotypes. In essence, the duration taken by Angaza and Metameta was significantly ($p \leq 0.05$) longer than that

recorded for both KAT B1 and Nyota. However, it was not significantly ($p \leq 0.05$) different from time taken to flowering by Angaza and GLP2 (Appendix 10).

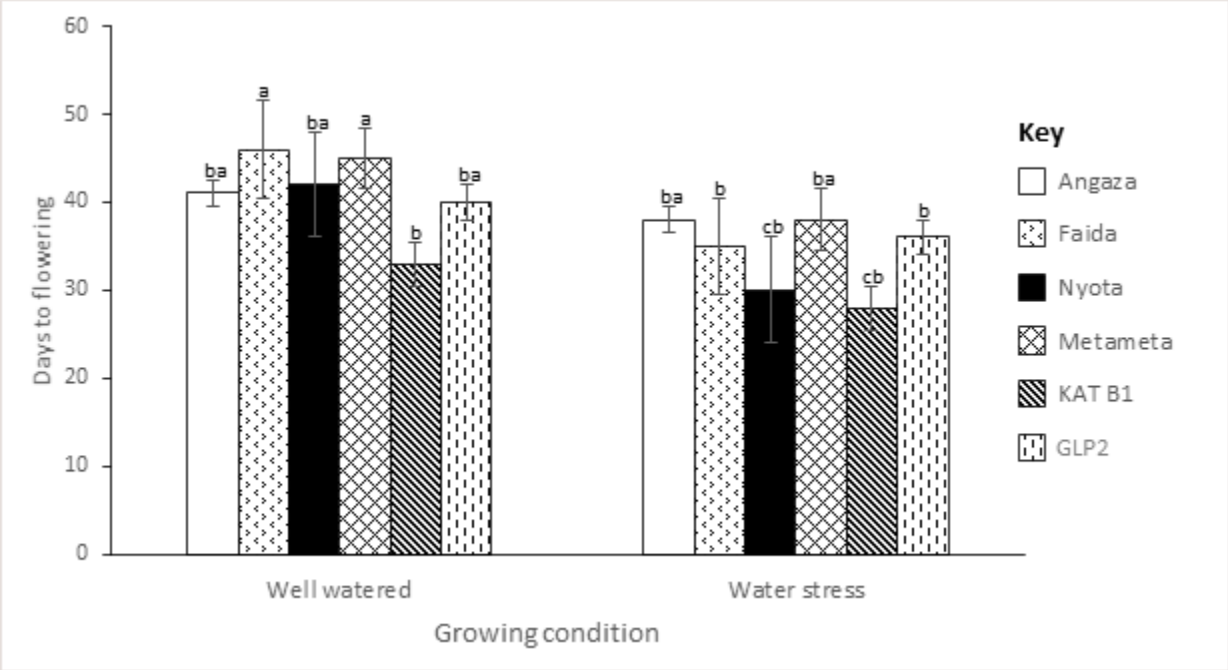


Figure 4.10: The effects of genotype and water treatment on days to flowering in common bean genotypes. Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

Water stress accelerated physiological maturity for all the genotypes. The mean number of days to physiological maturity under well watered treatment ranged between 80th and 84th day with two exceptions, KAT B1 and GLP2 that matured significantly ($p \leq 0.05$) earlier at 65th and 71st day respectively (Figure 4.11). Under well watered treatment, typically all genotypes matured between 55th and 72nd day. Maturity period was significantly ($p \leq 0.05$) lower under water stress treatment compared to well-watered conditions. The relative days to maturity of the different genotypes followed almost the same trend under each treatment (Appendix 11).

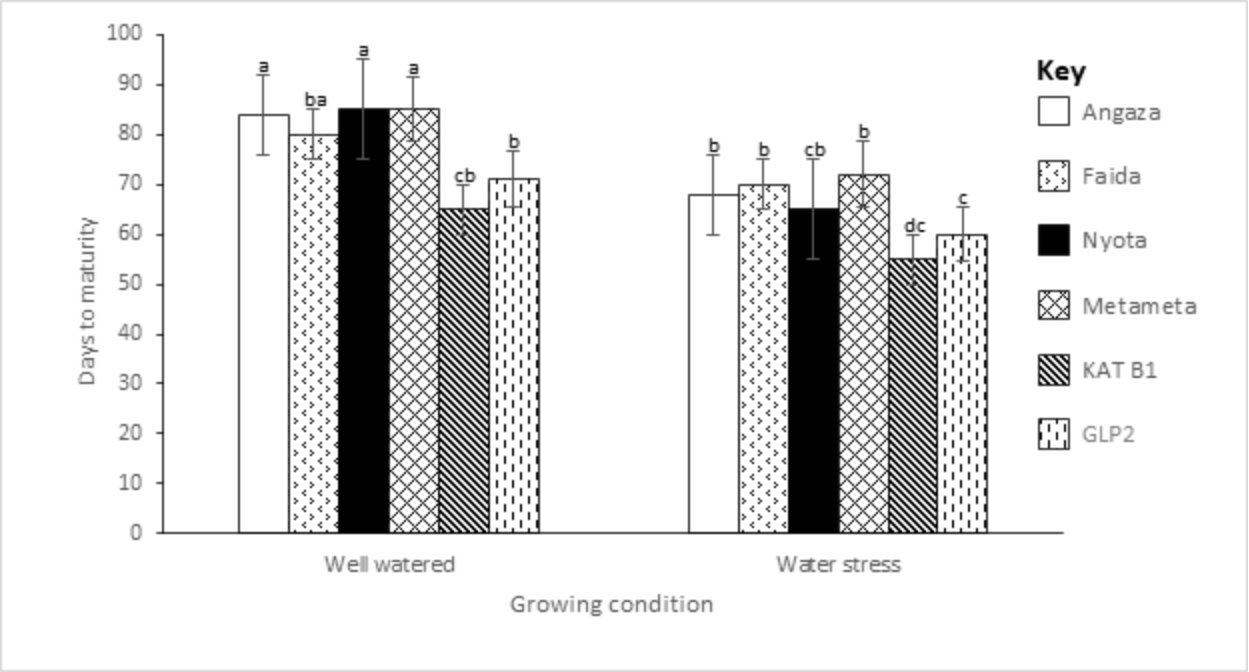


Figure 4.11: The effects of genotype and water treatment on days to physiological maturity in common bean genotypes. Bars with the same letters are not significantly different according to Tukey's honest significant difference at $p \leq 0.05$.

4.2 Characterization of F2 population (KAT B1 × GLP2) for drought tolerance using phenotypic and physiological characters traits

4.2.1 Variation in characters of the common bean population

Table 4.7 shows the mean squares and coefficients of variation (CV) for F2 population of common bean. Response to water stress treatment was highly significant ($p \leq 0.01$) for the number of pods per plant (NPP), number of grain per pod (NGP), yield per plant (YP) and 100-seed weight (HSW) while significant differences ($p \leq 0.05$) were observed under well watered and water stress treatment for all the other characters (Table 4.7).

Table 4.7: ANOVA for eleven characters of segregating population

Trait	Level	Replication Mean Squares (RMS)	Population Mean Squares (PMS)	Error Mean Squares (EMS)	Coefficient of Variation (CV %)
DF	ww	7.59	12.81*	0.42	7.75
	ws	4.56	9.1*	0.29	5.83
DM	ww	70.7	2.3 *	0.40	5.43
	ws	17.4	28.6*	0.40	3.70
NBP	ww	2.76	2.59*	0.35	12.37
	ws	0.44	8.58*	0.60	28.03
NPP	ww	12.91	8.1*	0.70	10.02
	ws	0.33	85.8**	0.76	26.95
SCO	ww	3.11	32.7*	0.48	13.29
	ws	0.29	46.37*	0.25	21.02
LWP	ww	10.93	1.72*	1.03	6.51
	ws	2.78	33.47*	0.15	10.51
HSW	ww	7.68	8.43*	6.41	10.79
	ws	0.14	4.17**	0.34	9.77
BG	ww	0.47	1.21*	0.29	14.54
	ws	0.92	9.99*	1.19	38.06
NGP	ww	0.18	0.38*	0.09	5.71
	ws	0.53	12.25**	0.33	21.85
YP	ww	0.023	0.2*	0.08	11.40
	ws	2.89	60.48**	1.09	16.86
HI	ww	0.075	0.14*	0.09	7.53
	ws	1.07	23.49*	1.77	24.80

*, ** Significant at $p \leq 0.05$ and $p \leq 0.01$ respectively. ww, well-watered; ws, water stress; DF, Days to flowering; DM, Days to maturity; NBP, Number of branches/plant; NPP, Number of pod/plant; SCO, Stomatal conductance; LWP, Leaf water potential; HSW, hundred seed weight; BG, Biomass above ground; NGP, Number of grain/pod; YP, Yield/plant; HI, Harvest index.

There was considerable variability among the various characters within population under consideration. The highest population diversity was recorded under water stress treatment in the biomass above ground (BG) (38.06 %), followed by number of branches per plant (NBP) (28.03 %), the number of pods per plant (NPP) (26.95 %), harvest index (HI) (24.80 %), the number of grains per pod (NGP) (21.85 %) and stomatal conductance (SCO) (21.02 %).

There was moderate diversity in yield per plant (YP) (16.86 %) under water stress treatment. Under well watered treatment there was moderate diversity in biomass above ground (BG) (14.54 %), stomatal conductance (SCO) (13.29 %) and number of branches per plant (NBP) (12.37 %). Incidentally, the lowest population diversity in either treatments was seen in days to maturity (DM); (3.70 %) under water stress and (5.43 %) under well watered treatment (Table 4.7).

4.2.2 Variance of components (σ^2)

There were small differences between phenotypic and genotypic variance for almost all characters (Table 4.8). The genotypic variance ranged from 0.03 (yield per plant) to 21.26 (number of pods per plant) under water stress treatment. In well watered conditions, it ranged between 0.05 (leaf water potential) and 14.85 (harvest index). Results showed that the highest shared genetic variance component was found to be the number of pods per plant (21.26) under water stress treatment.

Table 4.8: Estimates of variance of components for the studied characters

Characters	Level	σ^2g	σ^2e	σ^2p	GCV %	PCV %	h^2 %	GA	GAM %
DF	ww	3.10	0.42	3.52	17.61	19.20	88.07	2.46	22.41
	ws	2.45	0.29	2.74	15.65	11.23	89.42	2.19	19.92
DM	ww	2.03	0.4	2.43	14.25	15.63	83.54	1.99	18.13
	ws	7.55	0.4	7.95	27.48	25.58	94.97	3.85	34.97
NBP	ww	0.56	0.35	0.91	1.48	1.52	61.5	1.05	9.52
	ws	2.00	0.60	2.60	14.14	14.33	79.92	2.02	18.35
NPP	ww	9.60	0.70	10.30	30.98	27.17	73.20	4.34	32.16
	ws	21.26	0.76	22.02	36.11	36.70	96.54	6.45	22.32
SCO	ww	3.02	0.61	3.44	20.45	21.89	77.65	1.85	17.28
	ws	8.76	0.6	9.03	36.13	33.47	84.25	2.06	22.87
LWP	ww	0.05	0.09	0.17	2.37	2.28	43.40	0.29	2.86
	ws	2.73	0.21	2.29	16.80	14.37	60.14	4.27	25.46
HSW	ww	13.01	6.41	19.42	36.07	32.37	66.99	5.05	27.72
	ws	0.96	0.34	1.30	2.80	2.17	73.85	1.37	12.47
BG	ww	0.96	0.34	1.30	2.80	2.17	73.85	1.37	12.47
	ws	0.23	0.29	0.52	1.80	0.87	44.23	0.67	6.10
NGP	ww	2.20	1.19	3.39	4.83	5.65	64.90	2.08	28.88
	ws	0.07	0.09	0.16	2.65	2.27	43.75	0.37	3.37
YP	ww	2.98	0.33	3.31	17.26	15.52	90.03	2.42	21.97
	ws	0.03	0.08	0.11	1.73	1.18	67.27	0.24	2.20
HI	ww	14.85	1.09	15.94	28.54	26.57	93.16	5.39	26.32
	ws	0.09	0.09	0.04	1.00	2.01	50.00	0.06	5.57

ww, well watered; ws, water stress; σ^2_g, genotypic variance; σ^2_e, environmental variance; σ^2_p, phenotypic variance; GCV, genotypic coefficient of variation; PCV, phenotypic coefficient of variation; h^2, heritability; GA, genetic advance; GAM%, genetic advance as percentage of mean; DF, days to flowering; DM, days to maturity; NBP, number of branches/plant; NPP, number of pod/plant; SCO, stomatal conductance; LWP, leaf water potential; HSW, hundred seed weight; BG, biomass above ground; NGP, number of grain/pod; YP, yield/plant; HI, harvest index

However, under well watered conditions the highest shared genetic variance component was recorded for harvest index (14.85) and 13.01 hundred seed weight. High phenotypic variance was observed for the number of pods per plant (22.02) under water stress. In well watered conditions, hundred seed weight (19.42), harvest index (15.94) and the number of pods per plant (10.30) showed high phenotypic variance. Moderate phenotypic variance was exhibited by stomatal conductance (8.76) and days to maturity (7.55) under water stress conditions. None of the characters exhibited moderate phenotypic variance under well watered conditions (Table 4.8).

Low estimates of phenotypic variance were recorded for days to flowering (3.52), days to maturity (2.43), number of branches per plant (0.91), stomatal conductance (3.44), leaf water potential (0.17), biomass above ground (1.30), number of grains per pod (3.39) and yield per plant (3.31) under well watered conditions.

In water stress treatment, days to flowering (2.74), number of branches per plant (2.60), leaf water potential (2.29), harvest index (1.30), number of grains per pod (0.16), yield per plant (0.11) and harvest index (0.04) showed low estimates of phenotypic variance (Table 4.8).

4.2.3 Genotypic and phenotypic variation coefficient

There was a relatively large coefficient of variability within the genotypes with regard to all the characters studied (Table 4.8). The genotypic coefficient of variation (GCV)

ranged from 1.00 to 36.13 % while the phenotypic coefficient of the variation (PCV) ranged from 0.87 % for biomass above ground to 36.70 % for the number of pods per plant both under water stress. High genotypic coefficient of variation were observed in stomatal conductance (36.13 %), number of pods per plant (36.11 %) and days to maturity (27.48 %) under water stress treatment whereas moderate coefficient of variation was present on number of branches per plant (14.14 %), days to flowering (15.65 %) and leaf water potential (16.80 %) while low coefficient of variation was exhibited in harvest index (1.00 %), yield per plant (1.73 %), above ground biomass (1.80 %), number of grains per pod (2.65 %) and hundred seed weight (2.80 %). Under well watered treatment, high coefficient of variation was recorded for stomatal conductance (20.45 %), harvest index (28.54 %), number of pods per plant (30.98 %) and hundred seed weight (36.07 %).

Moderate genotypic coefficient of variation was present on the days to maturity (14.25 %), yield per plant (17.26 %) and number of days to flowering (17.61 %) while there was a low genotypic coefficient of variation on the number of branches plant (1.48 %), leaf water potential (2.37 %), above ground biomass (2.80 %) and number of grains per pod (4.83 %) (Table 4.8). The phenotypic coefficient of variation was high on stomatal conductance (21.89 %), harvest index (26.57 %), number of pods per plant (27.17 %) and hundred seed weight (32.37 %) but moderate for yield per plant (15.52 %), days to maturity (15.63 %) and days to flowering (19.20 %) whereas, low phenotypic coefficient of variation was recorded for branches per plant (1.52 %), above ground biomass (2.17 %), leaf water potential (2.28 %) and number of grains per pod (5.65 %) under well

watered treatment (Table 4.8). Under water stress, common bean showed high phenotypic coefficient of variation on days to maturity (25.58 %), stomatal conductance (33.47 %) and number of pods per plant (36.70 %) whereas moderate coefficient of variation was on days to flowering (11.23 %), number of branches per plant (14.33 %) and leaf water potential (14.37 %). Low phenotypic coefficient of variation was recorded for above ground biomass (0.87 %), yield per plant (1.18 %), harvest index (2.01 %), hundred seed weight (2.17 %) and number of grains per pod (2.27 %) (Table 4.8).

4.2.4 Heritability (h^2)

The estimates of heritability obtained from the study varied between 43.40 % and 96.54 % (Table 4.8). The moderate heritability estimates were recorded for leaf water potential (43.40 %) under well watered treatment while number of grains per pod (43.75 %) and above ground biomass (44.23 %) were obtained under water stress treatment. Characters that registered high heritability estimates (≥ 60 %) constitute the highest fraction of the total and were obtained either from well watered or water stressed conditions.

4.2.5 Expected genetic advance (GAM)

The expected genetic advance estimates (GAM) for all the characters studied are shown in Table 4.8. High GAM was recorded for days to maturity (34.97 %) followed by leaf water potential (25.46 %), stomatal conductance (22.87 %), and number of pods per plant (22.32) all under water stressed treatment. Under well watered treatment, the highest GAM was recorded for the number of pods per plant (32.16 %), then number of grains

per pod (28.88 %), hundred seed weight (27.72 %), harvest index (26.32 %), days to flowering (22.41 %) and yield per plant (21.97 %). Moderate GAM were recorded for days to flowering (19.92 %), number of branches per plant (18.35 %) and hundred seed weight (12.47 %) under water stress environment whereas days to maturity (18.13 %), stomatal conductance (17.28 %) and above ground biomass (12.47 %) were recorded under well watered condition.

The lowest values of GAM recorded were for the leaf water potential (2.86 %) and number of branches per plant (9.52 %) under well watered conditions whereas yield per plant (2.20 %), number of grains per pod (3.37 %), harvest index (5.57 %) and above ground biomass (6.10 %) under water stress conditions (Table 4.8).

4.2.6 Statistical parameters for characters of F2 population

The mean, skewness and kurtosis for the eleven characters was computed and depicted in Figures 4.12 to 4.24. The mean number of branches per plant under well watered treatment was 9.94 (Figure 4.12 A) while that for water stressed treatment was 8.78 respectively (Figure 4.12 B). The measure of skewness and kurtosis were found to be 0.537 and 1.59 for the population under well watered condition and 0.956 and 1.194 for those under stress. Frequency distribution graphs of the observed measurements from the two treatments were depicted in (Figure 4.12).

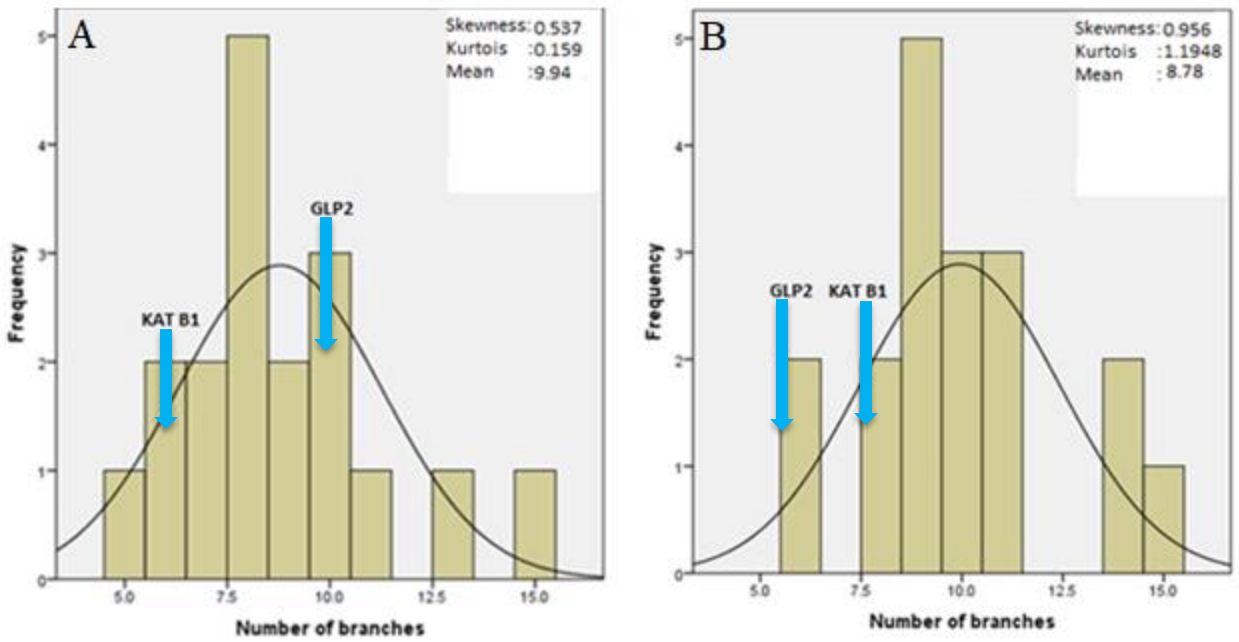


Figure 4.12: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for number of branches per plant in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

The mean value of the number of pods per plant was 8.17. This was below the ideal mean (central point of the scale being 11.5) which shows F2 population produced lower number of pods compared to the parents' (Figure 4.13 A). Kurtosis and skewness values for the number of pods per plant for the population under water stress treatment indicated that the distribution is asymmetric (0.965) and negatively skewed -0.024 as shown in Figure 4.13 B).

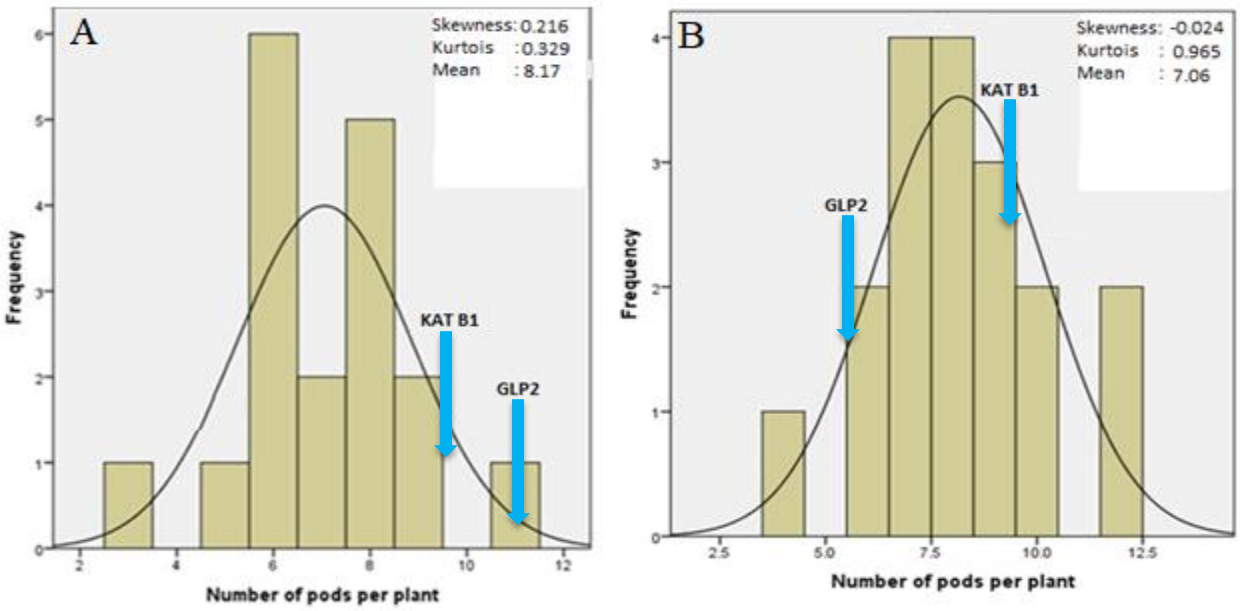


Figure 4.13: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for number of pods per plant in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

For the number of grains per pod the population distributions were near normal and symmetric while skewness was negative (-0.105) and almost near normal in population under well watered conditions (Figure 4.14 A) whereas under water stressed treatment skewness was positive (0.616) and the distribution of population was not normal (Figure 4.14 B). For stem biomass, mean of the population under well watered environment was 6.5 while that for water stress condition was 2.67 respectively.

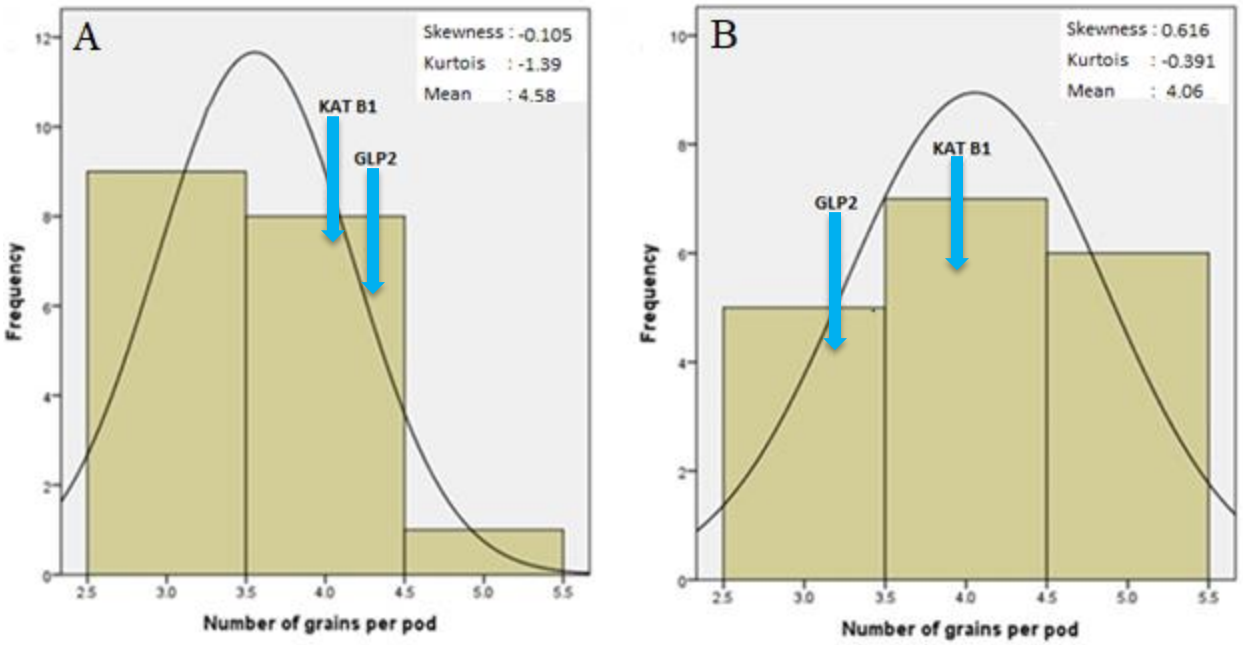


Figure 4.14: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for number of grains per pod in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

The distribution was not normal in the population (Figure 4.15A) and skewness was positive (0.276) under well watered condition while under water stress condition skewness was negative (-0.685) and frequency of individual with low values was higher and distribution was not normal (Figure 4.15 B).

The measure of skewness and kurtosis for pod biomass were found to be 0.842 and 0.537 for the population under well watered condition (Figure 4.16A) and 0.470 and -1.009 respectively for those under water stress environment (Figure 4.16 B). Frequency distribution of the observed measurements from the two treatments were depicted in Figure 4.16. The mean under well watered treatment was 2.2 while that for water stressed condition was 0.89.

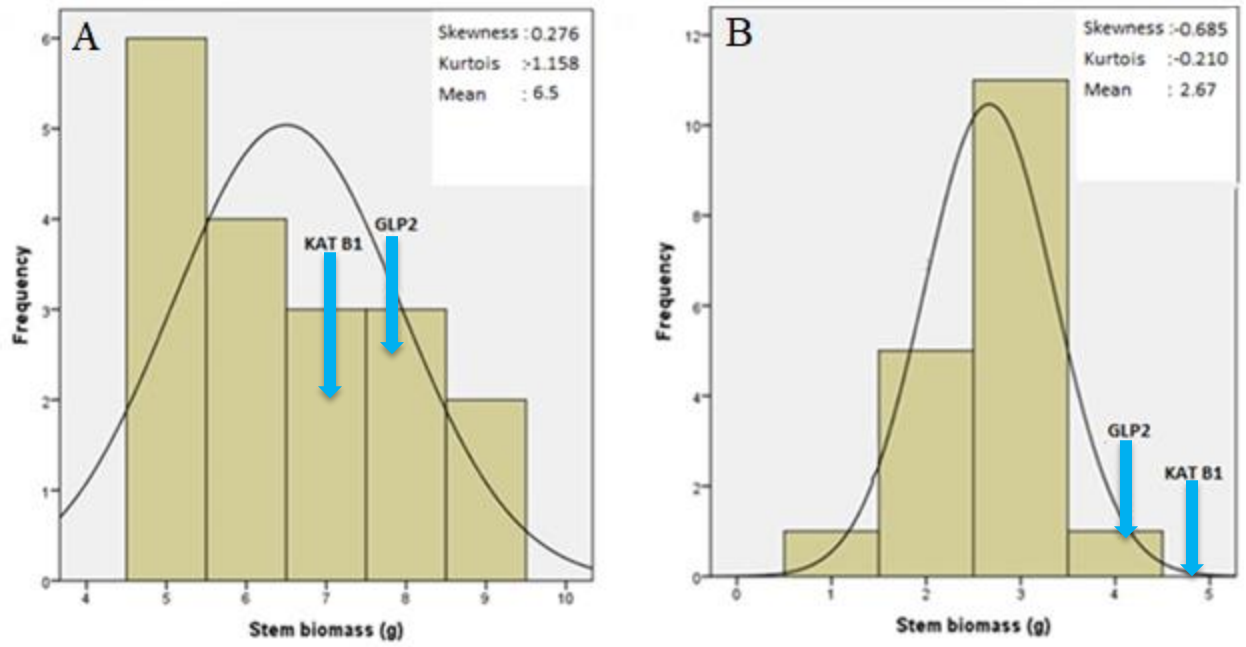


Figure 4.15: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for stem biomass in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

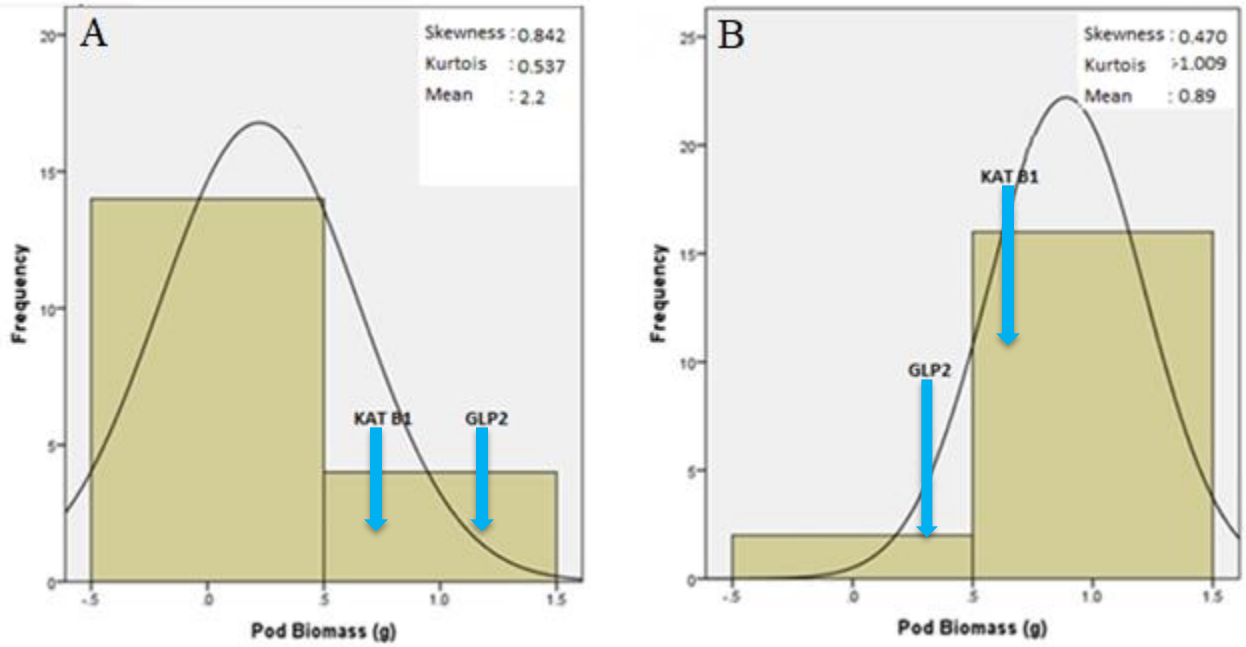


Figure 4.16: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for pod biomass in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

The frequency of individuals for leaf biomass was near normal in well watered condition (Figure 4.17 A) whereas the population distribution was unimodal and continuous under water stress conditions (Figure 4.17 B). The values of skewness and kurtosis (0.463 and -0.686) also confirm the above statement. The mean of 100-seed weight was 3.83g under well watered condition and in water stressed condition was 4.18. The population distribution was positively skewed (0.046) under well watered condition (Figure 4.18 A) but negatively skewed -0.448 under water stressed condition (Figure 4.18 B).

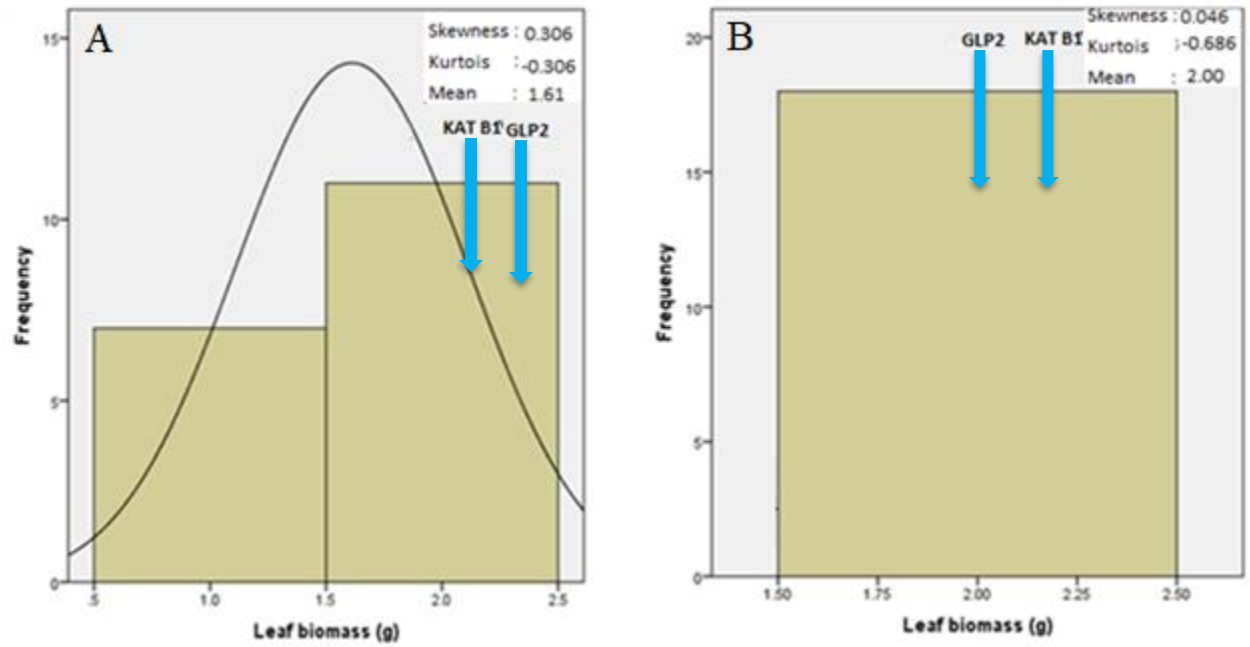


Figure 4.17: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for leaf biomass in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

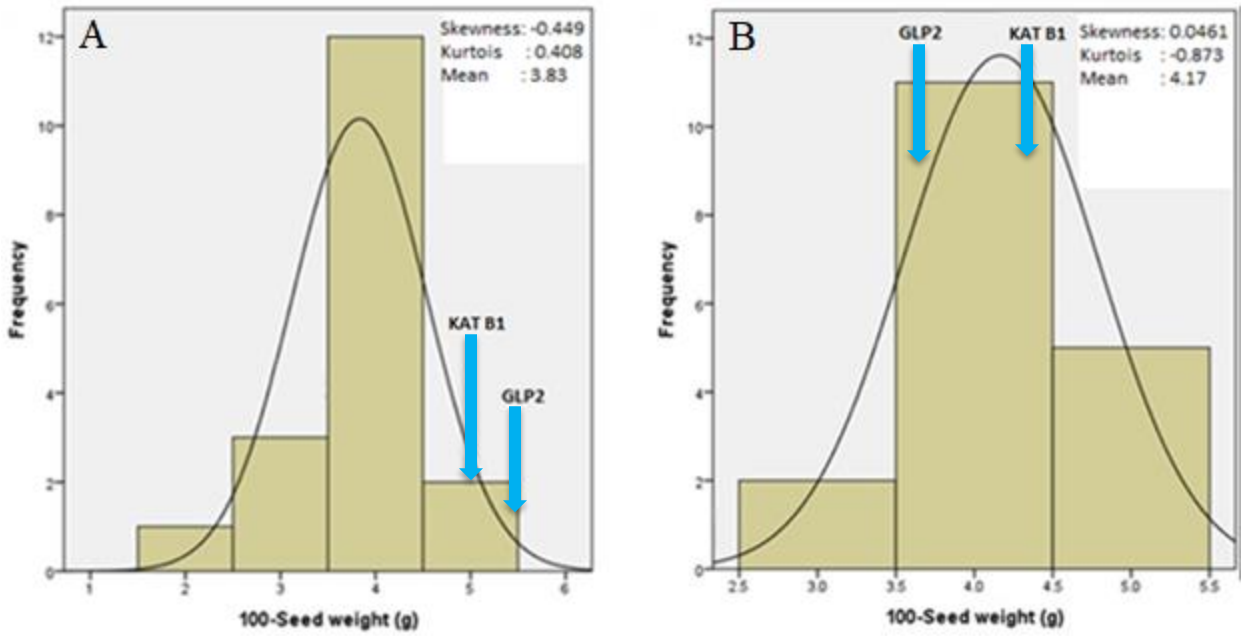


Figure 4.18: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for 100-seed weight in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

The population means for harvest index was 25.67 under well watered conditions while under water stress it was 23.61. The skewness value was 0.465 and kurtosis came to be -0.015 in well watered condition (Figure 4.19 A) and in water stress condition the values were 0.673 and 0.264 respectively (Figure 4.19 B).

The mean of stomatal conductance was 27.56 under well watered treatment while under water stress it was found to be 21.94 (Figure 4.20). Skewness and kurtosis were 0.429 and 0.294 respectively under well watered treatment.

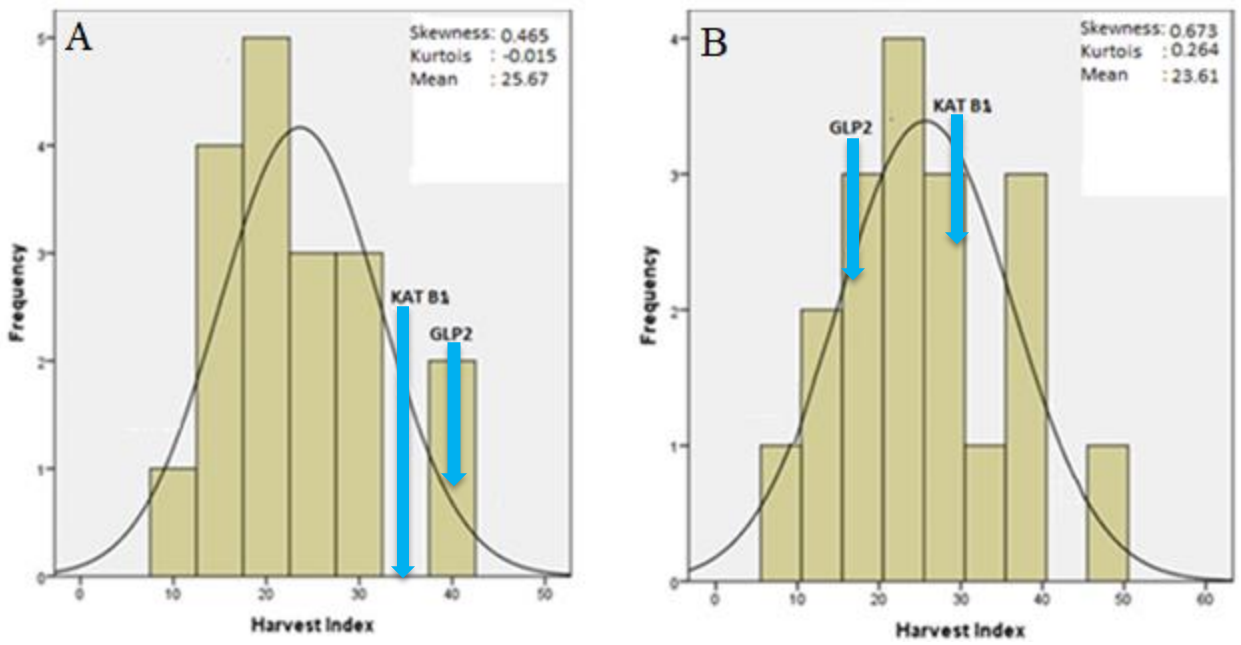


Figure 4.19: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for harvest index in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

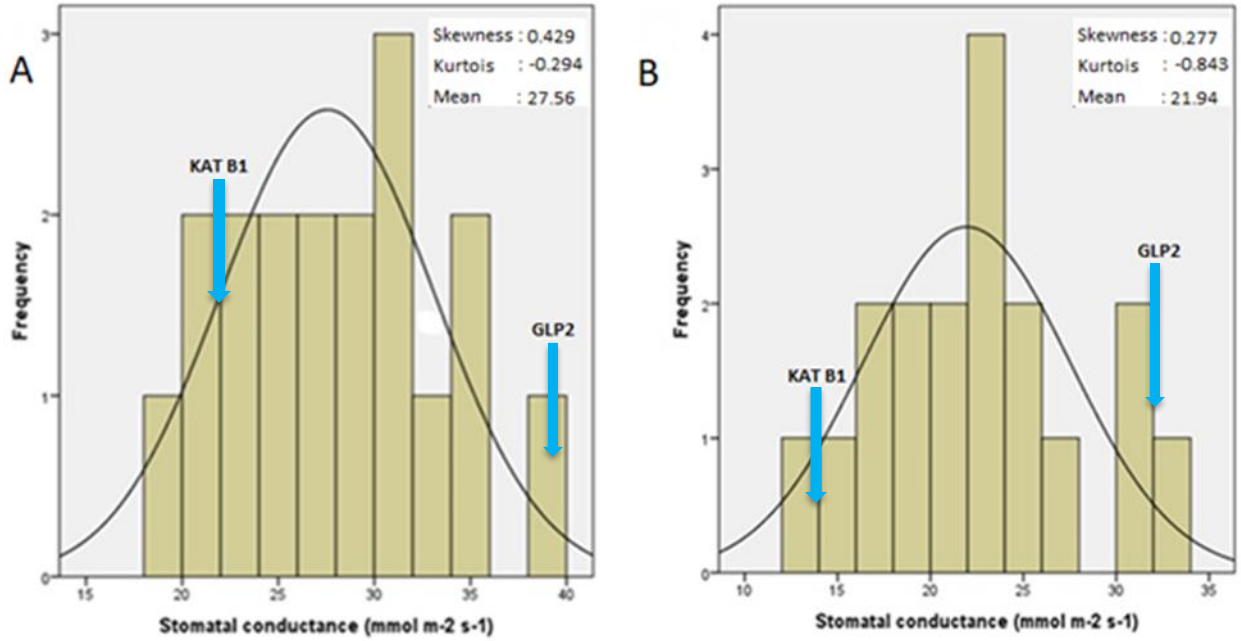


Figure 4.20: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for stomatal conductance in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

Information in Figure 4.21 shows that all the plants had similar leaf water potential (-0.48) under well watered condition. The mean was -0.48 under well watered condition while under water stress it was -0.691.

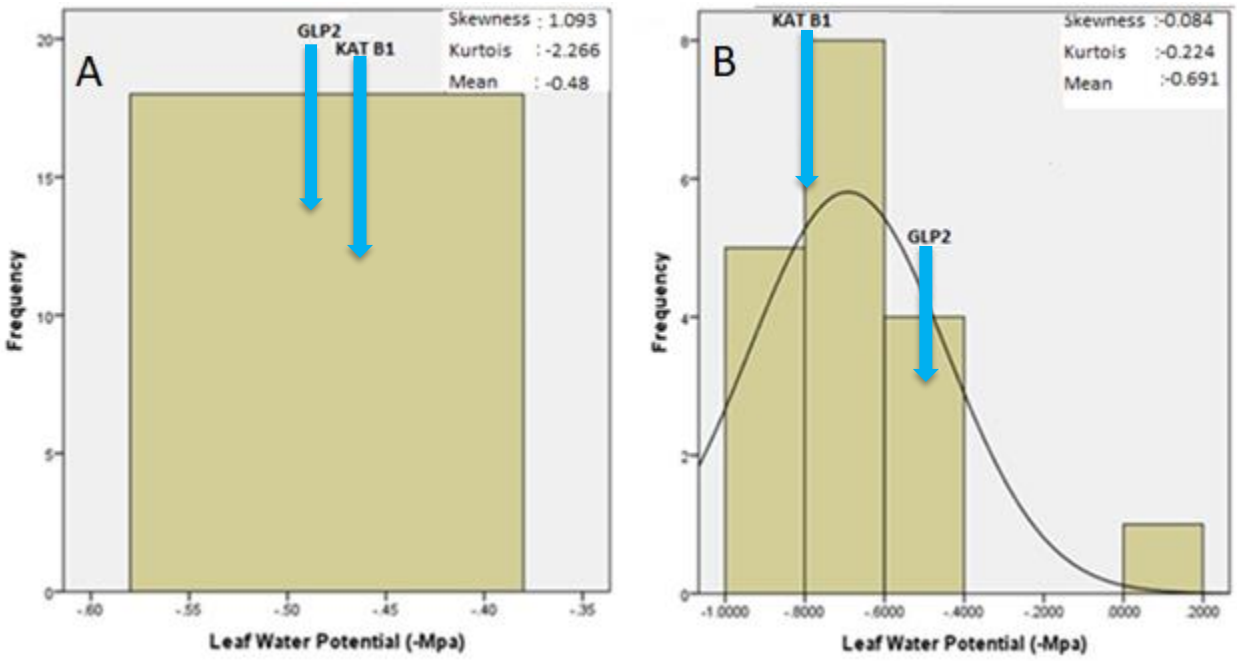


Figure 4.21: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for leaf water potential in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

The mean of water use efficiency was 5.56 under well watered treatment whereas under water stress it was 4.10. The skewness value was 0.280 and kurtosis -1.166 in well watered treatment whereas under water stress skewness was 0.421 while kurtosis was -0.761 (Figure 4.22).

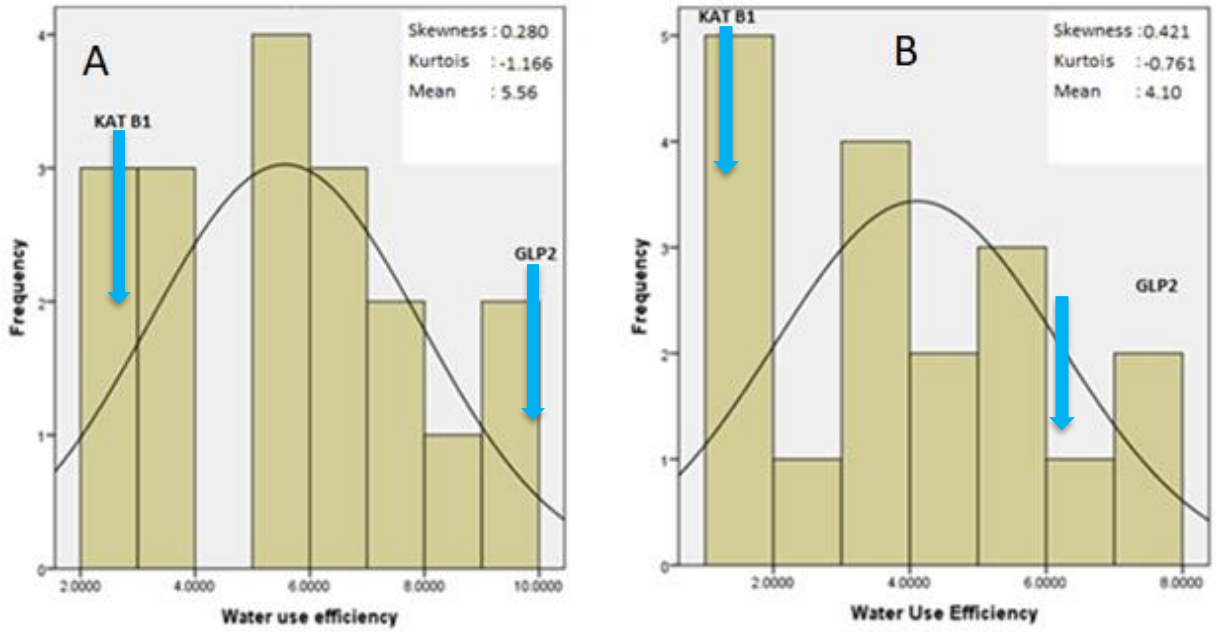


Figure 4.22: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for water use efficiency in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

The mean, range and skewness and kurtosis for days to flowering and days to maturity are presented in Figure 4.23 and Figure 4.24 respectively. The mean for days to flowering was 40.33 under well watered treatment while under water stress it was 38.83 (Figure 4.23). The distribution was negatively skewed in all cases, which means that all the F2 population flowered or matured earlier compared to the parents. However, for days to maturity (Figure 4.24), the measure of skewness shows that the curve was positively skewed 1.047 under water stress and 1.04 under well watered treatment.

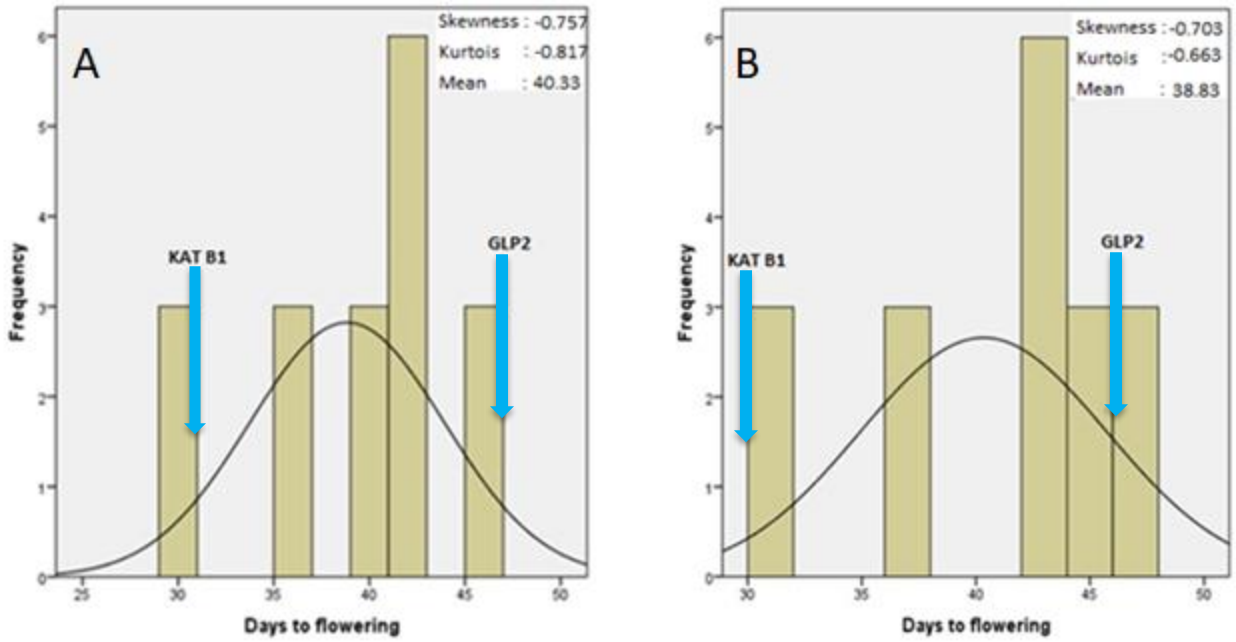


Figure 4.23: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for days to flowering in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

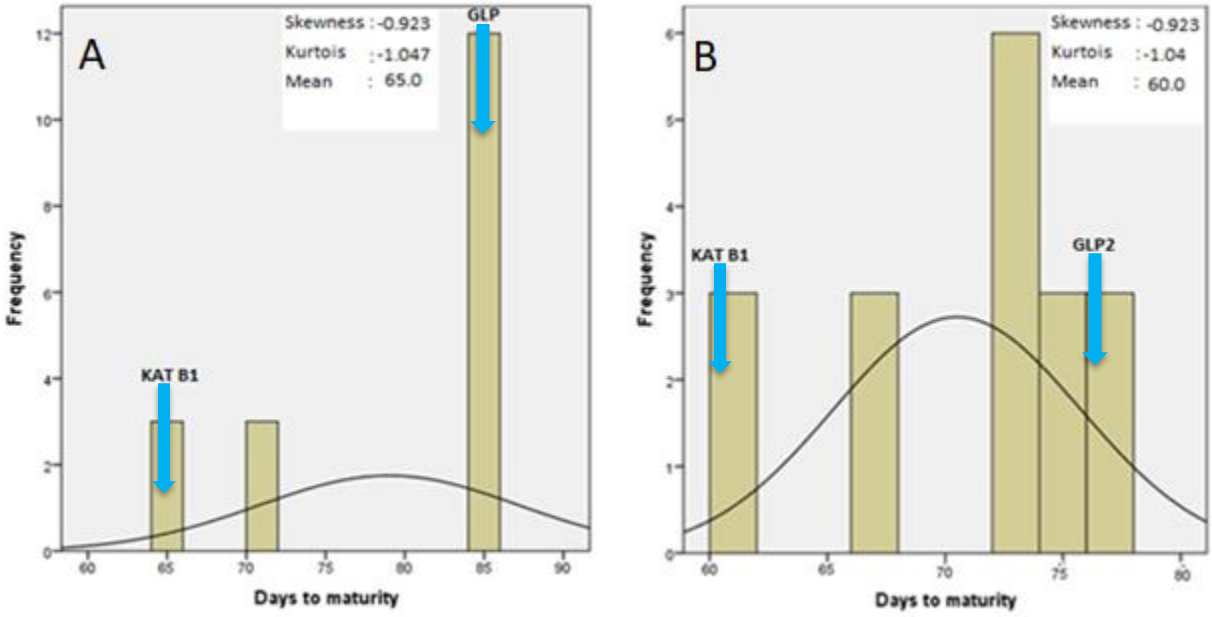


Figure 4.24: Frequency distribution of F2 population of a cross of KAT B1 × GLP2 for days to maturity in common bean under well watered (A) and water stress (B) conditions. The arrows show the position of both parents of the population in the distribution.

4.2.7 Pearson correlation analysis for eleven characters of the F2 population and parents

All the yield component characters; number of branches per plant, number of pods per plant and above ground biomass had positive and significant correlations with yield per plant under water stress regime whereas the same correlations were non-significant under well watered treatment (Table 4.9). The correlation between yield per plant and number of grains per pod was negative and non-significant ($r = -0.35$) under well watered, but positive and significant ($r = 0.86$) under water stress regime.

There was negative and non-significant correlation between days to flowering and number of grains per pod under well watered treatment ($r = -0.41$) whereas over water stressed treatment the correlation was positive and strongly significant ($r = 0.69$). Under well watered regime, hundred seed weight showed negative and non-significant correlations with days to flowering and maturity but it was significant and positively correlated with both characters under water stress conditions. Above ground biomass was found to be highly significant and positively ($p \leq 0.01$) correlated with the number of grains per pod over water stress regime whereas in well watered condition the correlation was positive but not significant. The correlation between number of branches per plant and number of pods per plant content was positive ($r = 0.14$) but non-significant in water stress condition. Significant and positive correlations were obtained between stomatal conductance and leaf water potential under water stress treatment ($r = 0.78$).

Table 4.9: Phenotypic correlations among different characters measured on progenies as result of crosses between GLP2 and KAT B1 under well-watered (ww) and water stress (ws)

Trait		DF	DM	NBP	NPP	SCO	LWP	HSW	BG	NGP	HI	YP
DF	ww	1	-0.41	0.30	-0.56	-0.21	-0.33	-0.29	-0.58*	-0.41	-0.26	-0.41
	ws	1	0.54*	0.62**	0.68**	0.57*	0.41	0.55*	-0.15	0.69**	0.58**	0.86**
DM	ww			-0.43	-0.21	-0.20	-0.43	-0.18	-0.23	-0.28	-0.34	-0.23
	ws			0.57*	0.63**	0.49	0.32	0.58*	0.66**	0.73**	0.55*	- 0.41
NBP	ww				-0.40	0.28	0.17	-0.24	-0.32	-0.17	-0.21	0.39
	ws				0.14	0.45	0.37	0.67**	0.65**	0.77**	0.72**	0.67**
NPP	ww					0.53*	0.40	-0.36	-0.34	0.38	-0.44	0.37
	ws					0.69**	0.55*	0.59*	0.47	0.69**	0.71**	0.86**
SCO	ww						0.33	0.45	0.44	0.58*	0.46	0.38
	ws						0.78**	0.50*	0.53*	0.64*	0.51**	0.73*
LWP	ww							-0.23	0.59*	0.63*	0.50*	0.41
	ws							-0.28	0.66**	0.70*	0.62*	0.68*
HSW	ww								0.71**	0.55**	0.56*	0.49
	ws								0.65**	0.67**	0.73**	0.85**
BG	ww									0.43	0.68**	0.36
	ws									0.75**	0.69**	0.86**
NGP	ww										-0.33	-0.35
	ws										0.58**	0.80**
HI	ww											-0.35
	ws											0.79**
YP	ww											1
	ws											1

* ** Significant at $p \leq 0.05$ and $p \leq 0.01$ respectively. DF, days to flowering; DM, days to maturity; NBP, number of branches/plant; NPP, number of pod/plant; SCO, stomatal conductance; LWP, leaf water potential; HSW, hundred seed weight; BG, biomass above ground; NGP, number of grain/pod; YP, yield/plant; HI, harvest index.

The correlation of the number of grains per pod with number of branches per plant(r = 0.77), number of pods per plant (r = 0.69) and hundred seed weight (r = 0.67) was significant and positive under water stress treatments while the relationship with days to maturity (r = -0.28) and days to flowering (r = -0.41) were not significant and negative under well watered treatment (Table 4.9).

The association of above-ground biomass with NBP (r = 0.65) and HSW (r = 0.65) under water stress treatment was significantly positive. Days to flowering exhibited a positive and significant correlation with DM (r = 0.54), NBP (r = 0.62), NPP (r = 0.68), SCO (r = 0.57), HSW (r = 0.55) NGP (r = 0.69), HI (r = 0.58) and YP (r = 0.86) under water stress treatment whereas under well watered treatment the only positive correlation was with the number of branches, while others were negative, but not always significant such as DM (r = -0.41), NPP (r = -0.56), SCO (r = -0.21), LWP (r = -0.33), HSW (r = -0.29), BG (r = -0.58) NGP (r = -0.41), HI (r = -0.26) and YP (r = -0.41)

4.3 Identifying quantitative trait loci (QTL) associated with tolerance to drought stress in common bean (*Phaseolus vulgaris* L.) using KAT B1 × GLP2 F2 population

4.3.1 Phenotypic character variation

Data for the various phenotypic characters measured among the F2 population and the parental lines are presented in Table 4.10. Results show that the response of F2 population to water treatment varied significantly for all characters studied.

Table 4.10: Statistical analysis for characters for KAT B1 × GLP2 and most susceptible (ss) and most drought tolerant (dt) F2 population (P) studied in a rain out shelter under well watered (ww) and water stress (ws) treatments (Trt)

* ** *** Significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ respectively, DF, Days to flowering; DM, Days to maturity; NBP, Number of branches/plant NPP, Number of pod/plant; SCO, Stomatal conductance; LWP, Leaf water potential; SB, stem biomass; LB, leaf biomass; PB, pod biomass; HSW, hundred seed weight; NGP, Number of grains/pod; YP, Yield/plant

		Parents		F2 population					
Trait	Trt	KAT B1	GLP2	Diff.	ss population	dt population	P	Trt	P × Trt
NBP	ww	10.8	11.2	ns	9.8	12.6	**	*	**
	ws	8.9	6.4	*	7.3	11.7	**	**	*
NPP	ww	13.6	14.2	ns	9.1	14.6	***	**	ns
	ws	11.8	9.7	*	7.5	12.5	**	***	*
NGP	ww	4.2	4.6	ns	2.5	5.7	**	ns	**
	ws	3.8	2.2	***	3.6	4.8	*	**	*
DF	ww	31.0	36.0	**	35.0	31.4	*	*	ns
	ws	30.5	35.4	**	33.0	30.2	*	**	ns
DM	ww	48.6	58.4	**	57.3	48.2	**	ns	*
	ws	46.7	54.2	**	50.6	46.1	**	ns	**
SB	ww	3.21	3.45	ns	3.35	4.1	**	*	**
	ws	3.01	2.65	ns	2.84	3.63	*	***	*
LB	ww	4.24	5.53	ns	4.05	5.24	*	**	***
	ws	3.75	3.08	ns	3.21	4.20	*	*	*
PB	ww	2.13	2.34	ns	1.18	3.45	**	**	**
	ws	2.05	1.12	*	1.18	3.18	**	***	*
HSW	ww	28.11	30.76	ns	26.21	34.24	**	*	ns
	ws	24.23	22.83	ns	23.42	32.93	*	**	ns
YP	ww	8.04	8.91	ns	8.12	9.55	*	***	*
	ws	7.63	5.49	*	4.48	8.79	**	*	**
SCO	ww	31.2	25.5	*	21.6	37.8	*	*	*
	ws	38.7	27.3	*	19.5	43.6	**	**	*
LWP	ww	-0.48	-0.49	*	-0.49	-0.49	ns	*	ns
	ws	-0.59	-0.63	*	-0.68	-0.55	*	*	**

Water treatment significantly affected all the characters except number of grains per plant and the number of days to maturity. Significant differences were found among all the characters for population-water treatment interactions except number of pods per plant (NPP), days to flower (DF), hundred seed weight (HSW) and leaf water potential (LWP). Under water stress treatment, parents differed significantly ($p \leq 0.05$) for all the characters except stem biomass (SB), pod biomass (PB) and hundred seed weight (HSW), however, the differences for those three characters within the F2 population were highly significant ($p \leq 0.01$) in water stress treatment. Meanwhile, KAT B1 flowered and matured earlier than GLP2 in either treatment. Similarly, among the F2s the tolerant population flowered and matured earlier compared with the susceptible group (Table 4.10).

For the susceptible F2 population, days to flowering (DF) varied from 33 to 35. Flowering occurred 2 days earlier in plants subjected to water stress compared to well-watered conditions. Within the same susceptible population of F2, difference of 6.7 days between well watered and water stress plants was also noticed on average for the number of days to maturity (DM) that varied from 50.6 to 57.3 days. Susceptible and drought tolerant F2 plants produced an average yield of 4.48 and 8.79 and per plant whereas those under well watered treatments produced 8.12 and 9.55 respectively. Among the parents, yield per plant (YP) varied from 5.49 to 7.63 under water stress conditions while the range was from 8.04 to 8.91 in well watered treatment.

The parental differences in terms of seed weight (SW) were not significant in well watered treatment while for the F2 population the differences were significantly in both treatments (Table 4.10). Average seed weight for susceptible F2 population across treatments was 23.42 g and 26.21 per 100 seeds for susceptible and 32.93 g and 34.24 per 100 seeds for tolerant population under well watered and water stress respectively. The seed weight across treatments for the parental lines ranged from 22.83 to 24.23 per 100 seeds under water stress conditions and from 28.11 to 30.76 per 100 under well watered conditions. Therefore, transgressive segregation for seed weight was therefore evident. Likewise, transgressive segregation was also observed for grain yield, number of branches, pod number and seed number as well as for the phenological characters (Table 4.10).

4.3.2 Correlation among characters studied

Correlation coefficients of various characters under well watered and water stress treatments are presented in Table 4.11. Negatively strong and significant correlations were found between stem biomass and days to flowering ($r = -0.53$) and days to maturity ($r = -0.56$) under water stress treatment however, under well watered treatment a weak and non-significant correlations was observed between them. Leaf and pod biomass also showed negatively strong and significant correlations with days to flowering and days to maturity respectively under water stress and a weak and non-significant relationship under well watered treatment. The number of pods per plant had significant correlation ($r = 0.60$ and $r = 0.51$) with yield per plant under both treatments. Days to flowering showed a significant correlation ($r = 0.47$) with yield per plant only under well-watered conditions.

Table 4.11: Pearson correlation coefficients between various characters in the F2 population

Trait	NBP	NPP	NGP	SB	LB	PB	HSW	YP	DF	DM	SCO	LWP
NBP	_	**0.56****	**0.52****	**0.44***	**0.08**	**0.16**	**0.03**	**0.71*****	**0.04**	**0.11**	**0.18**	**0.09**
NPP	0.62**	_	**0.49***	**0.58****	**0.11**	**0.11**	**0.08**	**0.60****	**-0.43***	**-0.52****	**0.07**	**0.12**
NGP	0.50*	0.48*	_	**0.40***	**0.05**	**0.10**	**0.02**	**0.60****	**-0.40***	**-0.39***	**0.22**	**0.02**
SB	0.39*	0.23	0.05	_	**0.15**	**0.03**	**0.17**	**0.05**	**-0.53****	**-0.56****	**0.24**	**-0.31***
LB	0.41*	0.25	0.14	0.11	_	**0.11**	**0.05**	**0.07**	**-0.66****	**-0.59****	**0.09**	**-0.44***
PB	0.54**	-0.22	0.13	0.03	0.10	_	**-0.15**	**0.10**	**-0.64****	**-0.55****	**0.25**	**0.21**
HSW	0.35*	0.26	-0.24	0.17	-0.21	-0.13	_	**0.38***	**-0.37***	**-0.31***	**0.33**	**0.24**
YP	0.68**	0.51**	0.51**	0.42*	0.45*	0.07	-0.10	_	**0.23**	**-0.45***	**0.71*****	**0.10**
DF	0.12	0.04	0.18	-0.29	-0.20	-0.22	0.57**	0.47*	_	**0.30***	**0.32***	**0.03**
DM	0.09	0.15	0.27	-0.17	-0.07	-0.16	0.63**	0.28	0.33*	_	**0.28**	**0.43***
SCO	0.15	0.26	0.22	0.17	0.11	0.13	0.05	0.20	0.07	-0.23	_	**0.33***
LWP	0.12	0.04	0.18	0.39*	0.20	0.22	0.17	0.37*	0.34*	0.30	0.22	_

* ** and *** represent the statistical significance at 0.05, 0.01 and 0.001 probability levels. NBP, number of branches per plant; NPP, number of pods per plant; NGP, number of grains per plant; SB, stem biomass; LB, leaf biomass; PB, pod biomass; HSW, hundred seed weight; YP, yield/plant; DF, days to flowering; DM, days to maturity; SCO, stomatal conductance; LWP, leaf water potential. The values bolded are correlation coefficients among characters under water stress treatment; Values not bolded are correlation coefficients among characters under well watered treatment.

During water stress treatment the correlation was not significant, in contrast, days to maturity was significantly correlated, though negative (r = -0.45) with yield per plant under water stress treatment while, the correlation was not significant (r = 0.28) under well watered condition. A positive and highly significant correlation (r = 0.71) was also observed between the stomatal conductance and with yield per plant under water stress treatment.

A negative but significant correlation was observed between the leaf water potential and stem biomass (r = -0.31) and leaf biomass (r = -0.44) under water stress treatment. Under well water treatment, non- significant negative correlation (r = -0.10) was recorded between hundred seed weight and yield per plant but significant positive correlation (r = 0.38) was observed under water stress (Table 4.11).

4.3.3 QTL analysis

The SNP profiling yielded 1578 polymorphic markers (Table 4.12). However, after omitting redundant and clustered markers, only a total of 229 of these markers were mapped in KAT B1/GLP2 F2 population across 11 chromosomes spanning a total length of 634 centiMorgan (cM) of the bean genome at an average distance of 2.02 cM between adjacent markers. The chromosomes sizes ranged between 11.9 cM and 116.5 cM with chromosome Pv09 being the shortest at 11.9 cM, while chromosome Pv06 was the longest with 116.5 cM (Table 4.12). The highest number of co-segregating markers were detected on chromosome Pv04 as indicated by the smaller genetic distances.

Table 4.12: Distribution of SNPs markers mapped across the eleven chromosomes of common bean in KAT B1 × GLP2 F2 population

Chromosome	No. of markers	Length (cM)	Average distance between markers (cM)
Pv01	34	107.9	3.10
Pv 02	49	77.1	2.72
Pv03	26	104.5	3.27
Pv04	18	23.4	1.21
Pv05	15	34.3	2.59
Pv06	30	116.5	3.01
Pv07	18	45.1	1.65
Pv08	21	65.0	1.76
Pv09	4	11.9	2.38
Pv10	9	26.7	4.54
Pv11	5	21.6	1.37
TOTAL	**229**	**634**	**2.02**

SNP, single nucleotide polymorphism; PV, *Phaseolus vulgaris*; cM, CentiMorgan.

Each QTL explained varied amount of phenotypic variance (between 6.45 and 23.28 %) among the characters while the values for the Logarithm of Odds (LOD) scores varied between 2.06 and 8.24 (Table 4.13). Five QTLs with major effect (LOD threshold ≥ 3.0) located on four chromosomes; Pv01, Pv02, Pv04 and Pv08 were detected in the experiment (Table 4.13), four under water stress while one was detected under well watered treatment (Figure 4.25). The four linkage groups contained QTL associated with stomatal conductance (SCO4.1^{KG}), days to maturity (DM4.1^{KG}), seed weight (SW8.1^{KG}) and number of pods per plant located in two chromosomal positions, (NP1.1^{KG}) and (NP2.1^{KG}).

Character	QTL	Trt	Pv	QTL position	Flanking marker	LOD score	LOD threshold	% explanation R^2
Number of branches/plant	**NB1.1^{KG}**	ws	1	5.56	sc00258ln515763_1856	3.1	2.28	11.25
Number of grains/ pod	**NG1.1^{KG}**	ws	1	84.52	sc00564ln943697_6815	4.31	2.82	10.65
	NG2.1^{KG}	ws	2	28.65	sc00117ln943287_1165	3.88	2.63	10.23
	NG8.1^{KG}	ws	8	4.7	sc00564ln856573_3755	2.06	1.82	12.64
Number of pods per plant	**NP1.1^{KG}**	ws	1	99.38	sc01264ln932625_2212	8.24	3.59	17.67
	NP3.1^{KG}	ww	3	47.02	sc00518ln692941_3545	5.31	2.51	17.01
	NP2.1^{KG}	ws	2	46.64	sc00339ln939425_4385	4.81	3.86	24.35
	NP3.1^{KG}	ws	3	7.02	sc00369ln874451_2254	5.31	2.66	6.84
Days to flowering	**DF1.1^{KG}**	ww	1	21.23	sc00023ln763611_6414	3.37	2.67	12.62
	DF2.1^{KG}	ws	2	66.56	sc00564ln882990_3214	3.52	2.91	6.45
	DF4.1^{KG}	ws	4	23.45	sc00439ln772650_2216	5.03	2.11	10.25
Days to maturity	**DM1.1^{KG}**	ww	1	81.2	sc00517ln941037_2554	3.26	2.08	16.00
	DM4.1^{KG}	ws	4	12.78	sc00325ln677312_7402	3.65	3.46	12.36
Stem biomass	**SB1.1^{KG}**	ws	1	27.1	sc00734ln523622_8765	3.47	2.64	21.49
	SB2.1 ^{KG}	ws	2	11.04	sc00693ln835947_5841	3.22	2.17	17.58
Leaf biomass	**LB2.1^{KG}**	ws	2	9.03	sc00890ln923464_1254	2.64	2.12	18.52
Pod biomass	**PB1.1^{KG}**	ws	1	45.8	sc00563ln950218_6547	3.77	2.96	23.28
seed weight	**SW8.1^{KG}**	ww	8	64.45	sc00064ln987325_7202	3.96	3.62	9.65
Seed yield per plant	**SY1.1^{KG}**	ws	1	25.78	sc00045ln763729_3325	4.08	2.90	12.06
	SY2.1^{KG}	ws	2	37.47	sc01585ln034402_5351	3.76	2.65	10.70
Stomatal conductance	**SCO3.1^{KG}**	ws	3	22.14	sc00004ln982325_3165	3.57	2.35	10.43
	SCO4.1^{KG}	ws	4	1.8	sc01235ln698241_2417	3.95	3.72	18.51
Leaf water potential	**LWP1.1^{KG}**	ws	1	5.54	sc00387ln936125_7264	4.35	3.41	12.01

Table 4.13: Identification of quantitative trait loci under well watered (ww) and water stress (ws) treatments

QTL, quantitative trait loci; Trt, treatment; Pv, *Phaseolus vulgaris*; R^2, coefficient of determination; LOD, logarithm of odd; NBP, number of branches/plant; NPP, number of pods/plant; NGP, number of grains/pod; DF, days to flowering; DM, days to maturity; SB, stem biomass; LB, leaf biomass; PB, pod biomass; SW, seed weight; SY, seed yield; SCO, stomatal conductance; LWP, leaf water potential in KAT B1/GLP2 F2 population.

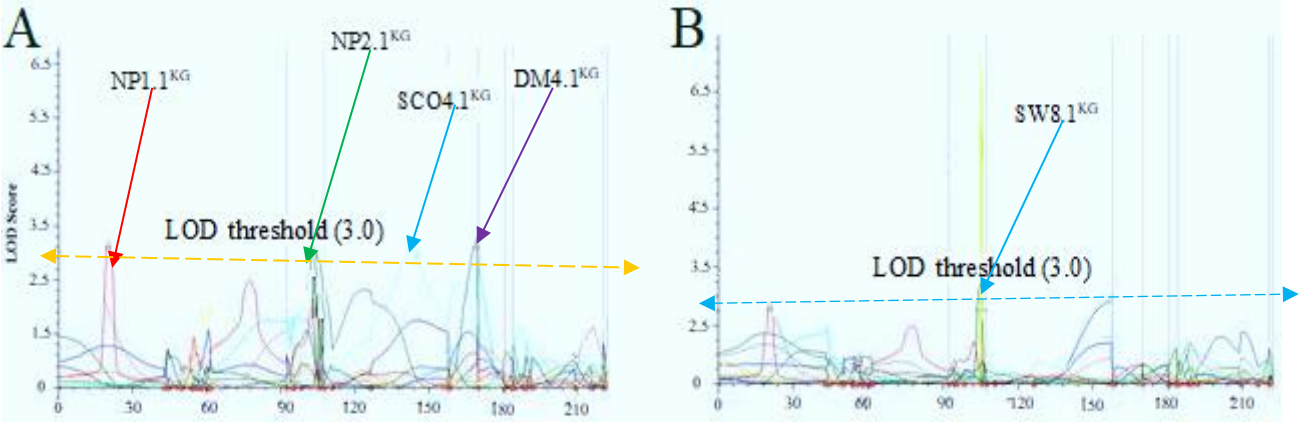


Figure 4.25: QTLs detected under water stress (A) and well watered (B) [control] treatment.

4.3.4 Linkage maps

The number of pods per plant had the highest number of QTLs (4) detected but spread on different chromosomes; Pv01, Pv02 and two on Pv03 (Figure 4.26). Two QTLs associated with the number of pods per plant and number of grains per pod (NG1.1^{KG} and NP1.1^{KG}) were detected under water stress conditions on chromosomes Pv01 (Table 4.13). Two other QTLs; NG2.1^{KG} and NG8.1^{KG} associated with the number of grains were expressed under water stress conditions but on different positions (NG2.1^{KG} was detected on Pv02 while NG8.1^{KG} was detected on Pv08). QTLs for pods per plant were also detected on chromosomes Pv02 and Pv03 (Figure 4.26). A constitutive QTL associated with days to maturity (DM1.1^{KG}) detected on chromosome Pv01 under well watered treatment was co-localized with other QTL associated with the number of branches per plant (NB1.1^{KG}), stem biomass (SB1.1^{KG}), pod biomass (PB1.1^{KG}), seed yield (SY1.1^{KG}) and leaf water potential (LWP1.1^{KG}) detected under water stress. Two

QTLs for seed yield mapped to chromosomes Pv01 and Pv02 were identified under water stress conditions (Table 4.13).

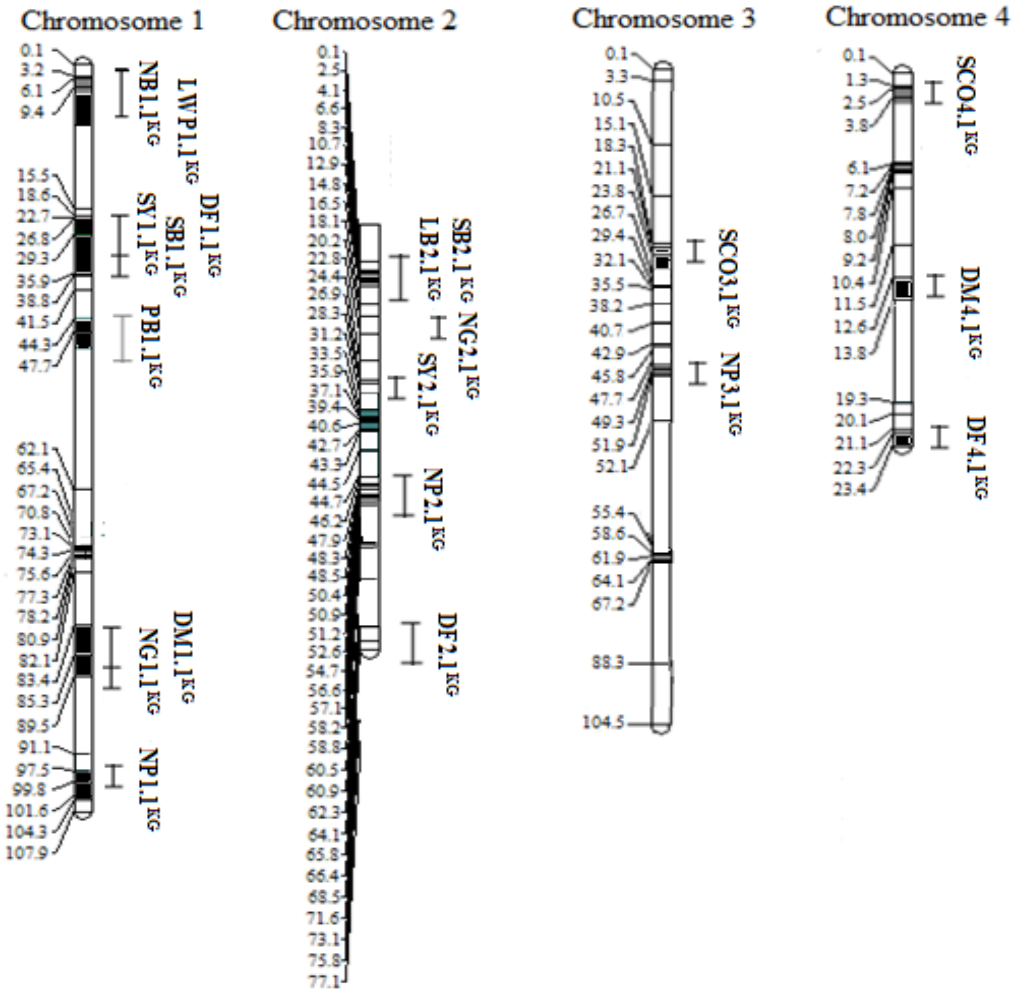
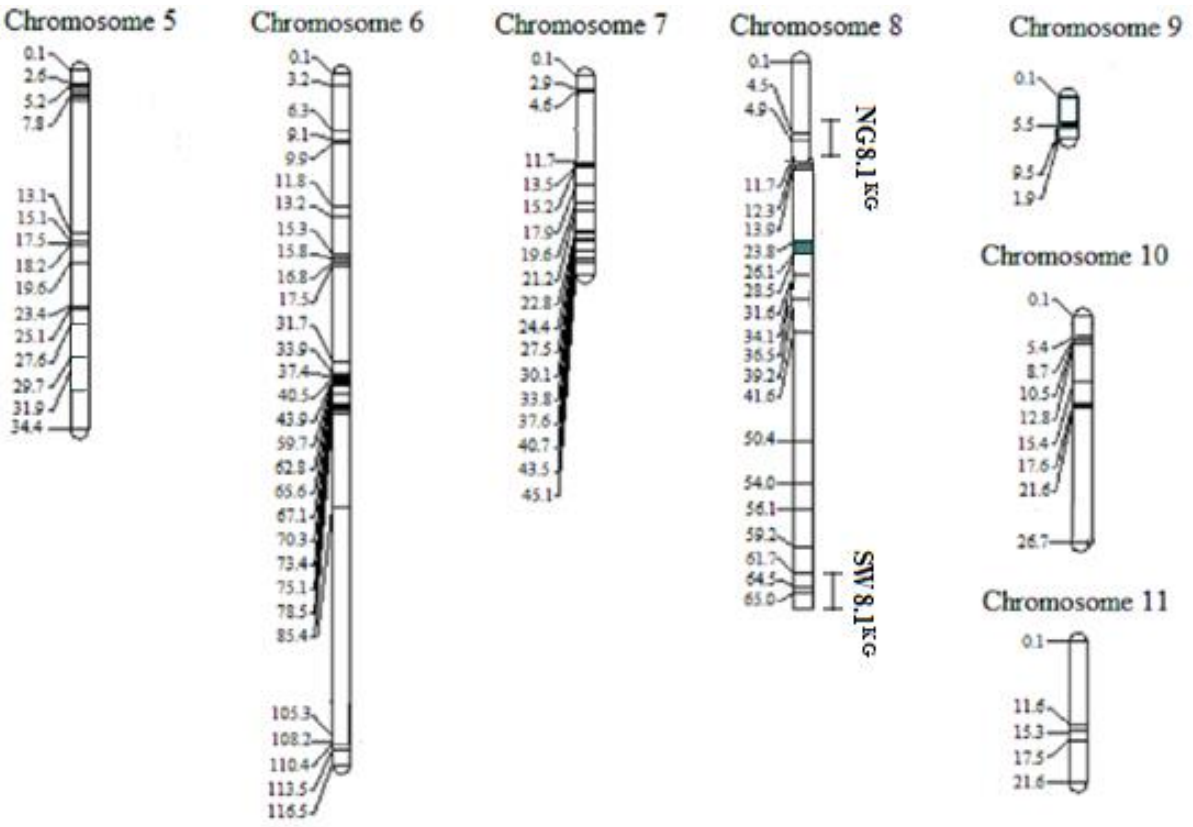


Figure 4.26: Linkage map of KAT B1 x GLP2 population indicating quantitative trait loci (QTLs). NB, number of branches per plant; NG, number of grains per pod; NP, number of pods per plant; SB, stem biomass; LB, leaf biomass; PB, pod biomass; LWP, leaf water potential; SY, seed yield; SW, seed weight; LB, leaf biomass; SCO, stomatal conductance; DF, days to flowering; and DM, days to maturity.



Linkage map of KAT B1 x GLP2 population indicating quantitative trait loci (QTLs). NB, number of branches per plant; NG, number of grains per pod; NP, number of pods per plant; SB, stem biomass; LB, leaf biomass; PB, pod biomass; LWP, leaf water potential; SY, seed yield; SW, seed weight; LB, leaf biomass; SCO, stomatal conductance; DF, days to flowering; and DM, days to maturity (Figure 4.26 continued).

The QTL for seed yield on chromosome Pv02 was associated with QTL for the number of grains per pod (NG2.1^{KG}) and co-localized with QTLs for days to flowering (DF2.1^{KG}) and leaf biomass (LB2.1^{KG}). Two stomatal conductance QTLs mapped on chromosomes Pv03 and Pv04. Under water stress treatment, QTLs for number of pods per plant and stomatal conductance were tagged near the SNP marker sc00369ln874451_2254 marker in Pv03 and SNP marker sc00004ln982325_3165 also in Pv03 (Figure 4.26).

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Identification of phenotypic and physiological characters associated with drought tolerance in selected common bean genotypes

The induced water stress on common bean in this experiment simulate the possible drought stress scenarios that are likely to occur in common bean producing areas in Kenya during growing seasons resulting from low and poorly distributed rainfall. In many common bean growing regions of Kenya, it is not unusual to have short rains at planting time giving a false start of a rainy season for a few days then followed by intermittent dry spells at various stages of growth of the growing cycle through the growing season. This exposes the crops to drought-stress. Therefore, in this study, bean crop was subjected to intermittent water stress periods to evaluate for drought tolerance traits in conditions similar to those encountered by farmers in rain-fed agricultural systems.

There was varied effect of water stress treatment on the expression of the various traits in this study. The drastic reduction in stem biomass accumulation under water stress treatment demonstrate that some traits are comparatively more sensitive to water deficit than others. Stomatal conductance and leaf water potential had a significant positive correlations with grain yield under water stress. These two traits were also observed to be

the moderately stable traits under genotype-treatment interaction confirming their reliability in distinguishing between the droughts tolerant and susceptible genotypes. Results, indicate that number of grains per plant and pod number remained significantly stable under genotype-treatment effects despite the fact that yield components have been found to be influenced by a number of external factors. The stability in performance of drought related traits under genotype-treatment interactions indicate their usefulness for phenotypic selection (Kang, 1997; Polania *et al*., 2016). Thus, the observed significantly positive correlations between number of grains per pod and number of pods per plant further support the possibility of these traits as key indicators of increased tolerance under water deficit stress.

Stomatal conductance showed a significant positive correlation with the number of grains under water stress but was negatively related with LWP under well watered treatments indicating that stomatal conductance and LWP could be used interchangeably in selecting common bean genotypes for drought tolerance. This stomatal conductance-LWP relationship also illustrates the role of stomatal conductance in the stability performance of genotypes whereas its association with the number of grains indicate that a higher stomatal conductance could lead to higher crop productivity. Similar relationship has been documented in barley (González *et al*., 1999; Gholipoor *et al*., 2013) and wheat (Blum *et al*., 1989; Ooro *et al*., 2009). This indicate the usefulness of stomatal conductance as a tool for selection against drought stress. Drought susceptible genotypes showed comparatively lower stomatal conductance compared to tolerant genotypes under stress treatment an indication that susceptible genotypes were unable to sustain sufficient

rate of transpiration under increased water deficit. Studies in various crops have also reported the association of higher yields with higher stomatal conductance under conditions of water deficit stress (Berger *et al*., 2006). As a strategy to avoid the effect of drought, most plants respond to water deficit through regulation of stomatal conductance (Cohen, 1970; Manzer *et al*., 2015). However, prolonged stomatal closure is counterproductive to the process of photosynthetic assimilation and plant growth due to reduced stomatal CO_2 uptake (Morgan, 1986). The correlation of stomatal conductance with the LWP under well watered and water deficit stress treatments suggest that the characters had significant association with each other under well watered and stress conditions.

The role of early flowering and maturity in allowing the crop to escape the effects of drought stress cannot be understated. In this study, genotypes with early flowering and maturity comparatively gave a better yield (number of grains per plant) than late flowering/maturing genotypes under water stress. Asfaw and Blair (2014) reported the positive association between grain yield and days to flowering under water stress conditions demonstrating the importance of early flowering of a crop under water stress environments. These authors also reported that selection for earliness enhances the probability to escape drought and also improve the partitioning of the water absorbed and transpired after anthesis consequently affecting the grain yield. Earliness as a mechanism of drought escape in beans under drought stress environments across India was confirmed by Berger *et al*. (2006). Thus, earliness could be an important indirect selection criterion in common bean improvement program. The early maturing genotypes showed

significant positive relationship between grain yield and early flowering under water stress treatment suggesting that they were more adapted to drought stress. Thus, to be indicating the significance of early flowering of a crop when drought conditions sets earlier. Though earliness is an essential trait in areas where terminal drought occurs quite often (Beebe *et al*., 2014), the choice between a shorter growth cycle and a reduced grain yield potential needs to be made (White and Singh, 1991). However, genetically manipulating the capacity of the plant to produce more seeds through better grain filling under drought stress could reduce the penalty of losing grain yield per day. The phenological plasticity and yield potential of a plant has been shown to be influenced by high stomatal conductance, water use efficiency and/or leaf water potential under drought stress (Polania *et al*., 2016). In this study, genotype Angaza displayed high values of pod biomass production under water stress, but presented moderate values of grain yield suggesting that biomass accumulation alone is not responsible for higher yield under water stress treatment. Above ground biomass is an important trait that has significant and positive correlation with grain yield in common bean (Trapp *et al*. 2016). Wondimu *et al.* (2018) also reported a positive association between an increase in biomass production and increase in grain yield under water stress.

Accordingly, selecting genotypes with greater sink strength indicated by greater values of biomass above the ground could improve the yield under drought conditions. Drought tolerant genotypes; Nyota and KAT B1 accumulated some of the highest values of stem, pod and leaf biomass under water stress conditions in this study. The genotype which registered the highest value of water use efficiency also presented the lowest mean values

of leaf water potential under conditions of water stress treatment, a clear indication of highest level of drought tolerance in comparison to other genotypes. The capacity to produce high number of grains per pod under stress treatment that was observed in Nyota could be explained by their ability to maintained high WUE and low LWP. This observation agrees with earlier findings which indicated that WUE could pass as a useful selection criteria (Polania *et al*., 2016) for enhancing drought tolerance in common bean because of its significant correlation with grain yield under water stress conditions. Beebe *et al*. (2014) also reported that drought tolerant common bean genotypes have an effective mechanisms that maintain water balance, ensuring sustained grain formation and filling during drought stress. Accordingly, when selecting for better yield and tolerance to drought in common bean, the potential amount and extent of variation of water use efficiency could be of great importance. Stepwise regression analysis revealed stomatal conductance had the largest contributions to grain yield under both water stress and well watered conditions. This observation was not, however, surprising given that other recent studies have reported significant variation for stomatal and leaf water potential traits among common bean lines in water stress conditions (Sofi *et al*., 2017). Traub *et al*. (2017) reported lower stomatal conductance values for drought tolerant common bean genotype (SER 16) compared to the more drought susceptible genotypes; Zorro and Jaguar bean.

Selection for drought tolerance in common beans based on grain yield alone is often not very effective due to the variability in the stress response by different genotypes. The efficiency of screening and selection for drought tolerance in common beans may be

improved by indirect selection for physiological traits having close relationship with stress response which can easily and quickly be measured (Koinange *et al*., 1996; Assefa *et al*., 2013; Masoumeh *et al*., 2018). Quantification of drought tolerance in common beans varieties through an experiment such as the one carried out would revealed trait variations in common beans. This may be exploited in breeding programs in the improvement of drought tolerance. In this study, common bean genotypes were evaluated for various attributes directly involved in drought tolerance and water use in crops.

There is a huge implication of this study in plant breeding. Knowledge of the relationships among the various traits under drought-stress is important in guiding the common bean breeders of the right combination of traits when selecting genotypes for drought stress environments. For instance, stomatal conductance and LWP were both stable under genotype-treatment interactions with strong positive correlations with number of grains under stress conditions. This relationship highlighted the importance of stomatal conductance in maintaining yield potential under drought. It also indicate that tolerance to drought could have been due to the ability of genotypes to maintain high level of stomatal conductance as well as retaining a good water balance during drought stress. The positively strong and significant association noticed between physiological and yield component traits under water stress conditions shows that multiple traits are responsible for drought adaptation in selected common bean genotypes. Therefore, the hypothesis associating the phenotypic and physiological characters with drought tolerance in selected common bean genotypes was accepted.

5.1.2 Characterization of F2 population (KAT B1 × GLP2) for drought tolerance using phenotypic and physiological characters

Significant differences were observed within the common bean population under consideration for all the characters suggesting the presence of high level of genetic diversity among them which can be useful in bean improvement programs. The relatively large population mean squares obtained for the number of pods per plant, harvest index, stomatal conductance, leaf water potential and yield per plant under stressed conditions revealed that the F2 population differed in their potential for these characters. Significant amount of phenotypic variability obtained indicated that all the F2 individuals varied with each other in regard to the characters implying there could be further improvement of the traits through multiple selections

According to Beebe *et al*. (2013), mechanisms associated with drought tolerance in different common bean genotypes have been identified. This confirms that the single trait selection concept is not the most reliable way to improve a trait. According to this study, it was observed that selection for grain yield improvement under drought stress conditions can be conducted by simultaneous selection for many characters such as stomatal conductance, number of branches per plant, grain yield per plant, number of pods per plant and harvest index. This can be attributed to the influence of the F2 generation by the interaction with the water treatment as shown by the results. The relatively higher CV % for some of the characters might have been due to this higher variability of genotype x treatment in the F2 performance. This suggests that the

evaluation of common bean population for drought tolerance traits under different levels of water stress rather than one level was quite satisfactory. The small differences existing between genotypic and the phenotypic variance for almost all characters indicated less influenced by environmental conditions suggesting a wider scope for plant breeders to select common bean genotypes tolerant to drought. This agrees with Abebe *et al*. (1998), Negash (2006) and Ambachew *et al*. (2015) whose findings showed that the contribution of the genetic effects outweighs the environmental effects for all characters in the estimation of phenotypic variance. In this study the highest estimates for the genotypic and phenotypic coefficient of variation under water stress condition were recorded for the number of pods per plant indicating that one of the most effective way to increase grain yield under drought stress is to increase the number of pods.

In this study, the grain yield and the number of branches showed a positively strong and significant correlation under water deficit indicating that under water stress condition, the grain yield was mainly influenced by the growth vigor of the plant and the amount of carbohydrate reserve available for remobilization. This positive correlation suggests that any improvement in the number of branches could result in a substantial increase in grain yield. These finding are consistent with those of Beebe *et al*. (2013) who reported the presence of strong and positive correlations of grain yield with plant vigor under drought conditions. However, Ambachew *et al*. (2015) established a negative and low correlation between branches per plant and yield per plant and contradicts with findings from the present study for the two characters. Asfaw *et al*. (2017) also reported that under well-watered conditions, the grain yield and the number of branches exhibited a negative and

non-significant correlation. Such negative relationships with the grain yield indicate that when grain yield is the major target of selection it would be futile to based selection only on the two characters. The utilization of genotypic and phenotypic correlations in the selection had previously been reported by several researchers (Van Oosteron and Acevedo, 1992; Marroig and Cheverud 2001; Kimani *et al*., 2007; Conner *et al*., 2014; Mwenda *et al*., 2018). Genotypic correlation coefficient provides the magnitude of the genetic association between characters and may offer an important selection criterion in plant breeding programs (Resende *et al*., 2017). Stomatal conductance, days to maturity, and pod numbers per plant for F2 population were positively skewed in distribution and platykurtic kurtosis in both water treatments, implying that larger number of F2's exhibited shorter days to maturity, higher stomatal conductance and pod numbers per plant than the parents and thus the shift in the mean towards the positive side which favours selection of progenies with drought tolerance characteristics.

A combined high estimates of heritability and high genetic variability with the genetic advance for days to maturity, stomatal conductance, and pod number per plant may suggest the additive gene action and their high genetic variability is due to less influence of the environment. Therefore, selection efforts based on the above characters aimed at identifying drought tolerant genotypes at early segregating generation may give positive result. These results are in apparent agreement with reports of Kimani *et al*., (2007), Dursun (2007), Barecha (2015) and Bagheri *et al*. (2015). Generally, for grain numbers per pod, hundred seed weight, water use efficiency, leaf water potential and days to flowering, negative skewness with platykurtic kurtosis distribution was evident in water

stress treatment indicating the unequal distribution of F2 progenies with decrease in trait values. The negative skewness of distribution in these characters was due to more F2's with reduced trait expression compared with the parents and that led to population mean reduction.

The estimates of heritability were low to medium and the genetic advance were also low in F2 population for all these characters suggesting that non-additive interaction and the low phenotypic variability among the F2 progenies implies that environment has the greatest influence on their expression. Therefore, selection for drought tolerance cannot be done for all the above characters in the early generation. In this study, the progenies of KAT B1 × GLP2 had platykurtic kurtosis and positive skewness distribution suggesting the existence of complementary gene interaction with increasing influence on characters including stomatal conductance, days to maturity and number of pods. Such positive interaction was confirmed by the presence of high estimates of genetic advance, genetic variability and broad sense heritability for all the three characters. It was on the basis of these findings that the hypothesis, on the characterization of F2 population (KAT B1 × GLP2) for drought tolerance can be achieved using phenotypic and physiological characters, was accepted.

5.1.3 Identification of quantitative trait loci (QTL) associated with drought tolerance in an F2 (KAT B1 × GLP2) population of common bean

The study was performed to detect various QTLs controlling traits linked to drought tolerance in common beans with the aim of providing valuable information that could

potentially be utilized in markers-assisted selection in common bean improvement. An F_2 population developed from a cross of drought tolerant (KAT B1) and drought susceptible (GLP2) genotypes was evaluated to map QTL for drought tolerance traits. Generally, the success of QTL mapping relies heavily on the marker density (Kapanigowda *et al*., 2014; Mukeshimana *et al*., 2014; Trapp *et al*., 2015). In this study, a fairly dense genetic map was developed from 229 SNPs mapped on KAT B1/GLP2 genome spanning a total length of 634 cM at an average distance of 2.02 cM between adjacent markers thus, compares favorably with previously constructed genetic maps of common bean (Langat *et al*., 2020). In essence, a highly saturated map can enhance the precision of QTL mapping (Trapp *et al*., 2015). Given that widely grown common bean varieties in Kenya are Andean derivatives, the successful construction of a genetic map using an Andean intragene cross population could immensely contribute to understanding the genetic control mechanism of drought tolerance among the local bean genotypes.

Although several QTLs related to drought tolerance have been identified in different studies (Blair *et al*., 2006; Asfaw *et al*., 2012; 2017), it is still a challenge to find QTLs with stable expression under different water stress environment due to the quantitative nature of drought which is highly influenced by prevailing environmental conditions. In this study, a total of 23 QTLs related to water stress tolerance in common bean genome with effects ranging between 6.45 and 24.35 % were identified, majority of which were in regions where other QTLs for similar traits were previously reported (Briñez *et al*., 2017). A greater number of the QTLs were detected under drought stress compared to well-watered conditions. This indicates that most of the identified QTLs has adaptive

nature to drought stress. Further, the parent KAT B1 contributed a higher number (78 %) of QTLs identified in this study. These findings therefore, confirms the suitability of KAT B1 as a source of drought tolerance. QTLs were detected on different locations throughout the genome with certain regions hosting more than one QTL associated with multiple traits. This could be as a result of correlation existing between these traits.

QTLs for correlated traits were localized on the same chromosome and the primary reason for this could be that these traits are associated on cause effect basis indicating that these traits are controlled by genes that are tightly linked (Tuberosa *et al*., 2002; Pérez-Vega *et al*., 2012). In this study, co-localization of QTLs for days to flowering and days to maturity, stem biomass and pod biomass was evident on chromosome Pv01. QTLs for seed yield and days to maturity were noticed to coincide on chromosome Pv02. In the study, QTLs conditioning days to flowering and maturity were mapped to chromosome Pv01 and PV04 under well watered and drought stress treatment respectively. Prior research by Mukeshimana *et al*. (2014) had reported the co-localization of the same QTL on chromosome Pv04 for flowering and maturity in the SEA5/CAL96 RIL population. A photoperiod gene on chromosome Pv04 with major effect on partitioning of assimilates between vegetative and reproductive growth that was previously described by Tar'an *et al*. (2002) is likely to be the same QTLs for days to flowering and maturity reported in this study. An important QTL ($NP3.1^{KG}$) (LOD score = 5.31) for number of pods per plant found in both water stress and well watered conditions responsible for between 6.84 and 17.01 % of the variance (R^2) was mapped to chromosome Pv03. This QTL may be related to stomatal conductance QTL ($SCO3.1^{KG}$)

(LOD score =3.57 and 10.43 % variance (R^2) explanation) on Pv03 detected under well watered conditions. This linkage could offer a chance to select simultaneously for number of pods per plant and stomatal conductance in an intragene pool crosses. Loci associated with leaf biomass and stem biomass ($LB2.1^{KG}$ and $SB2.1^{KG}$) were expressed in drought stress treatment and were linked to yield traits in chromosome Pv02. This overlapping is a strong indication of the important role stem and leaf biomass could play in controlling seed yield under drought stress.

Although some studies have detected QTL associated with stem biomass and some yield component traits (Asfaw *et al.*, 2012), co-localization with yield trait on the same region has not been reported in other studies. A 100-seed weight QTL ($SW8.1^{KG}$) with a LOD score of 3.96 and 9.65 % variance (R^2) appearing on chromosome Pv08 is another significant trait in the present study consistent under both water treatment. Trapp *et al.* (2015) reported QTLs controlling 100-seed weight located on chromosome Pv08 although those QTLs demonstrated higher genetic variance and were mapped towards the mid-point of chromosome Pv08. The variations could have been due to type of markers used, influence by the environmental factors or genetic background of the parents used to produce the F2 population. Lack of overlap between the QTLs for stomatal conductance and leaf water potential indicates the independence of these physiological parameters from each other. Days to maturity was negatively correlated with seed yield under drought stress treatment, implying that maturity is an important strategy in drought evasion. Plasticity as an adaptation mechanism to drought stress in common bean has been reported in other studies (Urrea *et al*., 2009 and Polania *et al*., 2016).

In this study, besides the occurrence of co-localization of QTLs for multiple traits, most of the QTLs detected were exclusively mapped to either well-watered or water stress treatment implying that QTLs showed different behavioural pattern for genetic control of characters such as stomatal conductance and leaf water potential under variable water conditions. In this study, stomatal conductance was strongly associated with yield under water stress treatment. This relationship supports similar findings on barley (Chloupek *et al*., 2010; González and Ayerbe 2010). The indirect use of stomatal conductance as selection criterion against drought tolerance has been reported in wheat (Blum, 2009; Bennett *et al*., 2012; Ouyang *et al*., 2017). In this study, some individuals in the population were able to maintain stomatal conductance as high as 43.6 mmol $m^{-2}s^{-1}$ while others as low as 19.5 mmol $m^{-2}s^{-1}$. The individuals that were able to maintain higher levels of stomatal conductance throughout their reproductive phase under drought stress conditions produced better yields. From the result, this trait has the potential to be used in screening and selection of drought tolerant common bean genotypes.

The study succeeded in identifying new QTLs; ($SCO4.1^{KG}$ and $LWP1.1^{KG}$) that are involved in drought adaptation mechanisms controlling a number of traits associated with productivity in common bean under drought stress conditions. Therefore, the hypothesis on the association between quantitative trait loci (QTL) and drought tolerance in an F2 population of common bean was accepted.

5.2 Conclusions

i. Drought tolerance is associated with the phenotypic character of higher number of stem branches as well as the physiological character of higher stomatal conductance in the selected common bean genotypes compared to drought susceptibility.

ii. Characterization of F2 population as either tolerant or susceptible to water deficit stress can be obtained using the following phenotypic and physiological characters: number of grains per pod (NGP), number of branches per plant (NBP), number of pods per plant (NPP), hundred seed weight (HSW), biomass above ground (BG), harvest index (HI), stomatal conductance (SCO), leaf water potential (LWP) and water use efficiency (WUE).

iii. QTLs associated with drought tolerance in an F2 (KAT B1 × GLP2) population of common bean are; $DM4.1^{KG}$, $SW8.1^{KG}$, $NP1.1^{KG}$, $NP2.1^{KG}$, $SCO4.1^{KG}$, and $LWP1.1^{KG}$. Two QTLs ($SCO4.1^{KG)}$) controlling stomatal conductance on Pv04 and ($LWP1.1^{KG}$) on Pv01 associated with leaf water potential, putatively involved in drought adaptation mechanisms have never been reported in other studies.

5.3 Recommendations

i. The identified QTLs should be incorporated into MAS program in order to increase the efficiency of screening and selection of drought tolerant bean genotypes in Kenya and the region.

ii. The identified QTLs ($SCO4.1^{KG}$ and $LWP1.1^{KG}$) linked to drought tolerance should be tried on genotypes of different genetic backgrounds

5.3.1 Suggestion for further study

i. It is recommended that, F2s populations larger than 120 developed either from parents of the same genotypes or from closely-related genotypes should be used to construct a denser linkage map to verify and confirm the possible linkage of detected QTLs.

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APPENDICES

Appendix 1: Analysis of Variance for number of branches per plant

1. Tests of Between-Subjects Effects

Dependent Variable: Number of branches per plant

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	227.222[a]	11	20.657	7.150	0.000
Intercept	2533.444	1	2533.444	876.962	0.000
Genotype	131.222	5	26.244	9.085	0.000
Treatment	87.111	1	87.111	30.154	0.000
Genotype * Treatment	8.889	5	1.778	0.615	0.689
Error	69.333	24	2.889		
Total	2830.000	36			
Corrected Total	296.556	35			

a. R Squared = .766 (Adjusted R Squared = 0.659)

2. Genotype

Estimates

Dependent Variable: Number of branches per plant

Genotype	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nyota	11.833	0.694	10.401	13.265
Angaza	7.833	0.694	6.401	9.265
Faida	8.333	0.694	6.901	9.765
KATB1	9.500	0.694	8.068	10.932
Metameta	7.000	0.694	5.568	8.432
GLP2	5.833	0.694	4.401	7.265

3. Pairwise Comparisons

Dependent Variable: Number of branches per plant

(I) Genotype	(J) Genotype	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Nyota	Angaza	4.000*	0.981	0.000	1.975	6.025
	Faida	3.500*	0.981	0.002	1.475	5.525
	KATB1	2.333*	0.981	0.026	0.308	4.359
	Metameta	4.833*	0.981	0.000	2.808	6.859
	GLP2	6.000*	0.981	0.000	3.975	8.025
Angaza	Nyota	-4.000*	0.981	0.000	-6.025	-1.975
	Faida	-0.500	0.981	0.615	-2.525	1.525
	KATB1	-1.667	0.981	0.102	-3.692	0.359
	Metameta	0.833	0.981	0.404	-1.192	2.859
	GLP2	2.000	0.981	0.053	-0.025	4.025
Faida	Nyota	-3.500*	0.981	0.002	-5.525	-1.475
	Angaza	0.500	0.981	0.615	-1.525	2.525
	KATB1	-1.167	0.981	0.246	-3.192	0.859
	Metameta	1.333	0.981	0.187	-0.692	3.359
	GLP2	2.500*	0.981	0.018	0.475	4.525
KATB1	Nyota	-2.333*	0.981	0.026	-4.359	-0.308
	Angaza	1.667	0.981	0.102	-0.359	3.692
	Faida	1.167	0.981	0.246	-0.859	3.192
	Metameta	2.500*	0.981	0.018	0.475	4.525
	GLP2	3.667*	0.981	0.001	1.641	5.692
Metameta	Nyota	-4.833*	0.981	0.000	-6.859	-2.808
	Angaza	-0.833	0.981	0.404	-2.859	1.192
	Faida	-1.333	0.981	0.187	-3.359	0.692
	KATB1	-2.500*	0.981	0.018	-4.525	-0.475
	GLP2	1.167	0.981	0.246	-.859	3.192
GLP2	Nyota	-6.000*	0.981	0.000	-8.025	-3.975
	Angaza	-2.000	0.981	0.053	-4.025	0.025
	Faida	-2.500*	0.981	0.018	-4.525	-0.475
	KATB1	-3.667*	0.981	0.001	-5.692	-1.641
	Metameta	-1.167	0.981	0.246	-3.192	0.859

Based on estimated marginal means

4. Treatment

Estimates

Dependent Variable: Number of branches per plant

			95% Confidence Interval	
Treatment	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Water stress	6.833	0.401	6.007	7.660
Well watered	9.944	0.401	9.118	10.771

5. Pairwise Comparisons

Dependent Variable: Number of branches per plant

					95% Confidence Interval for Difference[b]	
(I) Treatment	**(J) Treatment**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[b]**	**Lower Bound**	**Upper Bound**
Water stress	Well watered	-3.111*	0.567	0.000	-4.280	-1.942
Well watered	Water stress	3.111*	0.567	0.000	1.942	4.280

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

6. Univariate Tests

Dependent Variable: Number of branches per plant

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Contrast	87.111	1	87.111	30.154	0.000
Error	69.333	24	2.889		

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

7. Genotype * Treatment

Dependent Variable: Number of branches per plant

Genotype	Treatment	Mean	Std. Error
Nyota	1	8.667[b]	0.981
Angaza	1	6.333[cb]	0.981
Faida	1	5.133[c]	0.981
KATB1	1	7.667[b]	0.981
Metameta	1	5.333[c]	0.981
GLP2	1	4.666[c]	0.981
Nyota	2	10.666[a]	0.981
Angaza	2	8.666[b]	0.981
Faida	2	8.333[b]	0.981
KATB1	2	10.667[a]	0.981
Metameta	2	8.033[b]	0.981
GLP2	2	10.033[a]	0.981

1-water stress; 2-well watered

Appendix 2: Analysis of Variance for Number of pods per plant

1. **Tests of Between-Subjects Effects**

Dependent Variable: Number of pods per plant

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	161.333[a]	11	14.667	10.776	0.000
Intercept	1600.000	1	1600.000	1175.510	0.000
Genotypes	73.000	5	14.600	10.727	0.000
Treatment	81.000	1	81.000	59.510	0.000
Genotypes * Treatment	7.333	5	1.467	1.078	0.398
Error	32.667	24	1.361		
Total	1794.000	36			
Corrected Total	194.000	35			

a. R Squared = .832 (Adjusted R Squared = .754)

2. **Genotypes**

Estimates

Dependent Variable: Number of pods per plant

			95% Confidence Interval	
Genotypes	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Nyota	9.333	0.476	8.350	10.316
Angaza	6.500	0.476	5.517	7.483
Faida	6.500	0.476	5.517	7.483
KAT B1	7.333	0.476	6.350	8.316
Metameta	5.333	0.476	4.350	6.316
GLP2	5.000	0.476	4.017	5.983

3. Pairwise Comparisons

Dependent Variable: Number of pods per plant

(I) Genotypes	(J) Genotypes	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Nyota	Angaza	2.833*	0.674	0.000	1.443	4.224
	Faida	2.833*	0.674	0.000	1.443	4.224
	KAT B1	2.000*	0.674	0.007	0.610	3.390
	Metameta	4.000*	0.674	0.000	2.610	5.390
	GLP2	4.333*	0.674	0.000	2.943	5.724
Angaza	Nyota	-2.833*	0.674	0.000	-4.224	-1.443
	Faida	-2.498E-016	0.674	1.000	-1.390	1.390
	KAT B1	-0.833	0.674	0.228	-2.224	0.557
	Metameta	1.167	0.674	0.096	-0.224	2.557
	GLP2	1.500*	0.674	0.036	0.110	2.890
Faida	Nyota	-2.833*	0.674	0.000	-4.224	-1.443
	Angaza	2.498E-016	0.674	1.000	-1.390	1.390
	KAT B1	-0.833	0.674	0.228	-2.224	0.557
	Metameta	1.167	0.674	0.096	-0.224	2.557
	GLP2	1.500*	0.674	0.036	0.110	2.890
KAT B1	Nyota	-2.000*	0.674	0.007	-3.390	-0.610
	Angaza	0.833	0.674	0.228	-0.557	2.224
	Faida	0.833	0.674	0.228	-0.557	2.224
	Metameta	2.000*	0.674	0.007	0.610	3.390
	GLP2	2.333*	0.674	0.002	0.943	3.724
Metameta	Nyota	-4.000*	0.674	0.000	-5.390	-2.610
	Angaza	-1.167	0.674	0.096	-2.557	0.224
	Faida	-1.167	0.674	0.096	-2.557	0.224
	KAT B1	-2.000*	0.674	0.007	-3.390	-0.610
	GLP2	0.333	0.674	0.625	-1.057	1.724
GLP2	Nyota	-4.333*	0.674	0.000	-5.724	-2.943
	Angaza	-1.500*	0.674	0.036	-2.890	-0.110
	Faida	-1.500*	0.674	0.036	-2.890	-0.110
	KAT B1	-2.333*	0.674	0.002	-3.724	-0.943
	Metameta	-0.333	0.674	0.625	-1.724	1.057

4. Univariate Tests

Dependent Variable: Number of pods per plant

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	73.000	5	14.600	10.727	0.000
Error	32.667	24	1.361		

The F tests the effect of Genotypes. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment

Estimates

Dependent Variable: Number of pods per plant

			95% Confidence Interval	
Treatment	Mean	Std. Error	Lower Bound	Upper Bound
Water stress	5.167	0.275	4.599	5.734
Well watered	8.167	0.275	7.599	8.734

6. Pairwise Comparisons

Dependent Variable: Number of pods per plant

					95% Confidence Interval for Difference[b]	
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[b]	Lower Bound	Upper Bound
Water stress	Well watered	-3.000*	0.389	0.000	-3.803	-2.197
Well watered	Water stress	3.000*	0.389	0.000	2.197	3.803

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

7. Genotypes * Treatment

Dependent Variable: Number of pods per plant

Genotypes	Treatment	Mean	Std. Error
Nyota	1	8.333[ba]	0.674
Angaza	1	5.000[c]	0.674
Faida	1	5.667[c]	0.674
KAT B1	1	5.333[c]	0.674
Metameta	1	4.000[dc]	0.674
GLP2	1	3.333[dc]	0.674
Nyota	2	10.333[a]	0.674
Angaza	2	8.000[ba]	0.674
Faida	2	7.333[b]	0.674
KAT B1	2	9.667[a]	0.674
Metameta	2	6.666[b]	0.674
GLP2	2	9.333[a]	0.674

1- Water stress; 2-well watered

Appendix 3: Analysis of Variance for Number of grains per pods

1. **Tests of Between-Subjects Effects**

Dependent Variable: Number of grains per pod

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	28.972[a]	11	2.634	6.773	0.000
Intercept	406.694	1	406.694	1045.786	0.000
Genotypes	10.472	5	2.094	5.386	0.002
Treatment	17.361	1	17.361	44.643	0.000
Genotypes * Treatment	1.139	5	0.228	0.586	0.711
Error	9.333	24	0.389		
Total	445.000	36			
Corrected Total	38.306	35			

[a]. R Squared = .756 (Adjusted R Squared = 0.645)

2. **Genotypes**

Estimates

Dependent Variable: Leaf biomass

Genotypes	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nyota	2.148	0.057	2.032	2.265
Angaza	1.730	0.057	1.613	1.847
Faida	1.618	0.057	1.502	1.735
KAT B1	1.862	0.057	1.745	1.978
Metameta	1.637	0.057	1.520	1.753
GLP2	1.610	0.057	1.493	1.727

4. Univariate Tests

3. Pairwise Comparisons

Dependent Variable: Number of grains per pod

(I) Genotypes	(J) Genotypes	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Nyota	Angaza	1.333*	0.360	0.001	0.590	2.076
	Faida	1.167*	0.360	0.003	0.424	1.910
	KAT B1	0.167	0.360	0.648	-0.576	0.910
	Metameta	1.333*	0.360	0.001	0.590	2.076
	GLP2	0.833*	0.360	0.030	0.090	1.576
Angaza	Nyota	-1.333*	0.360	0.001	-2.076	-0.590
	Faida	-0.167	0.360	0.648	-0.910	0.576
	KAT B1	-1.167*	0.360	0.003	-1.910	-0.424
	Metameta	1.110E-016	0.360	1.000	-0.743	0.743
	GLP2	-0.500	0.360	0.178	-1.243	0.243
Faida	Nyota	-1.167*	0.360	0.003	-1.910	-0.424
	Angaza	0.167	0.360	0.648	-0.576	0.910
	KAT B1	-1.000*	0.360	0.010	-1.743	-0.257
	Metameta	0.167	0.360	0.648	-0.576	0.910
	GLP2	-0.333	0.360	0.364	-1.076	0.410
KAT B1	Nyota	-0.167	0.360	0.648	-0.910	0.576
	Angaza	1.167*	0.360	0.003	0.424	1.910
	Faida	1.000*	0.360	0.010	0.257	1.743
	Metameta	1.167*	0.360	0.003	0.424	1.910
	GLP2	0.667	0.360	0.076	-0.076	1.410
Metameta	Nyota	-1.333*	0.360	0.001	-2.076	-0.590
	Angaza	-1.110E-016	0.360	1.000	-0.743	0.743
	Faida	-0.167	0.360	0.648	-0.910	0.576
	KAT B1	-1.167*	0.360	0.003	-1.910	-0.424
	GLP2	-0.500	0.360	0.178	-1.243	0.243
GLP2	Nyota	-0.833*	0.360	0.030	-1.576	-0.090
	Angaza	0.500	0.360	0.178	-0.243	1.243
	Faida	0.333	0.360	0.364	-0.410	1.076
	KAT B1	-0.667	0.360	0.076	-1.410	0.076
	Metameta	0.500	0.360	0.178	-0.243	1.243

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

Dependent Variable: Number of grains per pod

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	10.472	5	2.094	5.386	0.002
Error	9.333	24	0.389		

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment

Estimates

Dependent Variable: Number of grains per pod

			95% Confidence Interval	
Treatment	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Water stress	2.667	0.147	2.363	2.970
Well watered	4.056	0.147	3.752	4.359

6. Pairwise Comparisons

Dependent Variable: Number of grains per pod

					95% Confidence Interval for Difference[b]	
(I) Treatment	**(J) Treatment**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[b]**	**Lower Bound**	**Upper Bound**
Water stress	Well watered	-1.389*	0.208	0.000	-1.818	-0.960
Well watered	Water stress	1.389*	0.208	0.000	0.960	1.818

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

7. Genotypes * Treatment

Dependent Variable: Number of grains per pod

				95% Confidence Interval	
Genotypes	**Treatment**	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Nyota	1	3.667	0.360	2.924	4.410
Angaza	1	2.000	0.360	1.257	2.743
Faida	1	2.333	0.360	1.590	3.076
KAT B1	1	3.333	0.360	2.590	4.076
Metameta	1	2.333	0.360	1.590	3.076
GLP2	1	2.333	0.360	1.590	3.076
Nyota	2	4.667	0.360	3.924	5.410
Angaza	2	3.667	0.360	2.924	4.410
Faida	2	3.667	0.360	2.924	4.410
KAT B1	2	4.667	0.360	3.924	5.410
Metameta	2	3.333	0.360	2.590	4.076
GLP2	2	4.333	0.360	3.590	5.076

1-Water stress; 2-Well watered

Appendix 4: Analysis of variance for leaf biomass

1. **Tests of Between-Subjects Effects**

Dependent Variable: Leaf biomass

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.732[a]	11	0.248	12.925	0.000
Intercept	112.466	1	112.466	5852.525	0.000
Genotypes	1.317	5	0.263	13.706	0.000
Treatment	1.273	1	1.273	66.252	0.000
Genotypes * Treatment	0.142	5	0.028	1.478	0.234
Error	0.461	24	0.019		
Total	115.659	36			
Corrected Total	3.193	35			

a. R Squared = .856 (Adjusted R Squared = 0.789)

2. **Genotypes**

Estimates

Dependent Variable: Leaf biomass

Genotypes	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Nyota	2.148	0.057	2.032	2.265
Angaza	1.730	0.057	1.613	1.847
Faida	1.618	0.057	1.502	1.735
KAT B1	1.862	0.057	1.745	1.978
Metameta	1.637	0.057	1.520	1.753
GLP2	1.610	0.057	1.493	1.727

3. Pairwise Comparisons

Dependent Variable: Leaf biomass

(I) Genotypes	(J) Genotypes	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Nyota	Angaza	0.418*	0.080	0.000	0.253	0.584
	Faida	0.530*	0.080	0.000	0.365	0.695
	KAT B1	0.287*	0.080	0.002	0.121	0.452
	Metameta	0.512*	0.080	0.000	0.346	0.677
	GLP2	0.538*	0.080	0.000	0.373	0.704
Angaza	Nyota	-0.418*	0.080	0.000	-0.584	-0.253
	Faida	0.112	0.080	0.176	-0.054	0.277
	KAT B1	-0.132	0.080	0.113	-0.297	0.034
	Metameta	0.093	0.080	0.255	-0.072	0.259
	GLP2	0.120	0.080	0.147	-0.045	0.285
Faida	Nyota	-0.530*	0.080	0.000	-0.695	-0.365
	Angaza	-0.112	0.080	0.176	-0.277	0.054
	KAT B1	-0.243*	0.080	0.006	-0.409	-0.078
	Metameta	-0.018	0.080	0.821	-0.184	0.147
	GLP2	0.008	0.080	0.918	-0.157	0.174
KAT B1	Nyota	-0.287*	0.080	0.002	-0.452	-0.121
	Angaza	0.132	0.080	0.113	-0.034	0.297
	Faida	0.243*	0.080	0.006	0.078	0.409
	Metameta	0.225*	0.080	0.010	0.060	0.390
	GLP2	0.252*	0.080	0.004	0.086	0.417
Metameta	Nyota	-0.512*	0.080	0.000	-0.677	-0.346
	Angaza	-0.093	0.080	0.255	-0.259	0.072
	Faida	0.018	0.080	0.821	-0.147	0.184
	KAT B1	-0.225*	0.080	0.010	-.0390	-0.060
	GLP2	0.027	0.080	0.742	-0.139	0.192
GLP2	Nyota	-0.538*	0.080	0.000	-0.704	-0.373
	Angaza	-0.120	0.080	0.147	-0.285	0.045
	Faida	-0.008	0.080	0.918	-0.174	0.157
	KAT B1	-0.252*	0.080	0.004	-0.417	-0.086
	Metameta	-0.027	0.080	0.742	-0.192	0.139

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

4. Univariate Tests

Dependent Variable: Pod biomass

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	1.317	5	0.263	13.706	0.000
Error	.461	24	0.019		

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment Estimates

Dependent Variable: Leaf biomass

			95% Confidence Interval	
Treatment	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Water stress	1.579	0.033	1.512	1.647
Well watered	1.956	0.033	1.888	2.023

6. Pairwise Comparisons

Dependent Variable: Leaf biomass

					95% Confidence Interval for Difference[b]	
(I) Treatment	**(J) Treatment**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[b]**	**Lower Bound**	**Upper Bound**
Water stress	Well watered	-0.376*	0.046	0.000	-0.471	-0.281
Well watered	Water stress	0.376*	0.046	0.000	0.281	0.471

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

7. Genotypes * Treatment

Genotypes	Treatment	Mean	Std. Error
Nyota	1	1.950	0.080
Angaza	1	1.507	0.080
Faida	1	1.550	0.080
KAT B1	1	1.710	0.080
Metameta	1	1.410	0.080
GLP2	1	1.350	0.080
Nyota	2	2.347	0.080
Angaza	2	1.953	0.080
Faida	2	1.687	0.080
KAT B1	2	2.013	0.080
Metameta	2	1.863	0.080
GLP2	2	1.870	0.080

1-Water stress; 2-Well watered

Appendix 5: Analysis of variance for pod biomass

1. Tests of Between-Subjects Effects

Dependent Variable: Pod biomass

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.738[a]	11	0.158	12.255	0.000
Intercept	9.881	1	9.881	766.594	0.000
Genotypes	0.689	5	0.138	10.689	0.000
Treatment	1.020	1	1.020	79.146	0.000
Genotypes * Treatment	0.029	5	0.006	0.443	0.814
Error	0.309	24	0.013		
Total	11.927	36			
Corrected Total	2.047	35			

a. R Squared = .849 (Adjusted R Squared = 0.780)

2. Genotypes

Estimates

Dependent Variable: Pod biomass

Genotypes	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nyota	0.735	0.046	0.639	0.831
Angaza	0.478	0.046	0.383	0.574
Faida	0.462	0.046	0.366	0.557
KAT B1	0.690	00.046	0.594	0.786
Metameta	0.375	0.046	0.279	0.471
GLP2	0.403	0.046	0.308	0.499

3. Pairwise Comparisons

Dependent Variable: Pod biomass

(I) Genotypes	(J) Genotypes	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Nyota	Angaza	0.257*	0.066	0.001	0.121	0.392
	Faida	0.273*	0.066	0.000	0.138	0.409
	KAT B1	0.045	0.066	0.499	-0.090	0.180
	Metameta	0.360*	0.066	0.000	0.225	0.495
	GLP2	0.332*	0.066	0.000	0.196	0.467
Angaza	Nyota	-0.257*	0.066	0.001	-0.392	-0.121
	Faida	0.017	0.066	0.801	-0.119	0.152
	KAT B1	-0.212*	0.066	0.004	-0.347	-0.076
	Metameta	0.103	0.066	0.128	-0.032	0.239
	GLP2	0.075	0.066	00.264	-0.060	0.210
Faida	Nyota	-0.273*	0.066	0.000	-0.409	0.138
	Angaza	-0.017	0.066	0.801	-0.152	0.119
	KAT B1	-0.228*	0.066	0.002	-0.364	-0.093
	Metameta	0.087	0.066	0.199	-0.049	0.222
	GLP2	0.058	0.066	0.382	-0.077	0.194
KAT B1	Nyota	-0.045	0.066	0.499	-00.180	0.090
	Angaza	0.212*	0.066	0.004	0.076	0.347
	Faida	0.228*	0.066	0.002	0.093	0.364
	Metameta	0.315*	0.066	0.000	0.180	0.450
	GLP2	0.287*	0.066	0.000	0.151	0.422
Metameta	Nyota	-0.360*	0.066	0.000	-0.495	-0.225
	Angaza	-0.103	0.066	0.128	-0.239	0.032
	Faida	-0.087	0.066	0.199	-0.222	0.049
	KAT B1	-0.315*	0.066	0.000	-0.450	-0.180
	GLP2	-0.028	0.066	0.669	-0.164	0.107
GLP2	Nyota	-0.332*	0.066	0.000	-0.467	-0.196
	Angaza	-0.075	0.066	0.264	-0.210	0.060
	Faida	-0.058	0.066	0.382	-0.194	0.077
	KAT B1	-0.287*	0.066	0.000	-0.422	-0.151
	Metameta	0.028	0.066	0.669	-0.107	0.164

4. Treatment

Estimates

Dependent Variable: Pod biomass

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Water stress	0.356	0.027	0.300	0.411
Well watered	0.692	0.027	0.637	0.747

5. Pairwise Comparisons

Dependent Variable: Pod biomass

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference [b]	
					Lower Bound	Upper Bound
Water stress	Well watered	-0.337*	0.038	0.000	-0.415	-0.259
Well watered	Water stress	0.337*	0.038	0.000	0.259	0.415

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

6. Univariate Tests

Dependent Variable: Pod biomass

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	1.020	1	1.020	79.146	0.000
Error	0.309	24	0.013		

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

7. Genotypes * Treatment

Dependent Variable: Pod biomass

				95% Confidence Interval	
Genotypes	**Treatment**	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Nyota	1	0.547	0.066	0.411	0.682
Angaza	1	0.363	0.066	0.228	0.499
Faida	1	0.290	0.066	0.155	0.425
KAT B1	1	0.507	0.066	0.371	0.642
Metameta	1	0.223	0.066	0.088	0.359
GLP2	1	0.203	0.066	0.068	0.339
Nyota	2	0.923	0.066	0.788	1.059
Angaza	2	0.593	0.066	0.458	0.729
Faida	2	0.633	0.066	0.498	0.769
KAT B1	2	0.873	0.066	0.738	1.009
Metameta	2	0.527	0.066	0.391	0.662
GLP2	2	0.603	0.066	0.468	0.739

1-Water stress; 2-Well watered

Appendix 6: Analysis of variance for stem biomass

1. **Tests of Between-Subjects Effects**

Dependent Variable: Stem biomass

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	141.743[a]	11	12.886	12.019	0.000
Intercept	745.381	1	745.381	695.221	0.000
Genotypes	5.953	5	1.191	1.110	0.381
Treatment	132.595	1	132.595	123.672	0.000
Genotypes * Treatment	3.195	5	0.639	0.596	0.703
Error	25.732	24	1.072		
Total	912.856	36			
Corrected Total	167.475	35			

a. R Squared = .846 (Adjusted R Squared = 0.776)

2. **Genotypes**

Estimates

Dependent Variable: Stem biomass

			95% Confidence Interval	
Genotypes	Mean	Std. Error	Lower Bound	Upper Bound
Nyota	4.875	0.423	4.003	5.747
Angaza	4.383	0.423	3.511	5.256
Faida	4.150	0.423	3.278	5.022
KAT B1	5.128	0.423	4.256	6.001
Metameta	3.990	0.423	3.118	4.862
GLP2	4.775	0.423	3.903	5.647

3. Pairwise Comparisons

Dependent Variable: Stem biomass

(I) Genotypes	(J) Genotypes	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference [a]	
					Lower Bound	Upper Bound
Nyota	Angaza	0.492	0.598	0.419	-0.742	1.725
	Faida	0.725	0.598	0.237	-0.509	1.959
	KAT B1	-0.253	0.598	0.676	-1.487	0.980
	Metameta	0.885	0.598	0.152	-0.349	2.119
	GLP2	0.100	0.598	0.869	-1.134	1.334
Angaza	Nyota	-0.492	0.598	0.419	-1.725	0.742
	Faida	0.233	0.598	0.700	-1.000	1.467
	KAT B1	-0.745	0.598	0.225	-1.979	0.489
	Metameta	0.393	0.598	0.517	-0.840	1.627
	GLP2	-0.392	0.598	0.519	-1.625	0.842
Faida	Nyota	-0.725	0.598	0.237	-1.959	0.509
	Angaza	-0.233	0.598	0.700	-1.467	1.000
	KAT B1	-0.978	0.598	0.115	-2.212	0.255
	Metameta	0.160	0.598	0.791	-1.074	1.394
	GLP2	-0.625	0.598	0.306	-1.859	0.609
KAT B1	Nyota	0.253	0.598	0.676	-0.980	1.487
	Angaza	0.745	0.598	0.225	-0.489	1.979
	Faida	0.978	0.598	0.115	-0.255	2.212
	Metameta	1.138	0.598	0.069	-0.095	2.372
	GLP2	0.353	0.598	0.560	-0.880	1.587
Metameta	Nyota	-0.885	0.598	0.152	-2.119	0.349
	Angaza	-0.393	0.598	0.517	-1.627	0.840
	Faida	-0.160	0.598	0.791	-1.394	1.074
	KAT B1	-1.138	0.598	0.069	-2.372	0.095
	GLP2	-0.785	0.598	0.202	-2.019	0.449
GLP2	Nyota	-0.100	0.598	0.869	-1.334	1.134
	Angaza	0.392	0.598	0.519	-0.842	1.625
	Faida	0.625	0.598	0.306	-0.609	1.859
	KAT B1	-0.353	0.598	0.560	-1.587	0.880
	Metameta	0.785	0.598	0.202	-0.449	2.019

Based on estimated marginal means

4. Univariate Tests

Dependent Variable: Stem biomass

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	5.953	5	1.191	1.110	0.381
Error	25.732	24	1.072		

The F tests the effect of Genotypes. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment

Pairwise Comparisons

Dependent Variable: Stem biomass

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Water stress	Well watered	-3.838*	0.345	0.000	-4.551	-3.126
Well watered	Water stress	3.838*	0.345	0.000	3.126	4.551

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

6. Univariate Tests

Dependent Variable: Stem biomass

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	132.595	1	132.595	123.672	0.000
Error	25.732	24	1.072		

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

7. Genotypes * Treatment

Dependent Variable: Stem biomass

Genotypes	Treatment	Mean	Std Error
Nyota	1	3.217	0.598
Angaza	1	2.407	0.598
Faida	1	2.177	0.598
KAT B1	1	3.053	0.598
Metameta	1	2.540	0.598
GLP2	1	2.393	0.598
Nyota	2	6.533	0.598
Angaza	2	6.360	0.598
Faida	2	6.123	0.598
KAT B1	2	7.203	0.598
Metameta	2	5.440	0.598
GLP2	2	7.157	0.598

1-Water stress; 2- Well watered

Appendix 7: Analysis of Variance for water use efficiency

1. **Tests of Between-Subjects Effects**

Dependent Variable: Water use efficiency

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	185.932[a]	11	16.903	129.682	0.000
Intercept	835.210	1	835.210	6407.851	0.000
Genotypes	162.789	5	32.558	249.787	0.000
Treatment	18.006	1	18.006	138.144	0.000
Genotypes * Treatment	5.138	5	1.028	7.884	0.000
Error	3.128	24	0.130		
Total	1024.271	36			
Corrected Total	189.061	35			

a. R Squared = .983 (Adjusted R Squared = 0.976)

2. **Genotypes**

Estimates

Dependent Variable: Water use efficiency

			95% Confidence Interval	
Genotypes	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Nyota	8.392	0.147	8.087	8.696
Angaza	4.235	0.147	3.931	4.539
Faida	5.920	0.147	5.616	6.224
KAT B1	5.613	0.147	5.309	5.918
Metameta	2.677	0.147	2.372	2.981
GLP2	2.063	0.147	1.759	2.368

3. Pairwise Comparisons

Dependent Variable: Water use efficiency

(I) Genotypes	(J) Genotypes	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Nyota	Angaza	4.157*	0.208	0.000	3.726	4.587
	Faida	2.472*	0.208	0.000	2.041	2.902
	KAT B1	2.778*	0.208	0.000	2.348	3.209
	Metameta	5.715*	0.208	0.000	5.285	6.145
	GLP2	6.328*	0.208	0.000	5.898	6.759
Angaza	Nyota	-4.157*	0.208	0.000	-4.587	-3.726
	Faida	-1.685*	0.208	0.000	-2.115	-1.255
	KAT B1	-1.378*	0.208	0.000	-1.809	-0.948
	Metameta	1.558*	0.208	0.000	1.128	1.989
	GLP2	2.172*	0.208	0.000	1.741	2.602
Faida	Nyota	-2.472*	0.208	0.000	-2.902	-2.041
	Angaza	1.685*	0.208	0.000	1.255	2.115
	KAT B1	0.307	0.208	0.154	-.124	0.737
	Metameta	3.243*	0.208	0.000	2.813	3.674
	GLP2	3.857*	0.208	0.000	3.426	4.287
KAT B1	Nyota	-2.778*	0.208	0.000	-3.209	-2.348
	Angaza	1.378*	0.208	0.000	0.948	1.809
	Faida	-0.307	0.208	0.154	-0.737	0.124
	Metameta	2.937*	0.208	0.000	2.506	3.367
	GLP2	3.550*	0.208	0.000	3.120	3.980
Metameta	Nyota	-5.715*	0.208	0.000	-6.145	-5.285
	Angaza	-1.558*	0.208	0.000	-1.989	-1.128
	Faida	-3.243*	0.208	0.000	-3.674	-2.813
	KAT B1	-2.937*	0.208	0.000	-3.367	-2.506
	GLP2	0.613*	0.208	0.000	0.183	1.044
GLP2	Nyota	-6.328*	0.208	0.000	-6.759	-5.898
	Angaza	-2.172*	0.208	0.000	-2.602	-1.741
	Faida	-3.857*	0.208	0.000	-4.287	-3.426
	KAT B1	-3.550*	0.208	0.000	-3.980	-3.120
	Metameta	-0.613*	0.208	0.007	-1.044	-0.183

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

4. Univariate Tests

Dependent Variable: Water use efficiency

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	162.789	5	32.558	249.787	0.000
Error	3.128	24	0.130		

The F tests the effect of Genotypes. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment

Estimates

Dependent Variable: Water use efficiency

			95% Confidence Interval	
Treatment	Mean	Std. Error	Lower Bound	Upper Bound
Water stress	5.524	0.085	5.348	5.700
Well watered	4.109	0.085	3.934	4.285

6. Pairwise Comparisons

Dependent Variable: Water use efficiency

					95% Confidence Interval for Difference[b]	
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[b]	Lower Bound	Upper Bound
Water stress	Well watered	1.414*	0.120	0.000	1.166	1.663
Well watered	Water stress	-1.414*	0.120	0.000	-1.663	-1.166

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

7. Genotypes * Treatment

Dependent Variable: Water use efficiency

Genotypes	Treatment	Mean	Std. Error
Nyota	1	8.443[a]	0.208
Angaza	1	6.757[b]	0.208
Faida	1	6.087[b]	0.208
KAT B1	1	7.903[a]	0.208
Metameta	1	6.333[b]	0.208
GLP2	1	5.110[cb]	0.208
Nyota	2	4.840[cb]	0.208
Angaza	2	5.083[cb]	0.208
Faida	2	4.333[c]	0.208
KAT B1	2	4.677[cb]	0.208
Metameta	2	4.110[c]	0.208
GLP2	2	4.000[c]	0.208

1-water stress; 2-well watered

Appendix 8: Analysis of variance for stomatal conductance

1. **Tests of Between-Subjects Effects**

Dependent Variable: Stomatal conductance

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1005.015[a]	11	91.365	6.480	0.000
Intercept	21973.121	1	21973.121	1558.545	0.000
Genotypes	728.089	5	145.618	10.329	0.000
Treatment	273.572	1	273.572	19.404	0.000
Genotypes * Treatment	3.355	5	0.671	0.048	0.998
Error	338.364	24	14.098		
Total	23316.500	36			
Corrected Total	1343.379	35			

a. R Squared = 0.748 (Adjusted R Squared = 0.633)

2. **Genotypes**

Estimates

Dependent Variable: Stomatal conductance

			95% Confidence Interval	
Genotypes	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Nyota	30.993	1.533	27.830	34.157
Angaza	27.138	1.533	23.975	30.302
Faida	21.793	1.533	18.630	24.957
KAT B1	28.752	1.533	25.588	31.915
Metameta	20.893	1.533	17.730	24.057
GLP2	18.663	1.533	15.500	21.827

3. Pairwise Comparisons

Dependent Variable: Stomatal conductance

(I) Genotypes	(J) Genotypes	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Nyota	Angaza	3.855	2.168	0.088	-0.619	8.329
	Faida	9.200*	2.168	0.000	4.726	13.674
	KAT B1	2.242	2.168	0.311	-2.233	6.716
	Metameta	10.100*	2.168	0.000	5.626	14.574
	GLP2	12.330*	2.168	0.000	7.856	16.804
Angaza	Nyota	-3.855	2.168	0.088	-8.329	0.619
	Faida	5.345*	2.168	0.021	0.871	9.819
	KAT B1	-1.613	2.168	0.464	-6.088	2.861
	Metameta	6.245*	2.168	0.008	1.771	10.719
	GLP2	8.475*	2.168	0.001	4.001	12.949
Faida	Nyota	-9.200*	2.168	0.000	-13.674	-4.726
	Angaza	-5.345*	2.168	0.021	-9.819	-0.871
	KAT B1	-6.958*	2.168	0.004	-11.433	-2.484
	Metameta	0.900	2.168	0.682	-3.574	5.374
	GLP2	3.130	2.168	0.162	-1.344	7.604
KAT B1	Nyota	-2.242	2.168	0.311	-6.716	2.233
	Angaza	1.613	2.168	0.464	-2.861	6.088
	Faida	6.958*	2.168	0.004	2.484	11.433
	Metameta	7.858*	2.168	0.001	3.384	12.333
	GLP2	10.088*	2.168	0.000	5.614	14.563
Metameta	Nyota	-10.100*	2.168	0.000	-14.574	-5.626
	Angaza	-6.245*	2.168	0.008	-10.719	-1.771
	Faida	-0.900	2.168	0.682	-5.374	3.574
	KAT B1	-7.858*	2.168	0.001	-12.333	-3.384
	GLP2	2.230	2.168	0.314	-2.244	6.704
GLP2	Nyota	-12.330*	2.168	0.000	-16.804	-7.856
	Angaza	-8.475*	2.168	0.001	-12.949	-4.001
	Faida	-3.130	2.168	0.162	-7.604	1.344
	KAT B1	-10.088*	2.168	0.000	-14.563	-5.614
	Metameta	-2.230	2.168	0.314	-6.704	2.244

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

4. Univariate Tests

Dependent Variable: Leaf water potential

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	728.089	5	145.618	10.329	0.000
Error	338.364	24	14.098		

The F tests the effect of Genotypes. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment

Estimates

Dependent Variable: Stomatal conductance

			95% Confidence Interval	
Treatment	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Water stress	27.462	0.885	25.636	29.289
Well watered	21.949	0.885	20.122	23.775

6. Pairwise Comparisons

Dependent Variable: Stomatal conductance

					95% Confidence Interval for Difference[b]	
(I) Treatment	**(J) Treatment**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[b]**	**Lower Bound**	**Upper Bound**
Water stress	Well watered	5.513*	1.252	0.000	2.930	8.097
Well watered	Water stress	-5.513*	1.252	0.000	-8.097	-2.930

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

7. Genotypes * Treatment

Dependent Variable: Stomatal conductance

Genotypes	Treatment	Mean	Std. Error
Nyota	1	33.787	2.168
Angaza	1	30.300	2.168
Faida	1	24.263	2.168
KAT B1	1	31.713	2.168
Metameta	1	23.150	2.168
GLP2	1	21.560	2.168
Nyota	2	28.200	2.168
Angaza	2	23.977	2.168
Faida	2	19.323	2.168
KAT B1	2	25.790	2.168
Metameta	2	18.637	2.168
GLP2	2	15.767	2.168

1-water stress; 2-well watered

Appendix 9: Analysis of variance for leaf water potential

1. **Tests of Between-Subjects Effects**

Dependent Variable: Leaf water potential

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.279[a]	11	0.025	48.120	0.000
Intercept	10.945	1	10.945	20738.026	0.000
Genotypes	0.048	5	0.010	18.169	0.000
Treatment	0.183	1	0.183	347.626	0.000
Genotypes * Treatment	0.048	5	0.010	18.169	0.000
Error	0.013	24	0.001		
Total	11.237	36			
Corrected Total	0.292	35			

a. R Squared = 0.957 (Adjusted R Squared = 0.937)

2. **Genotypes**

Dependent Variable: Leaf water potential

			95% Confidence Interval	
Genotypes	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Nyota	-0.517	0.009	-0.536	-0.497
Angaza	-0.565	0.009	-0.584	-0.546
Faida	-0.563	0.009	-0.583	-0.544
KAT B1	-0.497	0.009	-0.516	-0.477
Metameta	-0.610	0.009	-0.629	-0.591
GLP2	-0.557	0.009	-0.576	-0.537

3. Pairwise Comparisons

Dependent Variable: Leaf water potential

(I) Genotypes	(J) Genotypes	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Nyota	Angaza	0.048*	0.013	0.001	0.021	0.076
	Faida	0.047*	0.013	0.002	0.019	0.074
	KAT B1	-0.020	0.013	0.145	-0.047	0.007
	Metameta	0.093*	0.013	0.000	0.066	0.121
	GLP2	0.040*	0.013	0.006	0.013	0.067
Angaza	Nyota	-0.048*	0.013	0.001	-0.076	-0.021
	Faida	-0.002	0.013	0.901	-0.029	0.026
	KAT B1	-0.068*	0.013	0.000	-0.096	-0.041
	Metameta	0.045*	0.013	0.002	0.018	0.072
	GLP2	-0.008	0.013	0.536	-0.036	0.019
Faida	Nyota	-0.047*	0.013	0.002	-0.074	-0.019
	Angaza	0.002	0.013	0.901	-0.026	0.029
	KAT B1	-0.067*	0.013	0.000	-0.094	-0.039
	Metameta	0.047*	0.013	0.002	0.019	0.074
	GLP2	-0.007	0.013	0.620	-0.034	0.021
KAT B1	Nyota	0.020	0.013	0.145	-0.007	0.047
	Angaza	0.068*	0.013	0.000	0.041	0.096
	Faida	0.067*	0.013	0.000	0.039	0.094
	Metameta	0.113*	0.013	0.000	0.086	0.141
	GLP2	0.060*	0.013	0.000	0.033	0.087
Metameta	Nyota	-0.093*	0.013	0.000	-0.121	-0.066
	Angaza	-0.045*	0.013	0.002	-0.072	-0.018
	Faida	-0.047*	0.013	0.002	-0.074	-0.019
	KAT B1	-0.113*	0.013	0.000	-0.141	-0.086
	GLP2	-0.053*	0.013	0.000	-0.081	-00.026
GLP2	Nyota	-0.040*	0.013	0.006	-0.067	-0.013
	Angaza	0.008	0.013	0.536	-0.019	0.036
	Faida	0.007	0.013	0.620	-0.021	0.034
	KAT B1	-0.060*	0.013	0.000	-0.087	-0.033
	Metameta	0.053*	0.013	0.000	0.026	0.081

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

4. Univariate Tests

Dependent Variable: Leaf water potential

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	0.048	5	0.010	18.169	0.000
Error	0.013	24	0.001		

The F tests the effect of Genotypes. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment

Estimates

Dependent Variable: Leaf water potential

			95% Confidence Interval	
Treatment	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Water stress	-0.623	0.005	-0.634	-0.612
Well watered	-0.480	0.005	-0.491	-0.469

6. Pairwise Comparisons

Dependent Variable: Leaf water potential

					95% Confidence Interval for Difference[b]	
(I) Treatment	**(J) Treatment**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[b]**	**Lower Bound**	**Upper Bound**
Water stress	Well watered	-0.143*	0.008	0.000	-0.159	-0.127
Well watered	Water stress	0.143*	0.008	0.000	0.127	0.159

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

7. Genotypes * Treatment

Dependent Variable**: Leaf water potential**

Genotypes	Treatment	Mean	Std. Error
Nyota	1	-0.553[c]	0.013
Angaza	1	-0.603[cb]	0.013
Faida	1	-0.627[b]	0.013
KAT B1	1	-0.513[c]	0.013
Metameta	1	-0.650[ba]	0.013
GLP2	1	-0.740[a]	0.013
Nyota	2	-0.480[c]	0.013
Angaza	2	-0.480[c]	0.013
Faida	2	-0.480[c]	0.013
KAT B1	2	-0.480[c]	0.013
Metameta	2	-0.480[c]	0.013
GLP2	2	-0.480[c]	0.013

1-water stress; 2-well watered

Appendix 10: Analysis of variance for days to flowering

1. Tests of Between-Subjects Effects

Dependent Variable: Days to flowering

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	956.750[a]	11	86.977	.	.
Intercept	56406.250	1	56406.250	.	.
Genotypes	931.250	5	186.250	.	.
Treatment	20.250	1	20.250	.	.
Genotypes * Treatment	5.250	5	1.050	.	.
Error	0.000	24	0.000		
Total	57363.000	36			
Corrected Total	956.750	35			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

2. Genotypes

Dependent Variable: Leaf water potential

Genotypes	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nyota	-0.517	0.009	-0.536	-0.497
Angaza	-0.565	0.009	-0.584	-0.546
Faida	-0.563	0.009	-0.583	-0.544
KAT B1	-0.497	0.009	-0.516	-0.477
Metameta	-0.610	0.009	-0.629	-0.591
GLP2	-0.557	0.009	-0.576	-0.537

3. Pairwise Comparisons

Dependent Variable: Days to flowering

(I) Genotypes	(J) Genotypes	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference [a]	
					Lower Bound	Upper Bound
Nyota	Angaza	0.500	0.000	.	0.500	0.500
	Faida	-4.000	0.000	.	-4.000	-4.000
	KAT B1	11.000	0.000	.	11.000	11.000
	Metameta	-2.000	0.000	.	-2.000	-2.000
	GLP2	6.000	0.000	.	6.000	6.000
Angaza	Nyota	-0.500	0.000	.	-0.500	-0.500
	Faida	-4.500	0.000	.	-4.500	-4.500
	KAT B1	10.500	0.000	.	10.500	10.500
	Metameta	-2.500	0.000	.	-2.500	-2.500
	GLP2	5.500	0.000	.	5.500	5.500
Faida	Nyota	4.000	0.000	.	4.000	4.000
	Angaza	4.500	0.000	.	4.500	4.500
	KAT B1	15.000	0.000	.	15.000	15.000
	Metameta	2.000	0.000	.	2.000	2.000
	GLP2	10.000	0.000	.	10.000	10.000
KAT B1	Nyota	-11.000	0.000	.	-11.000	-11.000
	Angaza	-10.500	0.000	.	-10.500	-10.500
	Faida	-15.000	0.000	.	-15.000	-15.000
	Metameta	-13.000	0.000	.	-13.000	-13.000
	GLP2	-5.000	0.000	.	-5.000	-5.000
Metameta	Nyota	2.000	0.000	.	2.000	2.000
	Angaza	2.500	0.000	.	2.500	2.500
	Faida	-2.000	0.000	.	-2.000	-2.000
	KAT B1	13.000	0.000	.	13.000	13.000
	GLP2	8.000	0.000	.	8.000	8.000
GLP2	Nyota	-6.000	0.000	.	-6.000	-6.000
	Angaza	-5.500	0.000	.	-5.500	-5.500
	Faida	-10.000	0.000	.	-10.000	-10.000
	KAT B1	5.000	0.000	.	5.000	5.000
	Metameta	-8.000	0.000	.	-8.000	-8.000

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

4. Univariate Tests

Dependent Variable: Stem biomass

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	931.250	5	186.250	.	.
Error	0.000	24	0.000		

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment

Estimates

Dependent Variable: Days to flowering

			95% Confidence Interval	
Treatment	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Water stress	38.833	0.000	38.833	38.833
Well watered	40.333	0.000	40.333	40.333

6. Pairwise Comparisons

Dependent Variable: Days to flowering

					95% Confidence Interval for Difference [a]	
(I) Treatment	**(J) Treatment**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[a]**	**Lower Bound**	**Upper Bound**
Water stress	Well watered	-1.500	0.000	.	-1.500	-1.500
Well watered	Water stress	1.500	0.000	.	1.500	1.500

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

7. Genotypes * Treatment

Dependent Variable**: Days to flowering**

				95% Confidence Interval	
Genotypes	**Treatment**	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Nyota	1	41.000	0.000	41.000	41.000
Angaza	1	40.000	0.000	40.000	40.000
Faida	1	45.000	0.000	45.000	45.000
KAT B1	1	30.000	0.000	30.000	30.000
Metameta	1	42.000	0.000	42.000	42.000
GLP2	1	35.000	0.000	35.000	35.000
Nyota	2	42.000	0.000	42.000	42.000
Angaza	2	42.000	0.000	42.000	42.000
Faida	2	46.000	0.000	46.000	46.000
KAT B1	2	31.000	0.000	31.000	31.000
Metameta	2	45.000	0.000	45.000	45.000
GLP2	2	36.000	0.000	36.000	36.000

1-Water stress; 2- Well watered

Appendix 11: Analysis of variance for days to maturity

1. Tests of Between-Subjects Effects

Dependent Variable: Days to maturity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14958.750[a]	11	1359.886	.	.
Intercept	128522.250	1	128522.250	.	.
Genotypes	1508.250	5	301.650	.	.
Treatment	13340.250	1	13340.250	.	.
Genotypes * Treatment	110.250	5	22.050	.	.
Error	.000	24	0.000		
Total	143481.000	36			
Corrected Total	14958.750	35			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

2. Genotypes

Estimates

Dependent Variable: Days to maturity

Genotypes	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nyota	63.500	0.000	63.500	63.500
Angaza	63.000	0.000	63.000	63.000
Faida	65.500	0.000	65.500	65.500
KAT B1	48.000	0.000	48.000	48.000
Metameta	64.500	0.000	64.500	64.500
GLP2	54.000	0.000	54.000	54.000

3. Pairwise Comparisons

Dependent Variable: Days to maturity

(I) Genotypes	(J) Genotypes	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference [a]	
					Lower Bound	Upper Bound
Nyota	Angaza	0.500	0.000	.	0.500	0.500
	Faida	-2.000	0.000	.	-2.000	-2.000
	KAT B1	15.500	0.000	.	15.500	15.500
	Metameta	-1.000	0.000	.	-1.000	-1.000
	GLP2	9.500	0.000	.	9.500	9.500
Angaza	Nyota	-0.500	0.000	.	-0.500	-0.500
	Faida	-2.500	0.000	.	-2.500	-2.500
	KAT B1	15.000	0.000	.	15.000	15.000
	Metameta	-1.500	0.000	.	-1.500	-1.500
	GLP2	9.000	0.000	.	9.000	9.000
Faida	Nyota	2.000	0.000	.	2.000	2.000
	Angaza	2.500	0.000	.	2.500	2.500
	KAT B1	17.500	0.000	.	17.500	17.500
	Metameta	1.000	0.000	.	1.000	1.000
	GLP2	11.500	0.000	.	11.500	11.500
KAT B1	Nyota	-15.500	0.000	.	-15.500	-15.500
	Angaza	-15.000	0.000	.	-15.000	-15.000
	Faida	-17.500	0.000	.	-17.500	-17.500
	Metameta	-16.500	0.000	.	-16.500	-16.500
	GLP2	-6.000	0.000	.	-6.000	-6.000
Metameta	Nyota	1.000	0.000	.	1.000	1.000
	Angaza	1.500	0.000	.	1.500	1.500
	Faida	-1.000	0.000	.	-1.000	-1.000
	KAT B1	16.500	0.000	.	16.500	16.500
	GLP2	10.500	0.000	.	10.500	10.500
GLP2	Nyota	-9.500	0.000	.	-9.500	-9.500
	Angaza	-9.000	0.000	.	-9.000	-9.000
	Faida	-11.500	0.000	.	-11.500	-11.500
	KAT B1	6.000	0.000	.	6.000	6.000
	Metameta	-10.500	0.000	.	-10.500	-10.500

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

4. Univariate Tests

Dependent Variable: Days to maturity

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	1508.250	5	301.650	.	.
Error	0.000	24	0.000		

The F tests the effect of Genotypes. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment

Estimates

Dependent Variable: Days to maturity

			95% Confidence Interval	
Treatment	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Water stress	40.500	0.000	40.500	40.500
Well watered	79.000	0.000	79.000	79.000

6. Univariate Tests

Dependent Variable: Days to maturity

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	13340.250	1	13340.250	.	.
Error	0.000	24	0.000		

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

7. Genotypes * Treatment

Dependent Variable: Days to maturity

Genotypes	Treatment	Mean	Std. Error
Nyota	1	42.000	0.000
Angaza	1	42.000	0.000
Faida	1	46.000	0.000
KAT B1	1	31.000	0.000
Metameta	1	45.000	0.000
GLP2	1	37.000	0.000
Nyota	2	85.000	0.000
Angaza	2	84.000	0.000
Faida	2	85.000	0.000
KAT B1	2	65.000	0.000
Metameta	2	84.000	0.000
GLP2	2	71.000	0.000

1-Water stress; 2-Well watered