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RESEARCH ARTICLE

EVALUATION AND SELECTION OF DIFFERENT LEGUMES SPECIES BASED ON MORPHOLOGICAL AND NODULATION DIFFERENCES UNDER WATER DEFICIENT CONDITIONS OF LOWER EASTERN KENYA.

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ABSTRACT

Soil fertility depletion is a major limiting factor affecting crop production in Kenya's arid and semi-arid lands (ASALs). In lower eastern Kenya, low crop yield has been associated with moisture deficiency and low usage of commercial fertilizers. Amongst unexplored solutions that can mitigate these constraints includes potential role of rhizobia in crop performance under water deficit conditions. Thus the present study analyzed the effects of drought stress on nodule formation, growth and yield of four legumes (beans, cowpeas, dolichos lablab and green grams) commonly cultivated in Kenya's ASALs county of Kitui. The two seasonal field-based trials involved randomized complete block design with drought stress treatment (DST) induced through withholding total irrigation and well watered treatment (WWT) maintained as a control. Four blocks, each with four plots, were demarcated. The four legumes were randomly assigned to the plots and maintained under WWT. One month after planting (MAP), DST was randomly induced by withholding irrigation in two blocks while WWT was maintained in the other two blocks as controls. Upon termination of field experiment, root nodules were carefully harvested from each legume in both DST and WWT. The nodules were then cultured in the laboratory for isolations of rhizobia as well as preparation of an inoculant for specificity assays under greenhouse conditions. Results showed that plants subjected to DST had significantly ($p \leq 0.05$) less TND, NoP, lower LAI, more WIX and lower GYD compared to control or plants under WWT indicating the general deleterious impact of water deficit on legume nodulation, growth and yield. The reduced TND under DST could inhibit nitrogen fixation further lessening GYD in legumes. Amongst the legumes, green grams had significantly ($P \leq 0.05$) higher GYD, TND and least WIX, dolichos, lablab and cowpeas exhibited moderate performance of the three traits while beans showed the least TND, GYD and high WIX under DST. Under DST, Green grams had significantly ($p \leq 0.05$) the highest yield followed by Cowpeas, Dolichos lablab and Beans was significantly affected by water stress to give lowest yield. Generally, TND positively correlated with GYD and negatively with wilting (WIX), potentially implying that higher nodulation might have enhanced nitrogen fixation thus higher legume YLD and tolerance to water deficit. Based on observed performance i.e. wilting index, root nodules number per plant and grain yield, green grams was considered drought tolerant and beans drought susceptible, therefore this study recommends adoption and growing of green grams (variety KS-20) in the ASALs of Lower Eastern Kenya.

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INTRODUCTION

Poverty and hunger in Africa are mainly concentrated in the arid and semi-arid lands (ASALs) (UN, 2007) where agriculture is the dominant source of livelihood (GoK, 2010). Recent estimates (Nyage *et al.,* 2011) show that agriculture accounts for 35% of the continent's GDP, 40% of export earnings and 70% of employment. In Kenya, agriculture is the backbone of the economic and social development (Anon, 2010).

Kenya is food insecure even though it is the leading economy in East Africa as well as regional business center (Glopolis, 2013; GoK, 2011). Approximately 83% of Kenyan land is ASALs, more often characterized by chronic food insecurity, extreme poverty and episodic famine (GoK, 2010). It is estimated that over 50% of the Kenyan population does not have access to adequate food, and what they have is of poor nutritional value (Anon, 2010). Low farm productivity due to declining soil fertility (Gachimbi al., 2002) and soil moisture constraint (GoK, 2009a) has been cited as the dominant cause of food insecurity in these regions.

Decline in soil fertility is attributed to soil leaching, erosion and mining of nutrients mainly through continuous mono-cropping of cereal crops without adequate soil fertility replenishment (Gachimbi *et al.,* 2002). Food security has been an integral part of global efforts concerning development and reduction of poverty (Vink, 2012). In Kenya, many types of cereals and legumes are grown to alleviate food insecurity. Philips (1980) noted that leguminous crops continue to play a crucial role in agricultural production throughout history and attributes their success in N-deficient soils results from root nodules containing symbiotic Rhizobium bacteria that reduce N_2 to NH_3. Rhizobia are soil-inhabiting bacteria that form the root nodules where symbiotic biological nitrogen fixation occurs (Howieson and Brockwell, 2005; Weir, 2006). This process, where atmospheric N is captured for assimilation by plants is under-utilized by small-scale African farmers, in part because they do not understand its mechanism and management (Woomer *et al.,* 1997). For example, 95% of farmers in East and Southern Africa are familiar with legume root nodules but only 26% consider them beneficial (Woomer *et al.,* 1997). In another study, the percentage of farmers who use inoculants in Kenya is only 1% (Karanja *et al*., 2000).

The adaptability of indigenous rhizobia to their environment results in high levels of saprophytic competence, therefore continual identification of new, elite isolates offers the opportunity of improving Biological Nitrogen Fixation (BNF) with fine-tuned geographical targets (Zengeni et al., 2006; Appunu and Dhar, 2008). In this way, a wide diversity of rhizobial isolates ensures a sustainable source of strains for commercial application into the future (Musiyiwa *et al.,* 2005). One empirical approach to rhizobia strain selection focuses upon the stepwise collection, isolation and authentication of native rhizobia, screening of the isolates against reference strains for symbiotic effectiveness, assessment of their competitive abilities and evaluation of their performance under a range of field conditions (Howieson *et al*., 2000), with each step eliminating the worst performing isolates for further consideration. In this way, the identified elite rhizobial strains are likely to colonize the soil, tolerate environmental stresses, and compete with background populations (Slattery and Pearce, 2002). Nitrogen is the most abundant element on the earth and about 78 % of the earth's atmosphere is nitrogen gas (Sangakara *et al.*, 2003). There are hundreds of tons of nitrogen over every hectare of land surface. Despite the abundance of nitrogen in the atmosphere, plants are unable to use it directly because it is present in an inert form (N_2) and the nitrogen in the soil is lost through microbial dinitrification, soil erosion, leaching, chemical volatilization, removal of nitrogen containing crop residues from land and Nitrogen is therefore most limiting plant nutrient for crop production in Africa (Sangakara *et al.*, 2003). Most legumes, through symbiosis with rhizobia have the ability to reduce N_2 through biological nitrogen fixation (BNF) into a form usable for growth naturally (Mugwe *et al.,* 2007*)*. The amount of nitrogen fixed varies according to the legume species and variety. Within a species the amount of nitrogen fixed is directly related to (dry matter) yield (Delfin *et al*., 2008). Legumes can fix as much as 200 kg N ha^{-1} $year^{-1}$ under optimal field conditions (Giller, 2001). However, this can only be achieved in the presence of efficient *Rhizobial* strains, which can be native to the soil or introduced in-form of commercial inoculants (George *et al.,* 2007). The amount of N contributed to the soil system by the legume crops depends on the rate of symbiotic N fixing activity, growth and N harvest index of the legume crops (Zahran, 1999).

The rate of N fixation varies considerably, depending on type of legume cultivar, method of measurement, the presence of appropriate *Rhizobia*, and certain soil and environmental variables, including soil moisture, NO_3 level, soil acidity, and P nutrition (Danga *et al.,* 2009 & 2010; Zahran, 1999). However, the amount of biologically fixed N can be enhanced by different methods, including inoculation with proven strains, screening for improved microbial and host-plant materials, and introduction of improved cultural practices (Zengenia *et al.,* 2006; Giller, 2001). Farmers are in the process of advancing from subsistence to market-based agriculture and seeking to improve their field practices and yields (Woomer *et al*., 1998).A large population of farmers continues cultivating legumes including beans, lablab, cowpeas and green grams as increasingly important cash crops (Woomer *et al*., 1998).Most of these grain legumes can obtain between 50 - 80% of their nitrogen concentration requirements through biological fixation (Solomon *et al.*, 2012). Scientific data on nitrogen fixation by these legumes under water scarce conditions is either nascent or non-existent. This study aimed at analyzing legume nodulation, growth and yield under drought stress conditions in Kitui County, Kenya**.**

MATERIALS AND METHODS

Description of experimental sites: The field experiment was carried out in Kwa-Mulungu farm situated in Kitui County between January- March 2016 (first season) and June to August 2016 (second season). A plot experiment was conducted in Kwa Mulungu farm located at, latitude 1^0 21'45.78" S, longitude 37^0 52'48.18" E and altitude 1105m above sea level (Meteorological department Kitui, 2002). As a semi-arid region, Kitui County is among the most drought-vulnerable regions in Kenya with annual rainfall of 500 – 1050 mm and 40% reliability (GOK, 2010). The annual mean minimum temperatures range from 22 – 28ºC, while the annual mean maximum temperatures range from 28 – 32º C (GOK, 2010). The area is semi-arid under AEZ IV with very erratic and unreliable rainfall. The rainfall pattern is bimodal ,The 'long rains' fall in April-May; the 'short rains' last from October to December, and are more reliable. Overall, approximately 90% of the annual precipitation falls during the long rain season (Hoogmoed, 2007). Greenhouse experiment was carried out at Kenya Agricultural and Livestock Research Organization (KALRO) sub-station at Kitui County located at latitude 10 36'48.19" S, longitude 380 43'37.86" E and altitude 1148m above sea level (Management Hand Book Ministry of Agriculture, 2010).

Selected Legume varieties: The experiment comprised of four legume species namely: Beans (Variety - KAT 56) Cowpeas (Variety - K80), Dolichos lablab (Variety- 1001) and Green grams (Variety - KS20). These varieties had been researched, developed from KALRO in Machakos and recommended to farmers in ASALs field conditions (KALRO.org).

Field experiment**:** The field with no history of legume growth and which had not been fallowed for long was selected to ensure crops grew in field free from pests and diseases according to Lenne (2000). It was then cleared of grasses and other prevalent weeds using mechanical methods, followed by demarcation. The fields were ploughed to a depth of 30 cm. To ensure high viability and quality, legume seeds to be planted were carefully sorted to increase chances of uniform germination.

Plots measuring 1.5 m x4 m were marked out with a path of one meter between the plots. Recommended spacing of 45cm by 15 cm for each legume was followed in sowing. Before sowing seeds into the plots, field capacity was determined according to procedure by Zhen-tao Cong *et al* (2014). Field capacity was used as a reference value to guide on quantity of water required for watering (Rodríguez-Iturbe and Porporato, 2004). Watering was done every day to maintain the moisture at field capacity. The two seasonal field-based trials involved randomized complete block design with drought stress treatment (DST) induced through withholding total irrigation and well-watered treatment (WWT) maintained as a control. Four blocks, each with four plots, were demarcated. The four legumes were randomly assigned to the plots and maintained under WWT. One month after planting (MAP), DST was randomly induced in two blocks until the legumes wilted while WWT was maintained in the other two blocks as controls. Once project was terminated, nodules were harvested and taken to the laboratory for isolation and rhizobium cultured was used to prepare an inoculant for specificity assays under greenhouse conditions.

Laboratory design: Roots were harvested and thoroughly and carefully washed to remove soil. Ten nodules were collected from each plant by cutting the sever nodule about 0.5 cm on each side of the nodule. To reduce the risk of damaging the nodule, a forceps was used. The nodules were disinfected for thirty seconds in ethanol (95%) and for one minute in sodium hypochlorite (6%), adapted from Barrett and Parker (2006). They were then washed four times in sterilized water and finally crushed with a flame-sterilized glass rod. A loopful of the crushed nodule was then streaked across the surface of a Petri dish containing yeast mannitol agar (YMA; Vincent, 1970), and incubated at 25-30^{0}C in the dark. Typical well-isolated colonies were re-isolated on diagnostic media, adapted from Odee et al. (1997): test tubes containing liquid YM (YMA without agar) with 25 mg kg-1 (w/v) bromethymol blue (BTB) as pH indicator; Petri dishes containing YMA with 25 mg kg-1 (w/v) Congo red. The bacteria in test tubes were incubated at 28°C on a shaker (220 rpm) and classified according to their ability to change the pH of the growth medium (alkaline, neutral or acid). Petri dishes were incubated at 28°C in the dark, until the colonies were evaluated. They were characterized according to color (white, pink, translucid, yellow or white with a pink center), to the amount of extracellular polysaccharides (EPS) production (none to moderate or moderate to copious) and to colony size (the colony diameter measured with a ruler, after 3, 6 and 8 days of incubation).Three replicates for each isolate were analyzed, and the mean growth rate was used to separate different categories (Odee et al., 1997): very fast – colonies ≥ 5 mm in diameter after 3 days of incubation; fast – colonies ≥ 3 mm diameter after 3 days of incubation; intermediate – colonies ≥ 3 mm diameter after 6 days of incubation; slow – colonies ≥ 3 mm diameter after 8 days of incubation; very slow – colonies ≤ 3 mm diameter after 8 days of incubation. Color and EPS were only evaluated when the colonies reached the minimum diameter of 3 mm, except for the very slow growing ones, which did not reach this diameter until the eighth day.

Data collection and analysis

Days to seed germination: After land preparation, planting for the four legume genotypes were done at the same time and all blocks given similar treatment of watering and drought stress as assigned above. Data on germination was taken for each plot by physically counting the number of seeds that had germinated in the morning and evening and the results were tabulated according to Timson (1965).

Nodule number per plant: On termination of the experiment, ten plants from the two middle rows were randomly selected and gently dug out. The plants were then washed through a fine sieve with water to remove soil particles and organic debris according to procedure outlined by Geetha et al (2012). The number of nodules on each plant was counted and the average nodules per plant calculated.

Determination of shoot dry weight: Ten plants were randomly selected from the two border rows on each side of the treatment plot and cut at the ground level for shoot dry matter determination at the termination of the experiment. Total fresh shoot weight was measured using an electronic weighing balance. Plant materials were then put in brown envelopes and oven dried at 65^0 C for 72 hours as outlined by Ping Huang (2016). The dry materials were weighed and shoot dry weight recorded.

Leaf Area Index: Leaf area of third, fifth and seventh leaf of the ten legume plants selected from the experimental plot were measured and determined against their ground area. The leaf area was calculated as described by Xiong *et al*., 2006.

Leaf area (cm^2) = leaf length (cm) × leaf width (cm) × 0.83.

Leaf surface Index = Leaf area /ground area m^2.

Wilting Index: Leaf wilting is a fundamental trait used in drought tolerance evaluation. Signs of wilting were observed after one week of stress, a visual assessment of wilting was done since leaf water potentials cannot be measured in dead leaves. The following visual characteristics were used according to Bettina *et al* (2007).

Analysis of Data: The data collected on nodule number per plant, shoot dry weight, leaf area index, wilting index, pod numbers, pod yield or weight and grain yield were subjected to analysis of variance (ANOVA) using SAS (version 8.0) and Least Significant Difference (LSD) at P≤ 0.05 used to separate treatment means of significant treatments.

RESULTS

Soil analytical: The chemical characteristics of soil in the farm was analysed and results tabulated and indicated in table 4.1.

***Comparison of days to germination*:** The legumes varied significantly (P≤0.5) for days to seed germination (Fig.4.1). About 27% of Cowpeas (K80) germinated on 3rd day while only 5% of Green grams (KS20), 0.5% of Dolichos lablab (1001) and no beans germinated by that day. On the 4th day, 93% of planted Cowpeas (K80) had germinated while 66.2% of G/grams (KS20) and Beans (30.4%) had lowest seeds that had germinated. Cowpeas took shorter period of days to germinate followed by Green grams, Lablab and Beans in that order (Fig. 4.1).

Effects of water stress on leaf area index: The genotypes varied significantly (P≤0.05) for leaf area index (Fig. 4.2 Appendix 1 & 11). Plants which were not stressed had larger leaf area index compared to the plants which were stressed.

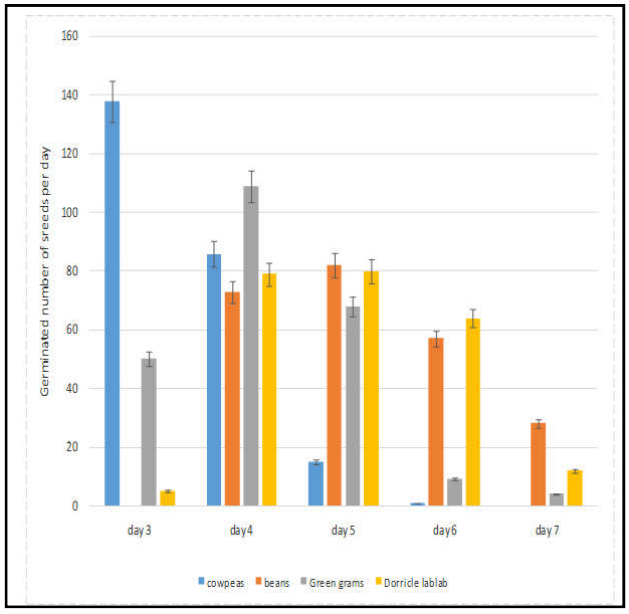

Figure 4.1. Mean germination of seeds in different legumes per day

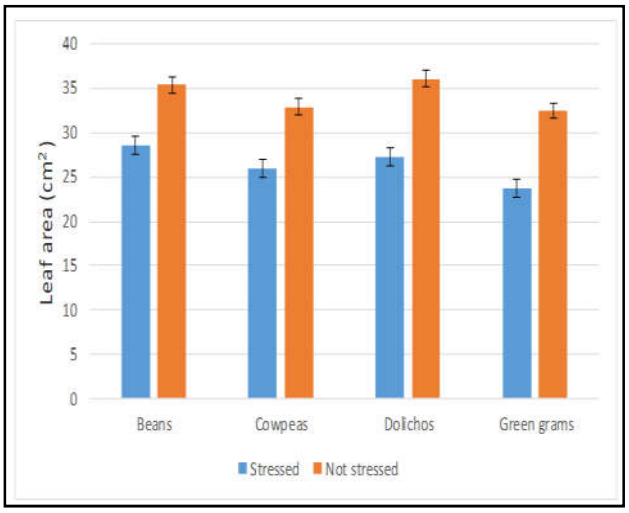

Figure 4.2 Mean leaf area index of stressed and unstressed legumes

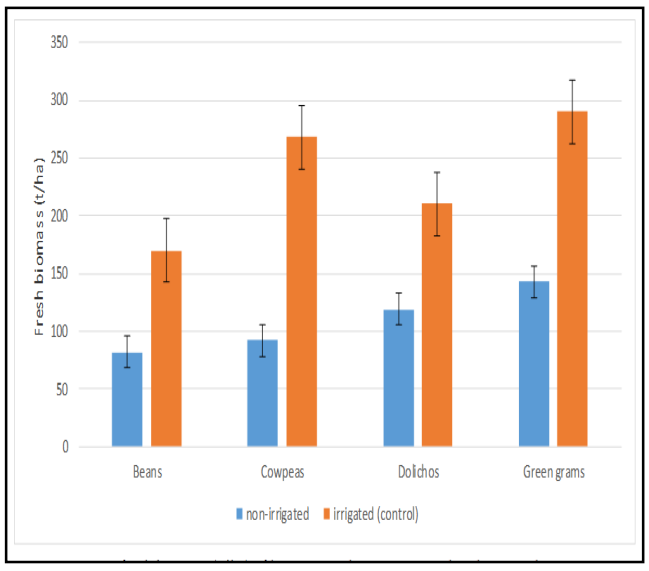

Figure 4.3 mean fresh biomass (t/ha) of legumes under non irrigated and irrigated treatments

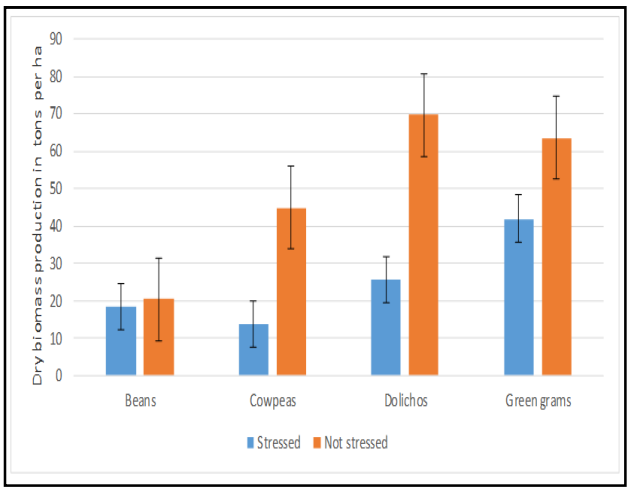

Figure 4.4. Mean dry biomass production per hectare

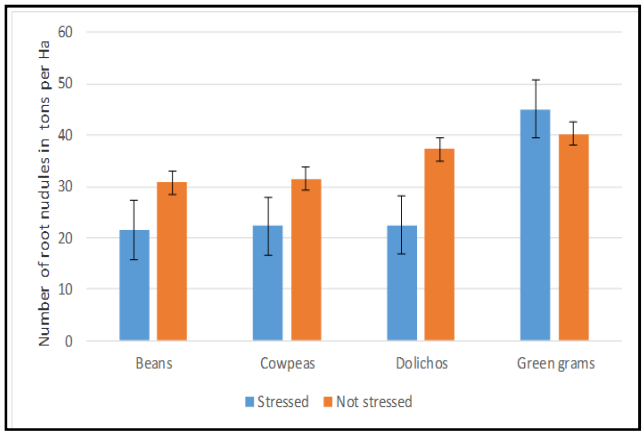

Figure 4.5. Mean number of root nodules in tons per ha

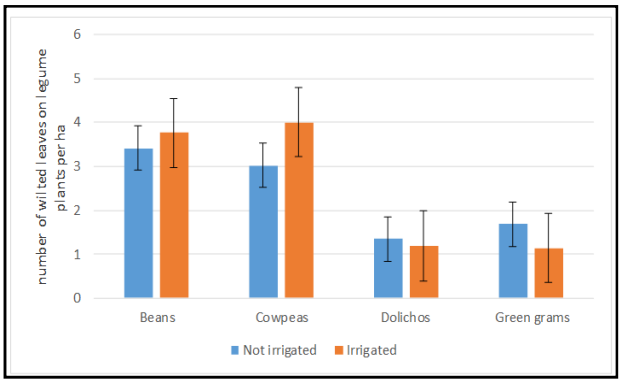

Figure 4.6 Mean number of wilted leaves on legume plants per hectare

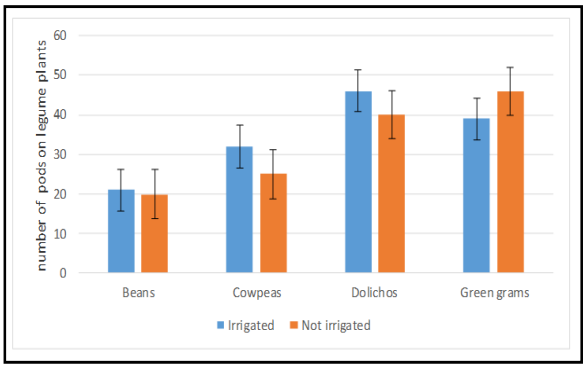

Figure 4.7. Mean number of pods on legume plants

Table 1.1. Scoring scale for above ground symptoms for wilting

Severity score	Severity Rating	Visual characteristics
1	Normal (not wilted)	*No signs of wilting* or drought stress
2	Slightly wilted	*Slight leaf angle* changes but no folding, rolling or changes in leaf surface structure
3	Wilted *Strong*	*leaf angle change* or *protrusion of veins on the leaf surface* but no cell death
4	Severely wilted	Very strong change of leaf angle or protrusion of veins on the leaf surface with *beginning necrosis*
5	Nearly dead	*Most leaves necrotic*, some young leaves still green near the midrib, leaf angles mostly near 0◦
6	Dead *All above*	*ground parts dead*, no re-sprouting after re-watering at the end of the experiment

Table 4.1 Soil chemical analysis for the farm

Parameter	Value	Class
Soil pH	4.4	Adequate
Acidity me %	5.90	Adequate
Total nitrogen %	0.34	Adequate
Organic carbon %	0.62	Adequate
Phosphorus ppm	108.2	Adequate
Potassium me %	1.45	Adequate
Calcium me %	7.90	Adequate
Magnesium me %	4.25	Adequate
Manganese me %	1.2	Adequate
Copper ppm	3.8	Adequate
Iron ppm	35	Adequate
Zinc ppm	8.6	Adequate
Sodium me %	0.85	Adequate

ppm = o parts per million; me% = metal percentage in the soil

Results indicated that beans and Dolichos had the largest leaf area index followed by cowpeas and green grams in that order.

***Effects of water stress on fresh biomass*:** The genotypes varied significantly (P≤0.05) in the production of fresh biomass (Fig. 4.3; Appendix 1V). Legume plants which were well watered had higher fresh biomass production compared to water stressed plants. Results showed that Green grams had the highest biomass production, cowpeas and Dolichos were not significantly (P≤0.05) different from each other. Beans had the lowest biomass production.

Effects of water stress on dry biomass production: There was a significant (P≤0.05) difference in production of dry biomass by the genotypes (Fig. 4.4 Appendix III). Genotypes which were not water stressed produced significantly higher dry biomass compared to the genotypes, which were not water stressed. Results reviewed that Green grams and Dolichos had the highest dry biomass and were not significantly (P≤0.05) different from each other. Beans and cowpeas were not significantly (P≤0.05) different from each other and their dry biomass production was lower than Green grams and Dolichos.

Effects of water stress on number of root nodules: There was a significant (P≤0.05) difference between droughted plants versus well watered plants in the number of root nodules in the respective roots of the genotypes (Fig. 4.5; Appendix VI). Unstressed genotypes had significantly more root nodules compared to stressed genotypes. Overall results reviewed that Green grams had the highest root nodules compared to other genotypes followed by Dolichos, cowpeas, and beans which were not significantly (P≤0.05) different from each other.

Effects of water stress on wilting index on legume varieties: Legumes varied significantly (P≤0.05) for leaf wilting. Overall, Beans and Cowpeas had the highest number of number of wilted leaves and were not significantly (P≤0.05) different from each other followed by Dolichos and green grams in that order. The irrigated plants showed significant (P≤0.05) differences in the number of wilted leaves with Beans having significantly (P≤0.05) the highest number of wilted leaves while green grams had the lowest number (Fig. 4.6). Those that were not water stressed showed significant differences in the number of wilted leaves with cowpeas having the highest number of wilted leaves while green grams had the lowest number of leaves, which wilted (Fig. 4.6).

Effects of water stress on number of pods on legume plants: Legumes varied significantly (P≤0.05) in the number of pods produced per treatment. Overall, Dolichos had the highest number of pods followed by cowpeas and green grams, which were not significantly different from each other (Fig. 4.7). Beans had the least number of pods. In irrigated plants. Dolichos had the highest number of pods while Beans had the least number of pods. The non-irrigated genotypes, Dolichos had the highest number of pods while beans had the least number of pods (Fig. 4.7).

Effect of water stress on grain yield: Cowpeas (K80) under irrigation produced significantly the highest yield (1.18 tons) followed by G/grams (KS20) Dolichos lablab (1001) and Beans (KAT 56) producing the lowest yields per plot (Fig. 4.8). Under water stress plot, Green grams had the highest yield (1.2 tons) followed by Cowpeas (K80) ,Dolichos lablab(1001) and Beans (KAT 56) was significantly affected by water stress to give lowest grain yield (Fig. 4.8).

DISCUSSION

Comparison of germination (days) between seeds of different legumes: The significant differences observed for days to germination in different legume species could be attributed to differences in the permeability of their seed coats. This is in agreement with the recent study conducted by Mwami *et al*. (2017) as well as earlier studies such as Baskin (2005) and Borji *et a*l., (2007) who attributed the differences in seed germination of different legumes to differences in seed coat permeability. From the results, it was deduced that the seed coat of cowpeas was more permeable and thus took shorter period to germinate compared to those of green grams, lablab and beans.

Fast germinators will take the advantage of the available moisture, nutrients and nitrogen flush besides reaching maturity early to evade moisture stress.

Effects of water stress on leaf area: The observed differences between leaf area of stressed plants and unstressed plants could be due to the role of water in the translocation of plants nutrients within the plant. This is in agreement with an earlier study by Gunton and Everson (1980) that attributed the differences in the leaf area of stressed and unstressed legumes to the nature of the environment, in regard to access to water, where the genotypes grew. Some studies on the transport of recent assimilates under water stress within the plants from leaves to sink organs (Li et al..,2003)found the translocation of newly assimilated carbon from source leave to be delayed under severe water stress. Differences in leaf area among different legumes could be related to the genotypical differences among the legumes as observed by Baskin (2005.in the present study, under water stressed conditions, Dollicles leaves had the largest leaf area an indication that it had larger surface area for photosynthesis and respiration which lowers the atmospheric temperature around the leaf.

Effects of water stress on fresh and dry biomass: Different quantities of biomass produced by legumes could be attributed to genotypical differences among the legumes. This is supported by a previous study by Asfaw (2014) and porch *et al*., (2009) who attributed differences in biomass production among bean varieties to legumes inherent characteristics. Besides genetic characteristics, as a key factor for different biomass production, water could be cited as a factor which determined the differences in biomass production, as it plays a role in nutrients mobilization especially nitrogen which aids in biomass production. A study conducted by Mitova and Stancheva, (2013) attributed differences in biomass production to the role of water in mobilizing plants' nutrients. In this study, under droughted plants, Green grams had the highest volumes of fresh and dry biomass production followed by cowpeas, Dolichos and Beans had the lowest biomass production. This implies that green gram is likely to give better yields under water stressed conditions because of high dry biomass as supported by Lee (2018) in his study which indicated a higher positive correlation between biomass and yield of crops.

Effects of water stress on number of leaves: Different number of leaves between stressed and unstressed legumes could be linked to differences in translocation of organic compounds which is necessitated by water. A study by Lazana *et al*., 2006 attributes differences between stressed bean genotypes and unstressed bean genotypes to differences in the translocation of organic compounds within the plant. Genetic differences could be cited as a factor responsible for differences in the number of leaves produced by bean legumes. A study conducted by porch *et al*., (2009) relates genetic differences as responsible for differences in the number of leaves produced by legumes. Under water stressed conditions, legumes had lower number of leaves compared to those of well watered conditions, reduction of number of leaves under droughted conditions can be explained as a, This implies that, legumes mechanism of avoiding excessive transpiration or water conservation strategy as supported by studies conducted by Jones, (1992).

Effects of water stress on number of wilted leaves: There was a significant difference in the number of wilted leaves between stressed and unstressed legume species. The observed trend could be due to the fact that drought or water stress prevents water movement from the root zone to other plant parts such as leaves. This concurs with previous studies (Hsiao 2000; Rahdari and Hoseini, 2012; Selvakumar *et al*., 2012) which observed that drought or water stress prevents the movement of water soluble nutrients up the plant and this leads to wilting of leaves. A similar study carried out by Jaleel *et al*., (2009) reported that wilting of leaves in legumes was due to unavailability of water in the root zone. Differences in number of wilted leaves among the legumes in water deficit environment and well-watered environment could be attributed to differences in some inherent and environmental factors leading to differences in their adaptations. This agrees with the study conducted by White (2005) who attributed the differences in the number of wilted leaves of bean genotypes to in both water deficit and well-watered environment to their genetic pool. Under water stressed conditions, Green grams had the least number of wilted leaves implying that it had higher photosynthetic area compared to other legumes and was drought tolerant, beans had highest wilted leaves thus drought susceptible. Cowpeas had the highest number of wilted leaves in well watered conditions, while green grams had the lowest number of leaves, which wilted, wilting reduces photosynthetic area according to Wang (2018).

Effects of water stress on root nodules: The observed differences in the number of nodules among the stressed and unstressed legumes could be due to genotypical differences. This is supported by a previous study conducted by Jaleel et al. (2009) which indicated that the differences in root nodules was due to genotypical differences existing among the bean genotypes. The reduction in root nodules in stressed legumes could be due to reduced nitrogenase activity within the plants. This is in agreement with two studies (Guerin et al., 1990; Kaur et al., 1985) that found out that reduced nodulation in the stressed legumes was as a result of low nitrogenase activity which resulted from low leaf water potential in water stressed legumes. Under drought stress green grams had the highest root nodules while beans had the least implying that green grams nodulated the most according to studies conducted by Janet and Sprent(1972) who noted a negative correlation between water stress in nitrogen fixation and root nodules. Further, according to studies done by Janet et al., (1972) indicated that reduced water content in the root nodules reduced nitrogen fixation by legumes. This implies that, green grams was drought tolerant due to high root nodules compared to beans which was susceptible to drought.

Effect of water stress on grain yield: The observed differences among water deficit and well-watered bean legumes could be attributed to the differences to their genetic and environmental adaptability. This agrees with the previous studies conducted by Akcura (2011) and Ali and Shakor (2012) who cited genotype and environmental interaction as a key factor determining differences in yields from water stressed and well watered environments. Peymaninia *et al*. (2012) noted variation in yields between water stressed and well watered environment due to environmental variation such as soil water which is essential in plants' nutrients movement. Under water stressed condition. Under water stressed conditions, green grams had the highest grain yield while under well-watered condition cowpeas had the highest grain yield, beans having the lowest grain yield, these differences agrees with latest study conducted by Jinpeng Li et al, (2018) who found out that irrigation

improved grain yield by increasing the uptake and utilization of water and nitrogen during grain filling.

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APPENDICES

Appendix 1-Leaf area analysis of variance for leaf 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	807.0	269.0	0.69	0.557
Treatment	1	842.2	842.2	2.18	0.142
Genotype.Treatment	3	10813.2	3604.4	9.31	<.001
Residual	152	58841.7	387.1		
Total159 71304.0					

Appendix II-Leaf area analysis of variance for leaf 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	25946.5	8648.8	15.26	<.001
Treatment	1	426.1	426.1	0.75	0.387
Genotype.Treatment	3	26660.5	8886.8	15.68	<.001
Residual	152	86173.9	566.9		
Total159 139207.0					

Appendix III-Dry mass analysis of variance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	1517.31	505.77	7.79	<.001
Treatment	1	112.90	112.90	1.74	0.189
Genotype.Treatment	3	314.70	104.90	1.62	0.188
Residual	152	9863.94	64.89		
Total159 11808.84					

Appendix IV-Fresh mass analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	32771.2	10923.7	13.79	<.001
Treatment	1	6616.5	6616.5	8.35	0.004
Genotype.Treatment	3	9029.7	3009.9	3.80	0.012
Residual	152	120388.2	792.0		
Total159 168805.5					

Appendix V-Number of leaves analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	984.319	328.106	48.36	<.001
Treatment	1	16.256	16.256	2.40	0.124
Genotype.Treatment	3	19.169	6.390	0.94	0.422
Residual	152	1031.350	6.785		
Total	159	2051.094			

Appendix VI-Root nodules analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	1696.82	565.61	16.12	<.001
Treatment	1	110.56	110.56	3.15	0.078
Genotype.Treatment	3	179.72	59.91	1.71	0.168
Residual	152	5334.15	35.09		
Total	159	7321.24			

Appendix VII-Pods analysis of variance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	50030.72	16676.91	661.65	<.001
Treatment	1	2287.66	2287.66	90.76	<.001
Genotype. Treatment	3	12154.22	4051.41	160.74	<.001
Residual	152	3831.15	25.20		
Total	159	68303.74			

Appendix VIII-wilting indexleaf analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	118.0688	39.3563	47.27	<.001
Treatment	1	31.5063	31.5063	37.84	<.001
Genotype. Treatment	3	20.3688	6.7896	8.16	<.001
Residual	152	126.5500	0.8326		
Total	159	296.4938			

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