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EFFECTS OF MOISTURE STRESS LEVELS AT DIFFERENT GROWTH STAGES ON NODULATION AND NITROGEN FIXATION IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) GENOTYPE

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Abstract

Moisture stress is among the limiting factors to crop yields. The objective of this study was to determine the effects of moisture stress imposed at different growth stages of bean plants on nodulation and nitrogen fixation. The experiment was conducted in greenhouse and in the field, at Sokoine University of Agriculture. The bean genotype "Kijivu" was used, the stages were; (i) VC (Cotyledonary and unifoliolate leaves visible), (ii) V2 (Second trifoliolate leaf unfolded), (iii) V4 (Fourth trifoliolates on the main stem, blossom clusters not opened) and (iv) R2 (Pods 1/2 inch long. Irrigation treatments were initiated to maintain moisture treatments of 100%, 75%, 50%, or 25% of the soils field capacity for each plant growth stage until plant maturity. Moisture stress significantly affected nodulation, nitrogen fixation, and finally grain yields. Numbers of nodules per plant were reduced by 56.0% in greenhouse and 69.2% in the field between V4 and VC at 25% moisture regime. Shoot biomass was reduced by 40.8% and 26.8% while root biomass was reduced 23.5% and 31.5% in greenhouse and field, respectively. These results suggest that for maximum nodulation and nitrogen fixation to be achieved, moisture stress must be avoided at the VC and V2 growing stages.

Keywords: Common bean, growth stage, N_2-fixation, moisture stress

1. INTRODUCTION

Biological nitrogen fixation is one of the most important sources of nitrogen in the production of leguminous crops (Mohammadi *et al*., 2012). A symbiotic relationship between rhizobia and legume plants can provide large quantities of N to the plant. Kimura *et al*. (2004) estimated contributions of N from N_2 fixation to range from 24 to 50% in field grown beans at different growth stages. The factors which can determine the amount of N_2 produced from legumes are the type of species of legume grown, the percentage of N_2 in the plant tissue and total biomass produced, hence, environmental circumstances that bound legume growth, such as moisture stress

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can reduce the amount of N_2 produced (Hirel *et al*., 2011). Marginal lands with low rainfall are among most problematic environments for rhizobia. The modification of rhizobial cells by water stress will eventually lead to a reduction in infection and nodulation of legumes (Worrall and Roughley, 1976). The rate of inhibition of the symbiosis depends on the stage of growth and development, as well as the severity of the stress (Williams and De Mallorca, 1984).

The production of common beans is undertaken commonly under rain-fed conditions; therefore, inadequate or unpredictable rainfall may limit the yields (Acosta-gallegos and Domingo, 2009). Erratic water availability during production exposes plants to water stress during every growth stage (Thangavel *et al*., 2011). About 60% of the common bean yield losses have been reported to be due to drought (Molina *et al*., 2001). Common bean is known as a plant that is vulnerable to water deficits, especially in pre-flowering and reproductive periods, producing significant negative impacts on seed yields (Singh, 2007). During dry spells, there is a decrease in populations of rhizobia which can be associated with poor nodulation of legumes, resulting in low levels of N_2 fixation and ultimately low yields (Mohammadi *et al*., 2012).

Moisture stress, which results from periodic dry spells during the growing season, is among the limiting factors for common bean production worldwide (Boutraa and Sanders, 2001). It influences symbiotic interactions by inhibiting growth, survival and metabolic activity of nitrogen fixing bacteria and ultimately growth of plants (Werner and Newton, 2005). Periods of water stress during the reproductive phase have been reported to be associated with significant reductions in nodulation and subsequently grain yields (Emam and Seghatoleslam, 2005).

The factors that influence growth, survival and metabolic activity of nitrogen fixing bacteria and plants and their ability to forge efficient symbiotic interactions include low pH, nutrient availability, temperature and low moisture status (Werner and Newton, 2005). Nitrogen fixation under P deficiency is often reduced, because low P supply induces changes in the relative growth of nodules and shoots (Hogh-Jensen *et al*., 2002). Nodules require relatively higher amounts of P and energy than do other plant tissues (Olivera *et al*., 2004).

A number of studies have reported the effects of water stress environment on growth of common bean. Molina *et al*. (2001) found that water deficits during flowering stages and beginning of pod development reduced most of the growth characteristics assessed. Getachew (2014) reported that flowering and pod filling stages of the crop are highly sensitive to drought, resulting in yield reductions. Kalima (2013) reported that the severity of effects of water stress depend on many factors, such as stage of development of the plant, duration and degree of stress. The influence of soil water activity on plant growth and hence nodulation in relation to growing stages should be taken into consideration especially in this era of climate change where many areas are becoming drier. The stage of the crop growth during which drought occurs can result in profound effects on nodulation and nitrogen fixation.

However, few investigations have studied the effects of moisture stress on nodulation and nitrogen fixation at early stages of development. Therefore, the objective of this study was to determine the effects of moisture stress at different growth stages of bean plants on nodulation and nitrogen fixation, beginning with early stages of plant development, so as to select best time for prediction of adverse water stress effect which could assist in developing mitigation measures.

2. MATERIAL AND METHODS

2.1. Experimental design

The experiment was conducted in a green-house and field, at Sokoine University of Agriculture (SUA), Latitude 6°45'S, Longitude 37°40'E, at the altitude of 547 meters above sea level. Maximum and minimum temperature was 27.6^{0}C and 17.9^{0}C respectively and 86.8% Relative

Humidity. The soil was (66.7% sand, 30.3% clay and 2.92 silt), pH in water 5.92, CEC 15.2 meq/100g, N 0.22%, P 5.80 ppm and B 0.49 ppm. The design of the experiment in green house was the Completely Randomized Design (CRD), with four replications. In the field the experiment was laid in Completely Randomized Block Design (CRBD) with three replications. The bean genotype "Kijivu" was used to assess the effects of water stress at different plant growing stages, which were: (i) Stage VC (Cotyledonary and unifoliolate leaves visible), (ii) Stage V2 (Second trifoliolate leaf unfolded), (iii) Stage V4 (Fourth trifoliolates on the main stem, but with blossom clusters still not visibly opened) and (iv) R1 (Pods 1/2 inch long at first blossom position (node 2 to 5 for most plants).

2.2. Plant establishment

Four-litre plastic pots were filled with 4 kg of field soil. TSP fertilizer at the rate of 60kg P/ha was incorporated into the soil prior to planting. Four bean seeds inoculated with NITROSUA rhizobia inoculant (10^8 cells/ml) were sown per pot 5 cm deep at 10 cm apart, later thinned to 2 seedlings per pot after germination. In the field, each plot had 2 rows of 4 m long and the plant spacing was 50 cm and 10 cm between and within rows, respectively

2.3. Imposition of different levels of moisture stress

Soil field capacity was calculated on soil dry basis. The soil moisture for all pots was initially maintained at field capacity (100% moisture regime). Then, for each of the growing stages specified above, irrigation treatments were initiated and maintained at moisture stress treatments of 100%, 75%, 50%, or 25% of the soil's field capacity for each plant growth stage until plant maturity.

2.4. Plant sampling and assessment

After flowering/early pod formation, plants from one set of pots covering all moisture stress levels were carefully dug out, washed free of soil, and nodules were detached and counted. Root and shoot biomass were dried and weighed. Other data collected were plant height, root length, and grain yield at maturity. Assessment of effectiveness of nodules was done by detaching randomly 30 nodules from each treatment cutting them open and observing the pink coloration and reporting as percentage effective. A score of 1 or 2 was used, where 1 = very pink (very effective), 2 = not pink (other colour, thus not effective).

2.5. Statistical analysis

Statistical analysis was carried out using the GenStat Statistical Package. Two way analysis of variance was performed and treatment means were compared using the Least Significance Difference (LSD) at the 5% level of significance.

3. RESULTS AND DISCUSSION

3.1. Effects of moisture stress and stage of growth in bean performance: Analysis of variance (ANOVA) summary

Summary of analysis of variance for the studied variables is shown in table 1 and 2 from green house and field experiment respectively. Significant differences between moisture stress levels were observed for number of nodules/plant, % effective nodules, root length and yield while growth stages also displayed significant variation in number of nodules/plant, % effective nodules and root biomass both in green house and in the field. Significant effects also observed on interaction of moisture stress levels and growth stage on number of nodules/plant and plant height in green house.

Table 1: Analysis of variance (ANOVA) (mean squares) from green house

Source of variation	df	Number of nodules/plant	% effective nodules	Plant height	Root length	Shoot biomass	Root biomasss	Shoot N%	Seed N%	Yield
Rep	3	294.02	470.22	347.10	26.10	10.33	0.063	0.015	0.012	10.33
Stress	3	314.06***	482.41***	330.10ns	54.52***	0.19*	0.006ns	0.189**	0.046**	0.19**
Stage	3	419.56***	1886.39***	136.50ns	23.76*	2.18**	0.004ns	0.617**	0.507**	2.18***
St x Sg	9	8.84*	72.68ns	423.90**	12.10	0.62	0.005ns	0.027*	0.040**	0.62
Resid.	45	13.45	73.71	174.20	7.71	0.59	0.006	0.012	0.013	0.59

St = Stress; Sg = Stage

Table 2: Analysis of variance (ANOVA) (mean squares) from the field

Source of variation	df	Number of nodules/plant	% effective nodules	Plant height	Root length	Shoot biomass	Root biomass	Yield
Rep	2	15.75	43.40	4.99	26.40	27.66	0.22	905.8
Stress	3	775.14***	2487.19***	26.70*	14.14ns	374.63***	0.85***	4357.4***
Stage	3	1371.81***	2651.79***	15.43ns	6.25ns	20.33ns	0.41*	200.4ns
St x Sg	9	121.90*	57.36ns	5.64ns	10.23ns	49.74ns	0.12ns	329.4ns
Residual	30	62.73	49.05	10.71	10.46	42.97	0.12	285.1

St = Stress; Sg = Stage

3.2. Main effect of moisture stress levels

The results from this study indicates that moisture stress has a substantial influence on common bean growth as all studied variables were reduced with increased moisture stress level (Table 3 and 4). The 25% moisture stress level showed the greater impact of reduction both in green house and in the field. This indicates that common bean is a sensitive plant to high moisture stress level (Emam *et al*., 2010). Moisture stress significantly affects nodulation, nitrogen fixation and finally yield. This may be because moisture stress causes biochemical and physiological changes hence affecting plant growth and development (Boutraa, 2010). It causes suppression in plant photosynthetic effectiveness (Lawlor and Cornic, 2002). Moisture stress is known to affect the physiological processes of plants as water is the primary component of actively growing plants and has a principal role in plant nutrient transport, chemical and enzymatic reactions.

Table 3: Main effect of moisture stress levels in green house

Moisture stress level %	Nodules/ Plant (no)	% Efficient nodules	Plant Height (cm)	Root Length (cm)	Root Biomass (g)	Shoot Biomass (g)	Yield/ plant (g)	Shoot N%	Seed N%
100	18.3b	66.1b	114.2b	15.0b	0.30b	4.69a	5.1b	2.62b	3.30b
75	18.1b	62.8b	111.9ab	14.8b	0.23ab	4.54a	4.5ab	2.58b	3.29b
50	15.6ab	56.5ab	111.4ab	14.8b	0.20ab	3.80b	4.2ab	2.56b	3.23ab
25	10,9a	55.1a	103.7a	11.2a	0.15a	3.63b	3.4a	2.38a	3.19a
Mean	15.7	60.1	110.3	13.9	0.21	4.20	4.40	3.25	3.25
CV%	24.3	16.9	4.2	9.2	17.4	21.4	15.6	4.4	3.5
SE	2.15	4.5	3.3	0.7	0.02	0.2	0.32	0.03	0.03

Different letters within each column indicate significant difference at 5% level

Table 4: Main effect of moisture stress levels in the field

Moisture stress level %	Nodules/ Plant (no)	% Efficient nodules	Plant Height (cm)	Root Length (cm)	Root Biomass (g)	Shoot Biomass (g)	Yield/plant (g)
100	21.8c	67.2b	38.9b	17.6a	1.2c	32.6b	84.7c
75	17.5bc	66.7b	36.8ab	16.5a	1.0bc	28.7b	78.7bc
50	17.5bc	61.4b	36.4ab	15.9a	0.8b	27.1b	66.9b
25	9.5a	44.0a	35.5a	15.1a	0.5a	19.3a	41.6a

Mean	16.6	59.8	36.9a	16.3	0.9	26.9	68.0
CV%	22.1	14.4	8.9	7.9	13.1	4.9	11.1
SE	0.9	2.2	1.0	0.9	0.1	1.9	4.9

Different letters within each column indicate significant difference at 5% level

3.3. Main effect of growth stages

The results from this study indicated that moisture stress imposed at different growth stages of common bean plant significantly affected number of nodules per plant, percentage of effective nodules, root biomass, shoot biomass and eventually yield (Table 5 and 6). Growth stage caused a reduction in almost all studied variables and the greater reduction occurred when moisture stress was imposed on VC and V1 growth stages. Manjeru *et al*. (2007) reported that yield was reduced most when water stress was imposed during the flowering and post flowering stages. At VC stage bean plant requires adequate water for root development. In adequate moisture during early stages result in reduced number of nodules per plant, percentage of effective nodules and biomass yield which eventually reduce final yield (Efetha, 2011).

Table 5: Main effect of growth stage in green house

Growth stage	Nodules/ Plant (no)	% Efficient nodules	Plant Height (cm)	Root Length (cm)	Root Biomass (g)	Shoot Biomass (g)	Yield/ plant (g)	Shoot N%	Seed N%
VC	13.4a	55.6a	106.4a	12.9a	0.16a	3.1a	3.8a	2.38a	3.11a
V1	13.8a	58.6a	109.7a	13.3a	0.18a	3.6ab	4.2ab	2.35a	3.08a
V4	18.8b	66.1b	112.5a	13.9ab	0.18a	3.7ab	4.5ab	2.67b	3.41b
R1	16.9b	60.2b	112.6a	15.7b	0.25b	4.1b	5.1b	2.73b	3.41b
Mean	16.9	60.1	110.3	13.9	0.18	3.6	4.36	2.54	3.25
CV%	24.3	16.9	12.0	9.2	17.4	21.4	15.6	4.4	3.5
SE	2.2	4.5	3.3	0.7	0.02	0.2	0.3	0.03	0.03

Different letters within each column indicate significant difference at 5% level

Table 6: Main effect of growth stage in the field

Growth stage	Nodules/ Plant (no)	% Efficient nodules	Plant Height (cm)	Root Length (cm)	Root Biomass (g)	Shoot Biomass (g)	Yield/plant (g)
VC	10.3a	51.7a	35.4a	16.2a	0.6a	25.5a	65.5a
V1	17.0b	61.0b	36.6a	15.4a	0.8ab	26.6a	65.7a
V4	18.8bc	63.1b	37.5a	16.4a	1.0b	27.1b	67.3b
R1	20.3c	63.4b	38.0a	17.2a	1.1b	28.6b	74.1c
MEAN	16.6	59.8	36.9	16.3	0.89	26.9	68.0
CV%	22.1	11.3	8.9	7.9	13.1	4.9	11.1
SE	0.9	2.2	0.9	0.9	0.1	1.9	4.9

Different letters within each column indicate significant difference at 5% level

3.4. Effect of moisture stress levels at different growing stages on nodulation

In the greenhouse, there was a significant increase ($P \leq 0.05$) in number of nodules per plant with advancing plant growth stages while nodulation was significantly ($P = 0.05$) reduced in the 25% moisture regime (Table 7). Generally, higher numbers of nodules were observed from plants that received 100% moisture regime. The least numbers of nodules were observed from plants that received 25% moisture regime, with the fewest nodules (5.5) obtained when moisture stress was imposed beginning the VC growth stage.

Table 7: Effect of moisture stress levels at different growth stages on number of nodules/plant in green house

Growing stage when Stress imposed	100% moisture regime	75% regime	50% regime	25% regime	Nodules/plant Mean
VC	24.6	9.5	11.3	5.5	12.7
V2	24.8	19.8	18.8	8.3	17.9
V4	25.8	21.8	21.3	12.5	20.4
R1	25.3	21.8	21.3	12.5	20.4
Mean	25.1	18.2	18.2	9.7	

LSD within Table is 5.2, SE 4.3

In the field, there was also a significant increase ($P \leq 0.05$) in number of nodules per plant with advancing plant growth stages only at the 75% moisture regime (Table 8). There were significant ($P = 0.05$) reductions in nodulation as moisture stress was increased, with all plant growth stages affected at the 50% and 25% moisture regimes (Table 8). The least number of nodules (4.0) was observed from plants when moisture stress was imposed beginning at the earliest plant growth VC.

Table 8: Effect of moisture stress levels at different growth stages on number of nodules/plant in the field

Growing stage when Stress imposed	100% moisture regime	75% regime	50% regime	25% regime	Nodules/plant Mean
VC	36.7	7.7	7.0	4.0	13.9
V2	43.3	18.0	10.7	8.0	20.0
V4	39.3	32.7	17.7	13.0	25.7
R1	36.7	29.7	18.3	8.3	23.3
Mean	39.0	22.0	13.4	8.8	

LSD within Table is 13.2, SE 1.8

These results show that the magnitude of the moisture stress effects depended on the growth stage of the crop at the time the moisture stress was initiated. When moisture stress was imposed to plants from earliest stage (VC), the plants produced few numbers of nodules per plant compared to when moisture stress was imposed in the later V4 or R1 stages. These observations imply that moisture stress was a limiting factor for proper initiation and development of nodules. Plants that received higher amounts of water from the beginning of the development of plants (VC) produced more root nodules. According to Werner and Newton (2005), water status influences survival and metabolic activity of nitrogen fixing bacteria and plants and their ability to enter into symbiotic interactions. Leung and Bottomley (1994) also reported that low soil water potentials, occurs during periods of moisture stress, inhibit growth of rhizobia and nodulation.

These results are in agreement with those by Onuh and Donald (2009) who reported reduced numbers of nodules in cowpea plants due to moisture stress imposed two weeks after planting. At all growth stages nodule numbers were reduced with increased moisture stress. In greenhouse the reduction of numbers of nodules per plant between plant growth stages V4 and VC was 56.0% while in the field reduction was 69.2%. The relatively smaller reductions in nodulation in the green house as compared to greater reduction in the field may be due to the fact that greenhouse conditions are better controlled than the more open field condition and may be also due to higher evaporation in the field. Thus, moisture stress becomes exacerbated in the open field than under greenhouse conditions.

3.5. Effect of moisture stress at different growth stages on proportions of effective nodules

Results showed some significant increases (P ≤ 0.05) in proportions of effective nodules in advanced growth stages in the moisture stressed plants in the greenhouse (Table 9). However, there was a general decrease in the proportions of effective nodules with increase in moisture stress, with the decreases particularly significant (P = 0.05) at the 25% moisture regime (Table 9) for all growth stages.

Table 9: Effect of moisture stress levels at different growth stages on % effective nodules in the greenhouse

Growing stage when Stress imposed	% Efficient nodules				
	100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	71.1	57.4	56.9	37.0	55.0
V2	69.1	66.3	58.4	48.4	60.6
V4	71.8	72.8	61.1	50.0	63.9
R1	72.3	70.2	69.2	40.7	63.1
Mean	71.1	66.7	61.4	44.0	

LSD within Table is 12.2, SE 8.9

Similarly in the field, there was significant increase (P ≤ 0.05) on proportions of effective nodules with different growth stages in the moisture stressed plants (Table 10). The proportions of effective nodules decreased significantly (P = 0.05) particularly in the 25% moisture regime and for all plant growth stages (Table 10).

Table 10: Effect of moisture stress levels at different growth stages on effective nodules % in the field

Growing stage when Stress imposed	% Efficient nodules				
	100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	70.4	47.4	43.2	29.3	47.6
V2	69.3	69.6	61.2	37.0	59.3
V4	88.1	85.8	76.4	47.1	74.4
R1	80.3	75.0	65.9	37.2	64.6
Mean	77.0	69.5	61.7	37.7	

LSD within Table is 11.7, SE 4.3

The effective nodules were shown by pink coloration. The pink color is caused by the pigment lephemoglobin that controls oxygen flow to the rhizobia. According to these results, the plants that received enough moisture in growth stage V2 and V4 had more effective nodules. The life span of the rhizobia is short, between 4 and 5 weeks after infection (Dupont *et al*., 2012). Thus, enough moisture at VC, V2 and V4 is very vital for proper initiation and development of nodules. According to Fresneau *et al*. (2007), drought induces changes in some physiological and biochemical processes. At the R1 stage is the time of pod filling, annual legumes, including beans, lose the ability to fix nitrogen as the plant feeds the developing seed rather than the nodules (Manjeru *et al*., 2007) this may account for the reduction of numbers or proportions of effective nodules at that stage. The results from this study and other studies show that imposition of severe drought for example 25% moisture, at any stage of plant growth can lead to drastic reductions in the proportions of effective nodules.

3.6. Effect of moisture stress levels at different growth stages on grain yields

The results (Table 11) showed some significant decrease ($P \leq 0.05$) in grain yields as a result of increased moisture stress. In greenhouse, the greatest decrease ($P = 0.05$) were recorded in the 25% moisture regime. In the 50% moisture regime, yields were higher ($P = 0.05$) when the stress was imposed beyond the V4 growth stage as compared to that in the VC stage. The least yield (2.2 g/plant) was observed in plants that received 25% moisture regime when imposed at VC growth stage.

Table 11: Effect of moisture stress levels at different growth stages on grain yield in the greenhouse

					Yield(g/plant)
Growing stage when Stress imposed	**100% moisture regime**	**75% regime**	**50% regime**	**25% regime**	**Mean**
VC	4.2	3.8	3.6	2.2	3.5
V2	5.3	4.7	4.5	2.5	4.3
V4	6.0	5.7	5.4	3.6	5.1
R1	6.8	5.5	5.9	2.7	5.2
Mean	5.6	4.9	4.9	2.8	

LSD within Table is 1.8, SE 0.6

In the field, significantly ($P = 0.05$) lower grain yields were recorded in the 50% moisture regime, and even lower yields at the 25% moisture regime (Table 12) relative to those at the 100% moisture regime. In the 25% moisture regime, yields were higher when the stress was imposed beyond the V4 plant growth stages as compared to when the stress was imposed at the earlier VC stage.

Table 12: Effect of moisture stress levels at different growth stages on grain yield in the field

		Grain yield (kg/ha)			
Growing stage when Stress imposed	**100% moisture regime**	**75% regime**	**50% regime**	**25% regime**	**Mean**
VC	525.5	427.5	360.0	165.0	344.5
V2	495.0	472.5	452.5	235.5	413.9
V4	517.5	550.0	525.0	355.0	486.9
R1	532.5	570.0	530.0	367.5	500.0
Mean	517.6	505.0	466.9	280.8	

LSD within Table is 180.1, SE 9.8

These observations indicate that moisture is an important factor for crop yield, and that extreme moisture stress drastically reduces yields. These results agree with those of Manjeru *et al*. (2007) who reported reduced grain yields of common bean due to water stress imposed at different growth stages. Marino *et al*. (2007) and Liu *et al*. (2003) also reported that the drought effect on BNF is the most important environmental factor resulting in crop yield losses. The present results showed that V2, V4 and R1 are the crucial stages which need enough moisture so as to attain high yields. According to CIAT (2004), drought reduces pod formation, seed setting and seed filling by reducing assimilate production, translocation and partitioning, thereby resulting in yield reduction. In the 50% and 25% moisture regime, especially in the field, yield was not affected when stress was imposed in later stages than in earlier stages. This may be due to the fact that at later stages plants have already established, hence the effect of moisture stress has relatively little effect on yield. Moisture stress is among of the limiting factors in crop growth and reduce yield (Beebe *et al*., 2008).

3.7. Effect of moisture stress levels at different growth stages on plant height

In the greenhouse, plant heights were significantly reduced ($P \leq 0.05$) with increased moisture stress (Table 13) when the stress (25% moisture regime) was imposed at the very early stages of plant growth but not in later stages. Plants were significantly ($P = 0.05$) taller when the moisture stress (75% and 25% regime) was imposed at the V4 stage.

Table 13: Effect of moisture stress levels at different growth stages on plant height in the greenhouse

Growing stage when Stress imposed	Plant height (cm) 100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	109.8	98.4	96.9	81.1	96.6
V2	109.2	99.9	96.4	87.5	98.3
V4	115.8	121.9	98.1	119.6	113.9
R1	112.8	114.8	106.8	104.5	109.7
Mean	111.9	108.8	99.6	98.2	

LSD within Table is 18.8, SE 6.6

In the field, plant heights were higher ($P \leq 0.05$) when moisture stress was imposed at later stages (Table 14). Compared with the 100% or 75% moisture regime, plants in the 50% and 25% moisture regimes were significantly ($P = 0.05$) shorter when the stress was imposed at the early plant growth stages (VC and V2).

Table 14: Effect of moisture stress levels at different growth stages on plant height in the field

Growing stage when Stress imposed	Plant height (cm) 100% moisture regime	75% regime	50 regime	25% regime	Mean
VC	36.7	36.6	30.3	30.6	33.6
V2	35.6	40.1	31.3	31.5	34.6
V4	37.5	38.4	37.6	33.9	36.9
R1	37.8	40.8	37.2	36.8	38.2
Mean	36.9	38.9	34.1	33.2	

LSD within Table is 5.5, SE 1.9

The reduction of plant height with increased moisture stress may be due to the fact that under moisture stress the physiological activities of the plant divert from functions of cell elongation and growth to those for coping with drought by slowing down the rate of assimilation of biosynthates (CIAT, 2004). Ohashi *et al*. (2000) also observed the reduction of plant height due to inadequate moisture as a result of decreased photosynthesis and translocation. Reduced mitosis, cell elongation and expansion result in reduced plant height, leaf area and crop growth under drought (Kaya *et al*., 2006; Hussain *et al*., 2008; and Kalima, 2013). Plant heights were higher when moisture stress was imposed at later plant growth stages. This might be because at early stages of vegetative stage, moisture stress reduced synthesis of the photosynthetic pigment; hence, reduced growth and alterations in physiological parameters including plant height (Baroowa and Gogoi, 2012).

3.8. Effect of moisture stress levels at different growth stages on root length

There was no significant increase ($P \leq 0.05$) in root length due to reduced moisture stress in the greenhouse (Table 15). Plants which received 25% moisture regime at VC and V2 had shorter roots compared to others.

Table 15: Effect of moisture stress levels at different growth stages on root length in the greenhouse

	Root length (cm)				
Growing stage when Stress imposed	100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	13.8	14.0	15.8	9.0	13.2
V2	13.0	15.8	13.5	9.5	13.0
V4	12.8	15.3	15.0	10.3	13.4
R1	19.8	15.0	15.0	13.0	15.7
Mean	14.9	15.0	14.8	10.5	

LSD within Table is 3.9, SE 1.4

In the field, generally there was no significant change ($P \geq 0.05$) in root length due to growth stage and moisture stress. The only significant decrease in root length was observed in the 25% moisture regime when moisture stress was imposed very early in the VC stage (Table 16).

Table 16: Effect of moisture stress levels at different growth stages on root length in the field

	Root length (cm)				
Growing stage when Stress imposed	100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	18.7	16.3	16.3	13.3	16.2
V2	16.3	16.0	17.3	12.0	15.4
V4	18.7	15.7	14.3	17.0	16.4
R1	17.0	15.0	15.7	18.0	16.4
Mean	17.7	15.8	15.9	15.1	

LSD within Table is 5.4, SE 1.9

These results of reduced root length when stress imposed early are in agreement with those by Ranawake *et al*. (2011) who reported reduced root length due to water stress. Water stress impairs root cell development, nutrient uptake and affects photosynthesis; hence, affect root elongation (Guo *et al*., 2013; Dhole and Reddy, 2010). However, the result from the field showed no significant difference for most moisture stress treatments. This may be due to the fact that in the field there is a large rooting space; hence, plants tend to modify their root growth and grow longer as a mechanism for avoiding drought stress by scavenging a larger soil volume for water.

3.9. Effect of moisture stress levels at different growth stages on shoot biomass

There was a significantly higher ($P \leq 0.05$) shoot biomass when stress was imposed in the V4 growth stages (Table 17). At 25% moisture regime the high shoot biomass (3.8g) was observed when moisture stress imposed at the V4 and R1 growth stages relative to the earlier stages (Table 17).

Table 17: Effect of moisture stress levels at different growth stages on shoot biomass in the greenhouse

	Shoot biomass (g/plant)				
Growing stage when Stress imposed	100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	3.06	3.01	3.01	2.15	2.8
V2	3.05	3.05	3.01	2.75	3.0
V4	4.18	4.13	4.08	3.63	4.0
R1	3.70	4.03	2.90	3.79	3.6

Mean	3.50	3.56	3.25	3.08

LSD within Table is 1.1, SE 0.4

In the field, there was a significant decrease ($P \leq 0.05$) in shoot biomass due to increased moisture stress at the 25% regime imposed at the VC and V2 growth stages (Table 18). At 25% moisture regime the high shoot biomass (20.9 g) was observed in plants when stress was imposed at the V4 and R1 growth stages.

Table 18: Effect of moisture stress levels at different growth stages on shoot biomass in the field

Growing stage when Stress imposed	Shoot biomass (g/plant) 100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	23.9	25.9	24.3	15.3	22.4
V2	27.4	28.1	25.2	18.6	24.8
V4	31.0	35.2	31.9	20.9	29.8
R1	32.6	38.1	35.1	19.8	31.4
Mean	28.7	31.8	29.1	18.7	

LSD within Table is 10.9, SE 3.8

This result indicates that moisture is an essential influence for shoot development. Greater accumulation of plant biomass is an important input to assure total translocation of photosynthate materials to seed. Emam *et al*. (2010) and Rosales-Serna *et al*. (2004) also reported that plant dry weight was decreased significantly by increasing water stress. Moisture stress also reduced shoot dry weight in forage legumes (Fening *et al*. 2009). Moisture stress is known to reduce dry matter production by limiting plant growth (Emam and Seghatoleslami, 2005).

3.10. Effect of moisture stress levels at different growth stages on root biomass

In the field, there was significant decrease ($P \leq 0.05$) in root biomass due to moisture stress (25% regime) and growth stages (Table 19).

Table 19: Effect of moisture stress levels at different growth stages on root biomass in the field

Growing stage when Stress imposed	Root biomass (g/plant) 100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	0.93	0.63	0.63	0.50	0.67
V2	1.00	1.07	1.00	0.47	0.89
V4	1.23	1.87	0.57	0.50	1.04
R1	1.23	1.57	0.67	0.73	1.05
Mean	1.10	1.30	0.72	0.55	

LSD within Table is 0.6, SE 0.2

In greenhouse there was no significant change ($P \geq 0.05$) in root biomass at different growth stages due to moisture stress (Table 20).

Table 20: Effect of moisture stress levels at different growth stages on root biomass in the greenhouse

Growing stage when Stress imposed	Root biomass (g/plant) 100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	0.20	0.18	0.15	0.13	0.17
V2	0.21	0.15	0.20	0.15	0.18
V4	0.18	0.18	0.18	0.17	0.18
R1	0.23	0.23	0.23	0.13	0.21
Mean	0.21	0.19	0.19	0.14	

LSD within Table is 0.10, SE 0.04

The lack of significant reduction in root biomass implies that when the moisture stress is imposed at VC and V2 stages, the plants are still young and able to scramble for nutrients and moisture around the rhizosphere. During V4 and R1 growth stages plants have developed enough and can modify themselves by elongating their roots around the growth environment in the attempt to capture nutrients and moisture.

3.11. Effect of moisture stress levels at different growth stages on shoot and seed N%

The results showed significant decrease ($P \leq 0.05$) in amount of N accumulated in shoots and seeds when moisture stress is increased (Table 21 and 22). The reduction is higher (3.09) when the stress is imposed at the earliest plant growth VC and V1 stages. This may be due to the fact that drought tend to weaken the uptake of N in the plants. Nakayama *et al*. (2007) also found the decrease in N accumulation in the leaves of studied cultivars under drought. The moisture stress imposed during VC and V1 was more disadvantageous to N accumulation than that imposed during the R1 stage. Moisture stress reduces nitrogenase activity through reductions in the formation and function of nodules (Ramos *et al*., 1999). Report by Fresneau *et al*. (2007) showed that drought effects are associated with changes in physiological and biochemical processes.

Table 21: Effect of moisture stress levels at different growth stages on shoot N%

Growing stage when Stress imposed	Shoot N% 100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	3.49	3.15	3.10	2.97	3.18
V2	3.44	3.23	3.10	3.14	3.23
V4	3.51	3.49	3.53	3.25	3.45
R1	3.48	3.34	3.44	3.22	3.37
Mean	3.48	3.30	3.29	3.15	

LSD within Table is 0.16, SE 0.03

Table 22: Effect of moisture stress levels at different growth stages on Seed N%

Growing stage when Stress imposed	Seed N% 100% moisture regime	75% regime	50% regime	25% regime	Mean
VC	3.39	3.13	3.15	3.09	3.19
V2	3.51	3.34	3.25	3.09	3.30
V4	3.48	3.49	3.44	3.23	3.41
R1	3.44	3.35	3.53	3.24	3.39
Mean	3.46	3.33	3.34	3.16	

LSD within Table is 0.16, SE 0.03

4. CONCLUSIONS

Moisture stress levels no matter of the degree of its severity has the capacity to affect/reduce nodulation, nitrogen fixation, root and shoot biomass and finally yield at different growing stages. These results indicate that when stress is imposed at a late stage of plant development, the effect is low because the plants are already well developed and they can modify themselves to cope with stress compared to the case when stress is imposed at early stages. Hence, for maximum nodulation and nitrogen fixation to be achieved, moisture stress must be avoided at the early VC and V2 growing stages. Since the period of nodulation is short, timing for planting is of great importance to escape dry periods at these early stages, otherwise irrigation scheduling should be undertaken to favour these growth stages. The water stress levels applied may not represent other areas, hence, multi-location and multi-year experiment is recommended.

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Reference

Acosta-gallegos, J. A., & Domingo, M. (2009). Adaptation traits in dry bean cultivars grown under drought stress. *Features Adaptation bean varieties under drought*, 35, 416-425.

Baroowa, B., & Gogoi, N. (2012). Effect of induced drought on different growth and biochemical attributes of black gram (Vigna mungo L.) and green gram (Vigna radiate L.). *Journal of Environmental Research and Developement*, 6, 584-593.

Beebe, S. E., Rao, I. M., Cajiao, C., & Grajales, M. (2008). Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. *Crop Science*, 48, 582-592.

Boutraa, T., & Sanders, F. E. (2001). Influence of water determinate growth habit, such as D81083, might have stress on grain yield and vegetative growth of twopotential as a dryland rotation crop for most dryland areas cultivars of bean (*Phaseolus vulgaris*, L.). *Journal of Agronomy of Iran Crop Science*, 187, 251-257.

Boutraa, T. (2010). Improvement of water use efficiency in irrigated agriculture. *Journal of Agronomy*, 9, 1- 8.

CIAT, International Center for Tropical Agriculture (2004). Coping with drought: Strategies to improve genetic adaptation of common bean to drought prone regions of Africa. *CIAT Occasional Publication Series, Addis Ababa, Ethiopia.* 38, 1-38.

Dhole, V. J., & Reddy, K. S. (2010). Gamma rays induced moisture stress tolerant long root mutant in mungbean (*Vignaradiata*L Wilczek). *Electronic Journal of Plant Breeding*, 1(5), 1299-1305.

Dupont, L., Alloing, G., Pierre, O., El Msehli, S., Hopkins, J., Hérouart, D., & Frendo, P., (2012). *The legume root nodule: From symbiotic nitrogen fixation to senescence*. Tetsuji Nagata. pp. 953.

Efetha, A. (2011). Irrigation scheduling for dry bean in Southern Alberta. *Food research international*, 44(8), 2497-2504.

Emam, Y., & Seghatoleslami, M. J. (2005). *Crop yield: Physiology and processes*. First edition. Shiraz University Inc., Shiraz. pp. 593.

Emam, Y., Shekoofa, A., Salehi, F., & Jalali, A. H. (2010). Water stress effects on two common bean cultivars with contrasting growth habits. *American-Eurasian Journal of Agriculture and Environmental Science* 9(5), 495-499.

Fening, J. O., Quansah, C., & Sarfo-Kantanka, A. (2009). Response of three forage legumes to soil moisture stress. *Journal of Science and Technology,* 29(3), 24-30.

Fresneau, C., Ghashghaie, J., & Cornic, G. (2007). Drought effect on nitrate reductase and sucrose-phosphate synthases activities in wheat (*Triticum durum* L.): Role of leaf internal CO_2. *Journal of Experimental Botany*, 58, 2983-2992.

Getachew, M. (2014). Influence of soil water deficit and phosphorus application on phosphorus uptake and yield of soybean (Glycine max L.) at Dejen, North-West Ethiopia. *American Journal of Plant Sciences,* 5, 1889-1906.

Guo, R., Hao, P., Zhi Gong, D., Li, Z., & XueGu, F. (2013). *Effects of water stress on germination and growth of wheat*. photosynthetic efficiency and accumulation of metabolites. [http://www.cdn.intechopen.com/pdfs-wm] site cited on 28/7/ 2014.

Hirel, B., Tétu, T., Lea, P. J., & Dubois, F. (2011). Improving Nitrogen Use Efficiency in Crops for Sustainable Agriculture. *Sustainability,* 3, 1452-1485.

Hogh-jensen, H., Schjoerring, J. K., & Soussana, J. F. (2002). The influence of phosphorus deficiency on growth and nitrogen fixation of white clover plants. *Annual Botany,* 90, 745-753.

Hussain, M., Malik, M. A., Farooq, M., Ashraf, M. Y., & Cheema, M. A. (2008). Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *Journal of Agronomy and Crop Science*, 194, 193-199.

Kalima, S. P. (2013). Physiological responses of common bean (*Phaseolus vulgaris* L.) genotypes to water stress. A thesis for Award of MSc. Degree at University of Zambia, Lusaka, Zambia, pp. 74.

Kaya, M. D., Okcu, G., & Atak, M. (2006). Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *European Journal of Agronomy,* 24, 291-295.

Kimura, D. S., Schmidtke, K., Tajima, R., Yoshida, K., Nakashima, H., & Rauber, R. (2004). Seasonal N uptake and N fixation by common and adzuki bean at various spacing. *Plant Soil*, 258, 91-101.

Lawlor, D. W., & Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environment*, 25, 275-294.

Leung, K., & Bottomley, P. J. (1994). Influence of phosphate on the growth and nodulation characteristics of *Rhizobium trifolii*. *Applied and Environmental Microbiology,* 53, 2098-2105.

Liu, F., Andersen, M. N., & Jensen, C. R. (2003). Loss of pod set caused by drought stress is associated with water status and ABA content of reproductive structures in soybean. *Functional Plant Biology*, 30, 271-280.

Manjeru, P., Madanzi, T., Makeredza, B., Nciizah, A., & Sithole, M. (2007). Effect of water stress at different growth stages on components and grain yield of common bean (*Phaseolus vulgaris* L.). *African Crop Science,* 8, 299-303.

Marino, D., Frendo, P., Gonzalez, E. M., Puppo, A., & Arrese, C. (2007). NADPA recycling systems in oxidative stressed nodules: A key role for the NADP + dependent isocitrate dehydrogenase. *Planta,* 225, 413-421.

Mohammadi, K., Sohrabi, Y., Heidari, G., Khalesro, S., & Majidi, M. (2012). Effective factors on biological nitrogen fixation. *African Journal of Agricultural Research,* 7(12), 17 1788.

Molina, J. C., Moda-Cirino, V., Da, N., Junior, S. F., De Faria, R. T., & Destro, D. (2001). Response of common bean cultivars and lines to water stress. *Crop Breeding and Applied Biotechnology,* 1, 363-372.

Nakayama, K., Okawa, K., Kakizaki, T., Honma, T., Itoh, H., & Inaba, T. (2007). Arabidopsis Cor15am is a chloroplast stromal protein that has cryoprotective activity and forms oligomers. *Plant Physiology,* 144, 513-523.

Ohashi, Y., Soneoka, H., & Fujita, K. (2000). Effect of water stress on growth, photosynthesis and photo assimilate, translocation in soybean and tropical pasture legume siratro. *Soil Science and Plant nutrition,* 46, 417-425.

Olivera, M., Tejera, N., Iribarne, C., Ocana, A., & Lluch, C. (2004). Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris*): Effect of phosphorus. Physiologia Plantarum, 121, 498-505.

Onuh, M., & Donald, K. M. (2009). Effects of water stress on the rooting, nodulation potentials and growth of cowpea (*Vignaunguiculata* (L) WALP). *Science World Journal*, 4(3), 31-34.

Ramos, M. L. G., Gordon, A. J., Minchin, F. R., Sprent, J. I., & Parsons, R. (1999). Effect of water stress on nodule physiology and biochemistry of a drought tolerant cultivar of common bean (*Phaseolus vulgaris* L.). *Annals of Botany*, 83, 57-63.

Ranawake, A. L., Dahanayaka, N., Amarasingha, U. G. S., Rodrigo, W. D. R. T., & Rodrigo, U. T. D. (2011). Effect of water stress on growth and yield of Mung bean (*Vignaradiata* L). *Tropical Agricultural Research and Extension*, 14(4), 76-79.

Rosales-Serna, R., Kohashi-Shibata, J., Acosta- Gallegos, J. A., Lopez, C. T., Ortiz-Cereceres, J., & Kelly, J. D. (2004). Biomass distribution, maturity acceleration and yield in drought - stressed common bean cultivars. *Field Crops Research*, 85, 203-211.

Singh, S. P. (2007). Drought resistance in the race Durango dry bean landraces and cultivars. *Agronomy Journal*, 99, 1219-1225.

Thangavel, P., Anandan, A., & Eswaran, R. (2011). AMMI analysis to comprehend genotype-by-environment (G*E) interaction in rainfed grown mungbean (*Vigna radiate*L.). *Astria Journal of Crop Science*, 5, 1767-1775.

Werner, D., & Newton, W. E. (2005). *Nitrogen fixation in agriculture, forestry, ecology and environment*. Springer, Netherlands pp. 255-276.

Williams, P. M. & De Mallorca, M. S. (1984). Effect of osmotically induced leaf moisture stress on nodulation and nitrogenase activity of *Glycine max*. *Plant Soil*, 80, 267-283.

Worrall, V. S., & Roughley, R. J. (1976). The effect of moisture stress on infection of *Trifolium subterraneum* L. by *Rhizobium trifolii* Dang. *Journal of Experimental Botany*, 27, 1233-1241.