



## RESEARCH PAPER

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## Influence of water stress and rhizobial inoculation on growth and yield of selected common bean cultivars (*Phaseolus vulgaris* L.)

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### Abstract

Two season's field experiment and single season screen house experiment were conducted to assess the effect of water stress periods and rhizobial inoculation in five *P. vulgaris* cultivars. The experiment consisted of two levels of rhizobia (with and without inoculation), two stress levels (with and without water stress) and five cultivars of *P. vulgaris* (*KAT B9*, *KAT B1*, *F9 Kidney Selection*, *F8 Drought line* and *JESCA*). Results showed that rhizobial inoculation significantly increased plant height (cm), leaf area (cm<sup>2</sup>), shoot and root dry weight (g<sup>-1</sup> plant) and seed yields (kg<sup>-1</sup> ha) at vegetative and flowering in field experiment. Furthermore, water stress treatments significantly reduced plant height (cm), stem diameter (mm), shoot and root dry weight (g<sup>-1</sup> plant) and seed yields (kg<sup>-1</sup> ha) in both growth stages at field experiment. For screen house experiment rhizobial inoculation significantly increased leaf area (cm<sup>2</sup>), number of leaves, stem girth (mm), shoot and root dry weight (g<sup>-1</sup> plant) at both growth stages. Additionally, water stress treatments significantly reduced number of leaves, stem diameter (mm), shoot and root dry weight (g<sup>-1</sup> plant) in both growth stages. Varieties *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* had significantly superior measurements reflected in increased plant height (cm), shoot and root dry weight (g<sup>-1</sup> plant) and seed yields (kg<sup>-1</sup> ha) as compared with *KAT B9* and *KAT B1*. Furthermore, significant interactive effects were also seen between rhizobial inoculation x stress level and tested bean cultivars on plant height, number of leaves, stem diameter, shoot dry weight and seed yields.

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## Introduction

Nitrogen is the major elements in all plants and constitutes a constructive effect on growth of legumes as it improves the quality and quantity of dry matter yields and proteins (Wood *et al.*, 1993; Caliskan *et al.*, 2008). The most important role of nitrogen in the plant is its presence in the structure of protein and nucleic acids, which are the most important building and information substances of every cell. For that reason, sufficient supply of nitrogen is necessary to attain high potential yields in crops. Nitrogen availability in the soil plays a positive significance functions on plant growth as it increases the leaf area of the plants and as a result influences photosynthesis activity of the plants (Uchida, 2000). Report by Namvar *et al.* (2013) on chick pea showed that plant height was increased with application of nitrogen fertilizer. However, inadequate (N) in the growth media/soil is the major limiting factor for crop growth in most areas of the world (Salvagiotti *et al.*, 2008; Fuzhong *et al.*, 2008). On the other hand, the source of N through fixation using beneficial soil bacterium (*Rhizobium*) can efficiently reduce the cost of production and improve crops production (Tairo and Ndakidemi, 2013). Common bean can acquire its N<sub>2</sub> requirement through N fixation when grown in association with effective and compatible *Rhizobium* strain (Makoi *et al.*, 2010). The inoculation of seeds with sufficient *Rhizobium* is known to enhance nodulation, nitrogen uptake, growth and yield parameters of legume crops (Sogut, 2006; Namvar, *et al.*, 2011). Therefore, determination of growth parameters of common bean crop in response to *Rhizobium* inoculation is very important to maximize yield and economic profitability of common bean production in a particular environment.

Plants experience water stress either when the water supply to their roots becomes limiting or when the transpiration rate becomes intense (Nielsen and Nelson, 1998). Water stress is one of the most restrictive features in crop growth which mainly decrease growth and finally the dry matter production. Water stress has been found to impair plant growth and development, whereby, the foremost effect of

water stress in plants impairs germination and poor stand establishment (Harris *et al.*, 2002). Cell growth is the one which is highly affected during water deficit in plants due to reduction in turgor pressure. Expansion of young cells is given by growth which is brought about by daughter-cell production by meristematic cell division. Nonami, (1998) reported that in severe water shortage, cell elongation of higher plants can be inhibited by disruption of water flow from xylem to the surrounding elongating cells. In general terms, water deficit diminish cell division, cell elongation and cell enlargement as a result reduce growth of the crop (Hussain *et al.*, 2008; Farooq *et al.*, 2009). At the same time, plant height, number of leaves per plant, leaf area, leaf longevity and soil water potential as well as fresh and dry biomass production are also reduced due to adverse effect of water deficit (Zhao *et al.*, 2006; Baroowa and Gogoi, 2012; Emam *et al.*, 2010). It has been reported that growth, development and performance of common bean is adversely affected if the quantity of water supplied is insufficient to meet the basic needs of plants (Seki *et al.*, 2002). Therefore, there is a need to assess the effects of water stress and *Rhizobium* inoculation on growth and yield of selected *P. Vulgaris* (L) cultivars and identify the potential ones which can perform well in water stressed environment.

## Materials and methods

### Narrative of Site Location

The trial was conducted at Agricultural Seed Agency (ASA) farm in Arusha, located at Latitude 3°18'S and Longitude 36°38'06.29"E. ASA receives the mean annual rainfall of 819mm, mean temperature of 19.15°C with relative humidity of about 94% and altitude of 1520 M.A.S.L. The field trial was carried out during dry season of January, to March 2014 and January, to March, 2015 while the screen house experiment was carried out from mid-January to March, 2016 under irrigation.

### Experimental Design and treatment application

The experiment was designed in a split, split plot arrangement with 3 replications. The plot size was 3 x

4m. The field experimental treatments consisted of 2 levels of Rhizobia (with and without inoculation) as the main factor followed by imposing of stress (sub factor) in vegetative and flowering stages of plant growth. Five cultivars of *P. vulgaris*: (KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line and JESCA) were assigned to sub-sub plots. The common bean seeds were sown at a spacing of 50 cm x 20 cm, making a plant population density of 200,000 plants per hectare. The BIOFIX legume inoculants were obtained from MEA Company Nairobi-Kenya, sold under license from the University of Nairobi. Common bean seeds were obtained from the breeding unit based at the Selian Agricultural Research Institute (SARI), Arusha, Tanzania. Land for field experiment was cleared and all the necessary practices like ploughing and harrowing were done before planting. Moreover, in the screen house experiment, the wooden box technique was used to establish the experiment. This was done by collecting the same soil used at field experiment and beans were planted using the protocol developed by (Agbicodo *et al.*, 2009) with some modifications. Common bean seeds were thoroughly mixed with *Rhizobium* inoculants to supply ( $10^9$  cells/g seed), following procedure stipulated by products manufacturer. To avoid contamination, all non-inoculated seeds were sown first, followed by inoculated seeds. Three seeds were sown and thinned to two plants per hill after full plant establishment. Stress period of 10 days were imposed at vegetative and flowering stages of plant growth by not irrigating.

#### *Study of Growth Parameters and yield in P. vulgaris (L.)*

Growth parameters in field and screen house experiment were collected in vegetative and flowering growth stages upon stress periods correspondingly. Plant height was taken using a meter rule. Plant height was measured from the base to the growing tip of the shoot in (cm) in each of the season at each growth stages. In field experiment, 10 plants were randomly selected in the two middle rows from each field plot for measuring the height of the plant at two stress periods. The same procedure was applied to the screen house experiment, whereby only two plants in

each row were measured for height. After recording the data, the average was worked out to get a representative plant height from each of the experimental unit. Number of leaves per plant was recorded in each of the growth stages of the *P. vulgaris* (L.). This was conducted in the same interval to the height of the plant at each stages of the common bean growth. The same exercise was also conducted for the glasshouse experiment and the average worked out as well. Stem girth (mm) was measured at each of the growth stages using a veneer caliper in both glasshouse and field experiments and values recorded. Leaf area (LA) was estimated according to Peksen, (2007), i.e.  $LA = 0.919 + 0.682LW$ , whereby LA = Leaf area ( $cm^2$ ), L = leaflet length (cm), W = maximum width of the leaflet (cm). Shoot and root dry weight ( $g^{-1}$  plants) was also measured after oven drying the plant samples at  $65^\circ C$  for 48hours and average worked out. Seed yields ( $g^{-1}$  plot) were evaluated by randomly sampling two middle rows from each stressed stages of the net plot threshed and then adjusted to constant moisture by air drying and weighs them. The plot yield was then converted to  $kg^{-1}ha$ .

#### *Statistical Analysis*

A 3-way ANOVA was used to analyze data collected. The analysis was done using STATISTICA software program of 2013. Fisher's least significant difference was used to compare treatment means at  $p = 0.05$  (Steel and Torrie, 1980).

#### **Results**

*Effect of inoculation with Rhizobium and stress periods on plant height (cm), number of leaves per plant, stem girth (mm), leaf area( $cm^2$ ), shoot dry weight ( $g^{-1}$  plant), root dry weight ( $g^{-1}$  plant) and seed yields ( $kg^{-1} ha$ ).*

Rhizobial inoculation increased plant height (cm) by 12 and 9% at vegetative and flowering growth stages respectively in field experiment (Table 1). Furthermore, rhizobial inoculation significantly increased leaf area by 17% in the second season at vegetative growth stage (Table 4).

Shoot dry weight ( $g^{-1}$  plant) and root dry weight ( $g^{-1}$  plant) were significantly increased through rhizobial inoculation by 32% in season one at vegetative stage and 31% and 20% in season two at vegetative and flowering stage both in field experiments respectively (Table 5). Rhizobial inoculation showed significant increase in seed yields ( $kg^{-1}$  ha) by 53%, 59% and 33%, 31% in season one and two both at vegetative and flowering growth stages under field experiment (Table 6). For screen house experiment, rhizobial inoculation significantly increased the number of leaves by 39% at vegetative stage and 30% at flowering stage (Table 8). Stem diameter were also increased as a result of rhizobial inoculation by 29% and 20% at vegetative and flowering growth stages respectively (Table 8). Rhizobial inoculation significantly increased the shoot dry weight ( $g^{-1}$  plant) and root dry weight ( $g^{-1}$  plant) by 28% at vegetative stage and 32% and 28% at vegetative and flowering stages respectively (Table 9).

For plants imposed with stress at vegetative stage, the water stress treatments significantly decreased plant height (Table 1), stem girth (Table 3) shoot and root dry weight (Table 5) in season one. Water stress imposed at vegetative stage also significantly decreased shoot and root dry weight (Table 5) and seed yield in season 2 (Table 6). Imposing water stress at flowering stage significantly reduced plant height (Table 1), shoot and root dry weight (Table 5) and seed yield (Table 6) for measurements taken in season 2. In the screen house experiment, water stress treatment imposed at vegetative stage significantly reduced number of leaves per plant, stem girth (Table 8) shoot and root dry weight (Table 9).

For the measured parameters, the performance of the varieties was as follows; Varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA* had superior measurements for girth (Table 3), shoot and root dry weight (Table 5) and seed yield (Table 6) in plants imposed with stress at vegetative stage in season one as compared with other tested varieties. Imposing stress at flowering stage in season one significantly

reduced number of leaves (Table 2), shoot and root dry weight (Table 5) and seed yield (Table 6) in varieties *KATB9* and *KATB1* as compared with varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA* which had better performances.

In season two, measurements taken from plants imposed with stress at vegetative stage indicated the superiority in varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA* with respect to number of leaves per plant (Table 2), shoot and root dry weight (Table 5) and seed yield (Table 6). Water stress imposed at flowering stage also significantly decreased plant height (Table 1), number of leaves per plant (Table 2), stem girth (Table 3), leaf area (Table 4), shoot and root dry weight (Table 5) and seed yield in season 2 (Table 6) in varieties *KATB9* and *KATB1* as compared with varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA*. In the screen house experiment, varieties *F9 Kidney Selection* and *JESCA* had superior root dry weight ( $g^{-1}$  plant) as compared to other varieties in plants imposed with stress at vegetative stage (Table 9).

*Interactive effect of inoculation with Rhizobium and stress periods on plant height (cm), number of leaves per plant, stem girth (mm), leaf area ( $cm^2$ ), shoot dry weight ( $g^{-1}$  plant), root dry weight ( $g^{-1}$  plant), and seed yields ( $kg^{-1}$  ha)*

There was significant interaction between *Rhizobium* and water stress in Plant Height (cm), shoot dry weight ( $g^{-1}$  plant) and seed yields ( $kg^{-1}$  ha) (Fig. 1, 5-8). Applying *Rhizobium* inoculants and stressing plants with water enhanced the growth parameters of plant height, shoot dry weight and seed yields in vegetative and flowering growth stages respectively compared with the un-inoculated treatments. Furthermore, significant interactive effects was also seen between stress level and varieties on plant height (cm) during flowering stage, number of leaves and stem girth (mm) (Fig. 2 - 4). Even under water stress treatments at vegetative and flowering stages of growth, varieties *JESCA*, *F9 Kidney Selection* and *F8 Drought Line* performed well in the above measured parameters as compared with varieties *KAT B9* and *KAT B1* respectively.

**Table 1.** Plant height (cm) in *P. vulgaris* influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons.

Treatments	1 <sup>st</sup> Season		2 <sup>nd</sup> Season	
	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage
R+	10.83±0.23a	17.63±0.46a	13.01±0.22a	19.95±0.45a
R-	9.57±0.32b	16.05±0.50b	12.90±0.23a	19.36±0.46a
Stress Levels				
S <sub>1</sub>	10.62±0.27a	17.14±0.48a	13.05±0.22a	20.82±0.38a
S <sub>2</sub> /S <sub>3</sub>	9.78±0.32b	16.53±0.51a	12.87±0.22a	18.49±0.42b
Varieties				
V <sub>1</sub>	10.39±0.30a	16.68±0.89a	13.32±0.37a	18.76±0.96b
V <sub>2</sub>	10.25±0.48a	17.03±0.66a	13.24±0.23a	18.73±0.76b
V <sub>3</sub>	10.82±0.41a	16.04±0.49a	12.56±0.21a	19.32±0.45b
V <sub>4</sub>	10.14±0.49a	17.78±0.69a	13.17±0.41a	20.19±0.62ab
V <sub>5</sub>	9.40±0.61a	16.68±1.10a	12.50±0.44a	21.26±0.48a
3-Way Anova (F-Statistics)				
Rhz	13.06***	5.06*	0.10ns	1.44ns
StrL	5.71*	0.75ns	0.32ns	22.32***
Vrty	1.74ns	0.66ns	1.19ns	3.83**
Rhz*StrL	4.70*	0.23ns	0.06ns	0.09ns
Rhz*Vrty	0.88ns	0.50ns	0.84ns	0.20ns
StrL*Vrty	0.86ns	0.54ns	0.95ns	4.86**
Rhz*StrL*Vrty	2.40ns	1.46ns	0.69ns	0.06ns

+R-: With *Rhizobium*; -R-: Without *Rhizobium* S<sub>1</sub>-: No water stress. S<sub>2</sub>-: Water stress imposed at Vegetative Stage. S<sub>3</sub>-: Water stress imposed at Flowering Stage. V<sub>1</sub>-: (KAT B9); V<sub>2</sub>-: KAT B1. V<sub>3</sub>-: F9 Kidney Selection. V<sub>4</sub>-: F8 Drought Line. V<sub>5</sub>-: JESCA. Values presented are means ± SE. \*, \*\*, \*\*\* = significant at  $p \leq 0.05$ , at  $p \leq 0.01$ , and at  $p \leq 0.001$  respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at  $p = 0.05$ .

**Table 2.** Number of leaves in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons.

Treatments	1 <sup>st</sup> Season		2 <sup>nd</sup> Season	
	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage
R+	6.23±0.31a	10.95±0.53a	6.80±0.18a	10.47±0.21a
R-	6.17±0.20a	10.10±0.41a	6.47±0.17a	10.37±0.20a
Stress Levels				
S <sub>1</sub>	5.97±0.26a	10.55±0.56a	6.57±0.19a	10.60±0.20a
S <sub>2</sub> /S <sub>3</sub>	6.43±0.24a	10.50±0.39a	6.70±0.16a	10.23±0.21a
Varieties				
V <sub>1</sub>	6.33±0.19a	8.58±0.43c	5.67±0.14d	9.33±0.19d
V <sub>2</sub>	6.00±0.58a	10.58±0.66b	6.17±0.17cd	10.17±0.24bc
V <sub>3</sub>	6.50±0.44a	12.00±0.72a	7.75±0.22a	11.83±0.27a
V <sub>4</sub>	6.50±0.34a	11.78±0.91a	7.17±0.21b	10.75±0.22b
V <sub>5</sub>	5.67±0.40a	9.68±0.61bc	6.42±0.23c	10.00±0.17c
3-Way Anova (F-Statistics)				
Rhz	0.04ns	2.37ns	3.70ns	0.25ns
StrL	2.11ns	0.01ns	0.59ns	3.36ns
Vrty	1.01ns	5.36**	18.20***	17.64***
Rhz*StrL	1.55ns	0.05ns	2.37ns	1.36ns
Rhz*Vrty	0.90ns	1.06ns	0.28ns	0.67ns
StrL*Vrty	3.72ns	4.06**	0.31ns	0.72ns
Rhz*StrL*Vrty	2.09ns	0.97ns	1.72ns	0.81ns

-R-: Without *Rhizobium*; +R-: With *Rhizobium*. S<sub>1</sub>-: No water stress. S<sub>2</sub>-: Water stress imposed at Vegetative Stage. S<sub>3</sub>-: Water stress imposed at Flowering Stage. V<sub>1</sub>-: KAT B9. V<sub>2</sub>-: KAT B1. V<sub>3</sub>-: F9 Kidney Selection. V<sub>4</sub>-: F8 Drought Line. V<sub>5</sub>-: JESCA. Values presented are means ± SE. \*, \*\*, \*\*\* = significant at  $p \leq 0.05$ , at  $p \leq 0.01$ , and at  $p \leq 0.001$  respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at  $p = 0.05$ .

**Table 3.** Stem girth (mm) in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons.

Treatments	1 <sup>st</sup> Season		2 <sup>nd</sup> Season	
	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage
R+	3.93±0.17a	6.91±0.11a	3.80±0.10a	6.64±0.10a
R-	3.75±0.08a	6.78±0.13a	3.69±0.11a	6.55±0.07a
Stress Levels				
S <sub>1</sub>	3.97±0.06a	6.95±0.13a	3.81±0.11a	6.66±0.09a
S <sub>2</sub> /S <sub>3</sub>	3.70±0.18b	6.75±0.11a	3.68±0.10a	6.53±0.08a
Varieties				
V <sub>1</sub>	3.02±0.35c	6.95±0.16a	3.79±0.18a	6.21±0.16d
V <sub>2</sub>	3.81±0.09b	6.93±0.23a	3.75±0.14a	6.35±0.04cd
V <sub>3</sub>	4.31±0.09a	6.75±0.16a	3.71±0.22a	7.12±0.08a
V <sub>4</sub>	4.15±0.08ab	6.84±0.14a	3.75±0.14a	6.76±0.09b
V <sub>5</sub>	3.91±0.07ab	6.78±0.25a	3.72±0.16a	6.51±0.08bc
3-Way Anova (F-Statistics)				
Rhz	1.91ns	0.54ns	0.48ns	0.91ns
StrL	4.24**	1.37ns	0.59ns	2.08ns
Vrty	11.68***	0.21ns	0.03ns	12.33***
Rhz*StrL	0.58ns	0.78ns	0.02ns	3.83ns
Rhz*Vrty	1.99ns	0.72ns	0.38ns	0.11ns
StrL*Vrty	3.44*	0.57ns	0.46ns	0.40ns
Rhz*StrL*Vrty	1.82ns	1.47ns	0.80ns	0.59ns

-R-: Without *Rhizobium*; +R-: With *Rhizobium*. S<sub>1</sub>-: No water stress. S<sub>2</sub>-: Water stress imposed at Vegetative Stage. S<sub>3</sub>-: Water stress imposed at Flowering Stage. V<sub>1</sub>-: KAT B9. V<sub>2</sub>-: KAT B1. V<sub>3</sub>-: F9 Kidney Selection. V<sub>4</sub>-: F8 Drought Line. V<sub>5</sub>-: JESCA. Values presented are means ± SE. \*, \*\*, \*\*\* = significant at  $p \leq 0.05$ , at  $p \leq 0.01$ , and at  $p \leq 0.001$  respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at  $p = 0.05$ .

**Table 4.** Leaf Area (cm<sup>2</sup>) in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons.

Treatments	1 <sup>st</sup> Season		2 <sup>nd</sup> Season	
	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage
R+	110.86±1.02a	149.29±9.57a	128.00±5.43a	163.71±5.61a
R-	113.54±1.02a	161.82±8.75a	105.86±5.41b	161.32±4.79a
Stress Levels				
S <sub>1</sub>	111.89±1.11a	144.84±9.12a	116.03±5.31a	164.22±4.92a
S <sub>2</sub> /S <sub>3</sub>	112.51±0.99a	166.27±8.93a	117.84±6.24a	160.81±5.49a
Varieties				
V <sub>1</sub>	114.47±1.68a	181.75±12.81a	126.44±5.65a	130.10±4.25e
V <sub>2</sub>	109.95±1.40a	131.06±14.30a	114.36±8.89a	142.72±3.47d
V <sub>3</sub>	112.32±1.66a	142.02±15.85a	107.64±7.94a	200.07±4.67a
V <sub>4</sub>	112.54±1.51a	157.77±11.97a	109.60±7.76a	178.15±3.76b
V <sub>5</sub>	111.72±1.95a	165.16±14.85a	126.61±13.36a	161.54±3.45c
3-Way Anova (F-Statistics)				
Rhz	3.79ns	1.01ns	8.08**	0.43ns
StrL	0.20ns	2.96ns	0.05ns	0.88ns
Vrty	1.11ns	2.02ns	1.09ns	47.02***
Rhz*StrL	3.51ns	0.01ns	0.62ns	1.40ns
Rhz*Vrty	2.03ns	0.67ns	0.46ns	0.80ns
StrL*Vrty	1.57ns	1.82ns	1.50ns	0.49ns
Rhz*StrL*Vrty	0.29ns	0.47ns	0.83ns	0.05ns

-R-: Without *Rhizobium*; +R-: With *Rhizobium*. S<sub>1</sub>-: No water stress. S<sub>2</sub>-: Water stress imposed at Vegetative Stage. S<sub>3</sub>-: Water stress imposed at Flowering Stage. V<sub>1</sub>-: KAT B9. V<sub>2</sub>-: KAT B1. V<sub>3</sub>-: F9 Kidney Selection. V<sub>4</sub>-: F8 Drought Line. V<sub>5</sub>-: JESCA. Values presented are means ± SE. \*, \*\*, \*\*\* = significant at  $p \leq 0.05$ , at  $p \leq 0.01$ , and at  $p \leq 0.001$  respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at  $p = 0.05$ .

**Table 5.** Shoot dry weight (g<sup>-1</sup> plant) and Root Dry weight (g/plant) in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons.

Treatments	Shoot dry weight (g <sup>-1</sup> plant)				Root Dry weight (g/plant)			
	1 <sup>st</sup> Season		2 <sup>nd</sup> Season		1 <sup>st</sup> Season		2 <sup>nd</sup> Season	
	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage
R+	1.36±0.04a	3.81±0.15a	3.37±0.21a	5.45±0.33a	0.33±0.01a	0.43±0.02a	1.59±0.04a	2.59±0.17a
R-	0.93±0.05b	3.70±0.19a	3.25±0.14a	5.12±0.32a	0.34±0.01a	0.41±0.01a	1.10±0.05b	2.06±0.19b
Stress Levels								
S <sub>1</sub>	1.20±0.05a	3.91±0.17a	4.05±0.15a	5.67±0.33a	0.36±0.01a	0.43±0.02a	1.43±0.06a	2.96±0.14a
S <sub>2</sub> /S <sub>3</sub>	1.09±0.06b	3.60±0.17a	2.57±0.08b	4.91±0.30b	0.33±0.01b	0.41±0.01a	1.26±0.07b	1.69±0.15b
Varieties								
V <sub>1</sub>	1.00±0.07cd	3.07±0.15b	2.83±0.25d	3.86±0.26b	0.29±0.01c	0.33±0.02b	1.17±0.08cd	1.89±0.28b
V <sub>2</sub>	0.95±0.06d	2.90±0.18b	2.95±0.20cd	3.86±0.37b	0.28±0.01c	0.37±0.02b	1.13±0.08d	1.80±0.21b
V <sub>3</sub>	1.42±0.09a	4.25±0.21a	3.96±0.32a	6.74±0.47a	0.40±0.02a	0.47±0.02a	1.65±0.10a	2.93±0.29a
V <sub>4</sub>	1.10±0.07c	4.36±0.27a	3.35±0.28bc	6.15±0.34a	0.35±0.01b	0.47±0.03a	1.32±0.09bc	2.47±0.26a
V <sub>5</sub>	1.25±0.08b	4.18±0.16a	3.47±0.28b	5.82±0.46a	0.35±0.02b	0.46±0.02a	1.45±0.11b	2.53±0.30a
3-Way Anova (F-Statistics)								
Rhz	97.64***	0.39ns	0.77ns	0.89ns	1.15ns	0.85ns	89.53***	10.05**
StrL	6.52*	2.78ns	123.64***	4.75*	26.05***	0.31ns	10.42**	56.80***
Vrty	15.47***	12.07***	9.20***	11.73***	19.05***	6.90***	13.86***	6.32***
Rhz*StrL	2.65ns	1.17ns	3.58ns	2.55ns	2.60ns	5.35ns	1.18ns	1.30ns
Rhz*Vrty	0.33ns	0.67ns	0.86ns	0.57ns	0.37ns	0.56ns	0.31ns	0.22ns
StrL*Vrty	0.48ns	0.67ns	1.21ns	0.48ns	0.95ns	0.18ns	0.19ns	0.41ns
Rhz*StrL*Vrty	0.15ns	0.77ns	0.65ns	0.26ns	0.13ns	0.09ns	0.28ns	0.97ns

+R-: With *Rhizobium*; -R-: Without *Rhizobium*; S<sub>1</sub>-: No water stress. S<sub>2</sub>-: Water stress imposed at Vegetative Stage. S<sub>3</sub>-: Water stress imposed at Flowering Stage. V<sub>1</sub>-: KAT B9. V<sub>2</sub>-: KAT B1. V<sub>3</sub>-: F9 Kidney Selection. V<sub>4</sub>-: F8 Drought Line. V<sub>5</sub>-: JESCA. Values presented are means ± SE. \*, \*\*, \*\*\* = significant at p ≤ 0.05, at p ≤ 0.01, and at p ≤ 0.001 respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at p = 0.05.

**Table 6.** Seed yields (kg<sup>-1</sup> ha) in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons.

Treatments	1 <sup>st</sup> Season		2 <sup>nd</sup> Season	
	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage
R+	674.97±33.91a	557.12±24.87a	979.33±64.63a	828.53±67.86a
R-	319.11±30.23b	227.04±13.77b	655.73±36.31b	575.60±44.43b
Stress Levels				
S <sub>1</sub>	509.29±48.92a	409.23±39.08a	941.60±69.44a	841.87±68.49a
S <sub>2</sub> /S <sub>3</sub>	484.78±42.94a	374.93±33.75a	693.47±37.65b	562.27±40.53b
Varieties				
V <sub>1</sub>	316.40±67.89b	291.78±45.05b	635.00±74.23c	427.33±92.58d
V <sub>2</sub>	400.98±59.79b	340.98±53.10b	628.00±96.43c	608.33±71.23c
V <sub>3</sub>	586.94±73.79a	446.33±63.47a	859.00±71.40b	706.33±71.67bc
V <sub>4</sub>	624.77±58.84a	459.18±58.75a	988.67±98.41a	778.00±81.67b
V <sub>5</sub>	556.10±67.51a	422.15±58.88a	977.00±84.28ab	990.33±97.16a
3-Way Anova (F-Statistics)				
Rhz	96.22***	189.27***	64.50***	43.67***
StrL	0.46ns	2.04ns	37.93***	53.36***
Vrty	10.60***	7.30***	15.48***	23.64***
Rhz*StrL	0.53ns	5.04*	50.01***	65.04***
Rhz*Vrty	0.32ns	0.46ns	0.71ns	0.45ns
StrL*Vrty	0.97ns	0.71ns	0.68ns	1.01ns
Rhz*StrL*Vrty	0.60ns	0.11ns	0.22ns	0.42ns

-R-: Without *Rhizobium*; +R-: With *Rhizobium*. S<sub>1</sub>-: No water stress. S<sub>2</sub>-: Water stress imposed at Vegetative Stage. S<sub>3</sub>-: Water stress imposed at Flowering Stage. V<sub>1</sub>-: KAT B9. V<sub>2</sub>-: KAT B1. V<sub>3</sub>-: F9 Kidney Selection. V<sub>4</sub>-: F8 Drought Line. V<sub>5</sub>-: JESCA. Values presented are means ± SE. \*, \*\*, \*\*\* = significant at p ≤ 0.05, at p ≤ 0.01, and at p ≤ 0.001 respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at p = 0.05.

**Table 7.** Plant height (cm) and Leaf area (cm<sup>2</sup>) in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in the screen house experiment.

Treatments	Plant height (cm)		Leaf area (cm <sup>2</sup> )	
	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage
R+	25.09±0.90a	33.09±0.82a	153.91±4.54a	171.98±3.75a
R-	24.33±0.92a	32.82±0.96a	118.44±1.45b	181.09±4.23a
Stress Levels				
S <sub>1</sub>	25.87±0.73a	33.46±0.84a	133.83±3.21a	177.43±4.05a
S <sub>2</sub> /S <sub>3</sub>	23.55±1.03a	32.44±0.94a	138.52±5.32a	175.64±4.08a
Varieties				
V <sub>1</sub>	24.49±1.24a	30.64±0.95a	130.14±6.22a	169.93±5.23a
V <sub>2</sub>	24.89±1.55a	32.29±1.35a	134.52±5.38a	180.49±5.46a
V <sub>3</sub>	22.30±1.85a	34.40±1.79a	139.83±6.90a	177.51±6.96a
V <sub>4</sub>	25.07±0.85a	33.56±1.51a	143.38±9.74a	175.60±8.38a
V <sub>5</sub>	26.79±1.43a	33.88±1.26a	133.01±5.94a	179.13±5.91a
3-Way Anova (F-Statistics)				
Rhz	0.35ns	0.04ns	61.82***	2.33ns
StrL	3.26ns	0.60ns	1.08ns	0.09ns
Vrty	1.24ns	1.04ns	1.13ns	0.38ns
Rhz*StrL	4.42ns	0.82ns	3.76ns	0.10ns
Rhz*Vrty	0.31ns	0.05ns	0.71ns	0.62ns
StrL*Vrty	0.46ns	0.55ns	1.74ns	0.87ns
Rhz*StrL*Vrty	0.60ns	0.97ns	2.04ns	0.64ns

-R-: Without *Rhizobium*; +R-: With *Rhizobium*. S<sub>1</sub>-: No water stress. S<sub>2</sub>-: Water stress imposed at Vegetative Stage. S<sub>3</sub>-: Water stress imposed at Flowering Stage. V<sub>1</sub>-: KAT B9. V<sub>2</sub>-: KAT B1. V<sub>3</sub>-: F9 Kidney Selection. V<sub>4</sub>-: F9 Drought Line. V<sub>5</sub>-: JESCA. Values presented are means ± SE. \*, \*\*, \*\*\* = significant at  $p \leq 0.05$ , at  $p \leq 0.01$ , and at  $p \leq 0.001$  respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at  $p = 0.05$ .

**Table 8.** Number of leaves and Stem girth (mm) in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in the screen house experiment.

Treatments	Number of leaves/plant		Stem girth (mm)	
	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage
R+	7.17±0.16a	10.38±0.28a	4.03±0.43a	5.41±0.37a
R-	4.35±0.22b	7.22±0.27b	2.87±0.26b	4.31±0.29b
Stress Levels				
S <sub>1</sub>	6.16±0.32a	10.06±0.31a	4.56±0.42a	5.16±0.23a
S <sub>2</sub> /S <sub>3</sub>	5.36±0.25b	7.53±0.32b	2.34±0.18b	4.56±0.42a
Varieties				
V <sub>1</sub>	5.59±0.57a	8.76±0.68a	3.07±0.55a	4.79±0.55a
V <sub>2</sub>	5.87±0.45a	9.02±0.51a	3.63±0.82a	5.70±0.68a
V <sub>3</sub>	5.95±0.48a	8.40±0.57a	2.96±0.37a	4.21±0.46a
V <sub>4</sub>	6.25±0.39a	8.92±0.65a	3.22±0.38a	4.58±0.39a
V <sub>5</sub>	5.16±0.42a	8.90±0.61a	4.36±0.64a	5.01±0.54a
3-Way Anova (F-Statistics)				
Rhz	134.06***	122.62***	7.07*	5.62*
StrL	10.77**	78.94***	25.99**	1.68ns
Vrty	2.29ns	0.58ns	1.38ns	1.15ns
Rhz*StrL	2.57ns	0.01ns	1.47ns	1.63ns
Rhz*Vrty	0.73ns	1.01ns	1.04ns	1.25ns
StrL*Vrty	0.94ns	0.96ns	0.73ns	0.87ns
Rhz*StrL*Vrty	1.13ns	0.66ns	0.66ns	0.57ns

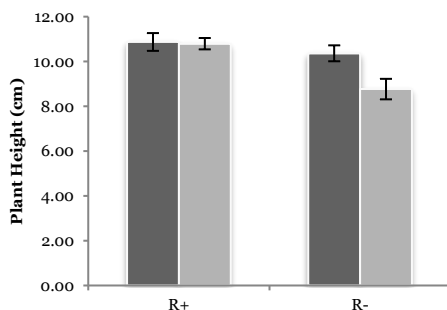
-R-: Without *Rhizobium*; +R-: With *Rhizobium*. S<sub>1</sub>-: No water stress. S<sub>2</sub>-: Water stress imposed at Vegetative Stage. S<sub>3</sub>-: Water stress imposed at Flowering Stage. V<sub>1</sub>-: KAT B9. V<sub>2</sub>-: KAT B1. V<sub>3</sub>-: F9 Kidney Selection. V<sub>4</sub>-: F8 Drought Line. V<sub>5</sub>-: JESCA. Values presented are means ± SE. \*, \*\*, \*\*\* = significant at  $p \leq 0.05$ , at  $p \leq 0.01$ , and at  $p \leq 0.001$  respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at  $p = 0.05$ .



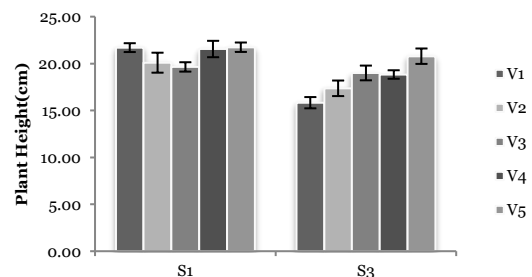
**Table 9.** Shoot Dry weight (g/plant) and Root dry weight (g/plant) in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in the screen house.

Treatments	Shoot Dry weight (g/plant)		Root dry weight (g/plant)	
	Vegetative Stage	Flowering Stage	Vegetative Stage	Flowering Stage
R+	1.34±0.14a	2.21±0.29a	0.19±0.01a	1.11±0.08a
R-	0.96±0.09b	2.14±0.15a	0.13±0.01b	0.80±0.03b
Stress Levels				
S <sub>1</sub>	1.52±0.14a	2.83±0.26a	0.18±0.01a	0.98±0.07a
S <sub>2</sub> /S <sub>3</sub>	0.78±0.06b	1.52±0.14b	0.13±0.01b	0.93±0.06a
Varieties				
V <sub>1</sub>	1.02±0.18a	1.96±0.30a	0.11±0.01c	0.79±0.08a
V <sub>2</sub>	1.21±0.27a	2.31±0.41a	0.10±0.02c	0.83±0.11a
V <sub>3</sub>	0.99±0.12a	2.03±0.35a	0.23±0.02a	1.06±0.11a
V <sub>4</sub>	1.07±0.13a	1.79±2.23a	0.16±0.02b	1.07±0.10a
V <sub>5</sub>	1.45±0.21a	2.78±0.49a	0.19±0.02ab	1.03±0.10a
3-Way Anova (F-Statistics)				
Rhz	7.07*	0.04ns	25.44***	10.97**
StrL	25.99***	18.88***	22.93***	0.27ns
Vrty	1.38ns	1.32ns	15.64***	1.75ns
Rhz*StrL	1.47ns	4.27*	0.26ns	0.34ns
Rhz*Vrty	1.04ns	0.29ns	0.39ns	0.06ns
StrL*Vrty	0.73ns	0.37ns	1.10ns	0.19ns
Rhz*StrL*Vrty	0.66ns	0.79ns	2.50ns	0.06ns

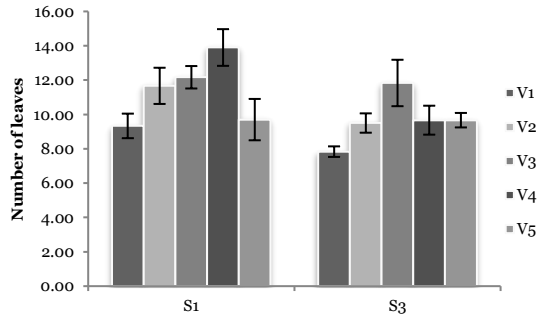
-R-: Without *Rhizobium*; +R-: With *Rhizobium*. S<sub>1</sub>-: No water stress. S<sub>2</sub>-: Water stress imposed at Vegetative Stage. S<sub>3</sub>-: Water stress imposed at Flowering Stage. V<sub>1</sub>-: KAT B9. V<sub>2</sub>-: KAT B1. V<sub>3</sub>-: F9 Kidney Selection. V<sub>4</sub>-: F8 Drought Line. V<sub>5</sub>-: JESCA. Values presented are means ± SE. \*, \*\*, \*\*\* = significant at p ≤ 0.05, at p ≤ 0.01, and at p ≤ 0.001 respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at p = 0.05.



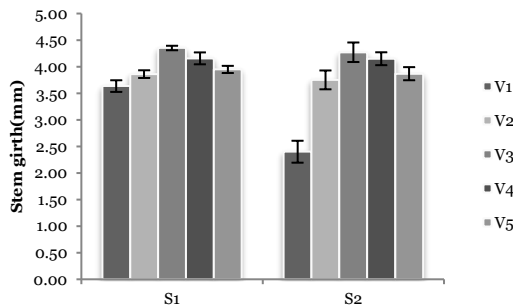
**Fig. 1.** Interactive effects of *Rhizobium* and stress levels on plant height (cm) in season one at vegetative stage (+R-: With *Rhizobium*, -R-: Without *Rhizobium*., S<sub>1</sub>-: Control, S<sub>2</sub>-: Water stress imposed at vegetative stage).



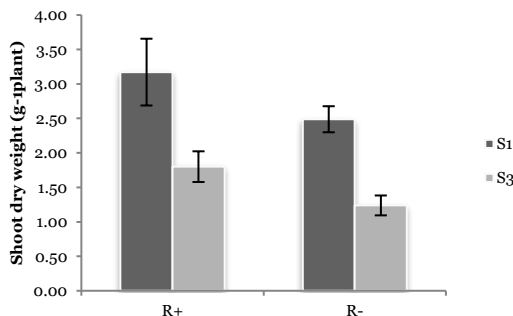
**Fig. 2.** Interactive effects of stress level and five (5) *P. vulgaris* on Plant height (cm) in season two at flowering stage (S<sub>1</sub>-: Control, S<sub>3</sub>-: Water stress imposed at flowering stage, V<sub>1</sub>-: KAT B9, V<sub>2</sub>-: KAT B1, V<sub>3</sub>-: F9 Kidney Selection, V<sub>4</sub>-: F8 Drought Line, V<sub>5</sub>-: JESCA).



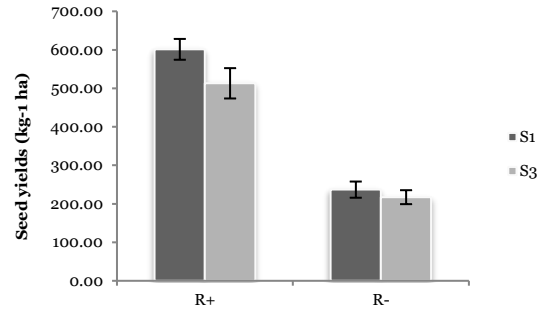
**Fig. 3.** Interactive effects of stress level and five (5) *P. vulgaris* (L.) on number of leaves in season one at flowering stage (S1-: Control, S3-: Water stress imposed at flowering stage, V1-: KAT B9, V2-: KAT B1, V3-: F9 Kidney Selection, V4-: F8 Drought Line, V5-: JESCA).



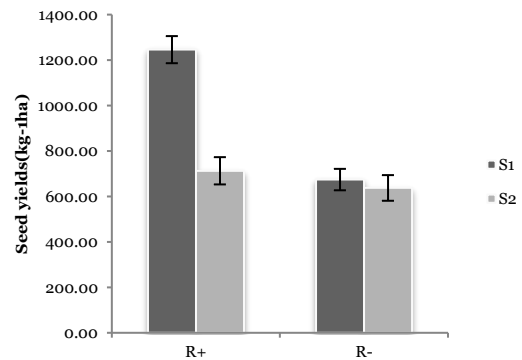
**Fig. 4.** Interactive effects of stress level and five (5) *P. vulgaris*(L.) on stem girth (mm) in season one at vegetative stage (S1-: Control, S2-: Water stress imposed at vegetative stage, V1-: KAT B9, V2-: KAT B1, V3-: F9 Kidney Selection, V4-: F8 Drought Line, V5-: JESCA).



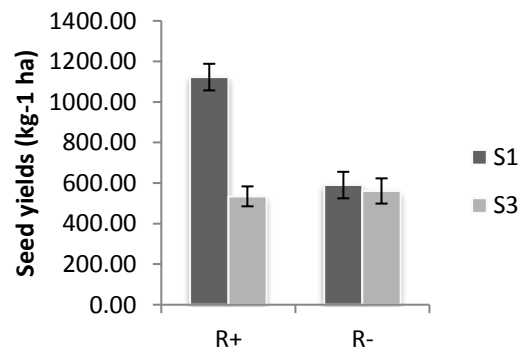
**Fig. 5.** Interactive effects *Rhizobium* and stress level on Shoot Dry weight (g/plant) in screen house experiment at flowering stage (+R-: With *Rhizobium*, -R-: Without *Rhizobium*., S1-: Control, S3-: Water stress imposed at flowering stage).



**Fig. 6.** Interactive effects of *Rhizobium* and stress level on seed yields (kg<sup>-1</sup> ha) in season one at flowering stage (+R-: With *Rhizobium*, -R-: Without *Rhizobium*, S1-: Control, S3-: Water stress imposed at flowering stage).



**Fig. 7.** Interactive effects of *Rhizobium* and stress level on seed yields (kg<sup>-1</sup> ha) in season two at vegetative stage (+R-: With *Rhizobium*, -R-: Without *Rhizobium*, S1-: Control, S2-: Water stress imposed at vegetative stage).



**Fig. 8.** Interactive effects of *Rhizobium* and stress level on seed yields (kg<sup>-1</sup> ha) in season two at flowering stage (+R-: With *Rhizobium*, -R-: Without *Rhizobium*, S1-: Control, S3-: Water stress imposed at flowering stage).

## Discussion

In the present study, we assessed the effects of *Rhizobium* inoculation and water stress periods on growth parameters in common bean (*P. vulgaris*). This study clearly showed that *Rhizobium* inoculation was supportive in improving growth parameters of the common bean. *Rhizobium* inoculation had great positive effects on plant height at vegetative and flowering stages, number of leaves per plant, stem girth (mm) in both growth stages at screen house experiment and Leaf area (Table 1, 4-6) as compared with the control. Significant observations were also observed in shoot dry weight ( $\text{g}^{-1}$  plant) and root dry weight ( $\text{g}^{-1}$  plant) as well as seed yields ( $\text{kg}^{-1}$  ha) (Table 1-2 & 4) as compared with the control. These improvements in inoculated treatments could be attributed to the legume inoculants BIOFIX, which increased nitrogen supply to the plants and consequently improved the growth parameters of the plant. Our results are similar to those reported by Uchida, (2000) in which plant growth potential was enhanced as a result of Biological Nitrogen Fixation; and Tairo and Ndakidemi, (2013) who reported the improvement of growth parameters in *B. japonicum* inoculated soybeans. The plant height was significantly affected by *Rhizobium* inoculation. The least plant height was recorded in non-inoculated control. Findings by (Amany, 2007; Caliskan *et al.*, 2008; Aminifard *et al.*, 2010; Tairo and Ndakidemi, 2013; Nyoki and Ndakidemi, 2014; Mfilinge *et al.*, 2014) showed that plant height was increased by rhizobial inoculation in different legumes. Moreover inoculated plants showed more dry matter and seed yields than non-inoculated plants. Inoculation with *Rhizobium* bacteria increased the shoot and root dry weight ( $\text{g}^{-1}$  plants) and seed yields ( $\text{kg}^{-1}$  ha) as compared with the non-inoculated control. Nitrogen is known to be an essential nutrient for plant growth and development. In this study, rhizobial inoculation increased the production of total dry matter in plants (Salvagiotti *et al.*, 2008) which enhanced the potential of the plant growth and ultimately resulted in higher seed yields.

Water stress significantly reduced plant height (cm), number of leaves, stem girth (mm) and Leaf area ( $\text{cm}^2$ ), shoot and root dry weight ( $\text{g}^{-1}$  plant) as well as

seed yields ( $\text{kg}^{-1}$  ha) as compared with control treatments which received adequate water supply. These findings are in line with studies by Hiler *et al.* (1972); Afolabi, (1998) and Aderolu, (2000) which showed decreased in plant height (cm) and number of leaves as a result of water stress. The decrease in the assessed growth parameters may be due to the impairment of cell division, cell enlargement caused by loss of turgor and inhibition of various growth metabolisms (Farooq *et al.*, 2012; Yordanov *et al.*, 2003). Common bean has been reported to respond differently to soil moisture stress during various stages depending on the severity of water stress (Emman *et al.*, 2010). For example, in a study by Hayatu and Mukhtar, (2010) in cowpea genotypes, it was reported that drought affected dry matter production and many other aspects of plant growth such as plant height, stem diameter, leaf area and number of leaves, results similar to our study. In closely related studies involving maize, Khan *et al.* (2001) conducted a study comprising of six irrigation treatments and concluded that plant height, stem diameter, leaf area decreased noticeably with increasing water stress. The reduction in plant height could be attributed to decline in the cell enlargement and more leaf senescence in the plant under water stress (Manivannan *et al.*, 2007a). Furthermore, Akinci and Losel (2009) also reported that water stress caused major reductions in plant height, leaf number and leaf area index of some *Cucurbitaceae* members. Apart from diseases, water stress has been reported to be the second major constraints in the legume seed yields (Rao, 2001). The reduced seed yields in bean yields as a result of water stress is mainly attributed to reduction in individual yield components such as dry matter yields, number of pods per plant, number of seeds per pod, seed weight as well as harvest index (Ramirez-Vallejo & Kelly, 1998; Shenkut & Brick, 2003). Report by Nielsen and Nelson (1998) on bean showed that seed yields were reduced due to reduced number of pods per plant and seeds per pod during water stress at flowering and/or reproductive stage. Similarly, in a study by Remenyik and Nemeske, (2010) in French bean (*Phaseolus vulgaris* (L.)) great variation was reported in yields as a result of irregular occurrence of drought periods accompanied by high temperature.

Our findings are also similar with studies by (Molina *et al.*, 2001; Nielsen and Nelson, 1998; Emam, 1985; Emam and Seghatoleslami, 2005). They all reported a reduction in grain yields and dry weight following water stress and this is attributed by lower percentage of pod production when the water stresses occurring especially during flowering.

*P. vulgaris* (L.) varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA* showed significant increase in seed yields ( $\text{kg}^{-1}$  ha), shoot and root dry weight ( $\text{g}^{-1}$  plant) compared with varieties *KAT B9* and *KAT B1*. The reduced yields in varieties *KAT B9* and *KAT B1* might be attributed by their low genetic potential to deal with water stress imposed at either vegetative or flowering growth stages. Study by Singh (1995) showed that water stress during flowering and grain filling reduced seed yield and seed weight and accelerated maturity of dry bean. It has been reported that the quality and the yield of beans were negatively affected by short periods of water shortage (Ramirez-Vallejo and Kelly 1998).

There was a significant interaction between *Rhizobium* water stress treatments and varieties in plant height, number of leaves per plant, stem girth, shoot dry weight and seed yields of *P. vulgaris*. The interactions between inoculations showed that *Rhizobium* inoculation in water stress treatment imposed at vegetative and flowering stage had greater effect on the above parameters as compared with un-inoculated treatments. These results suggest that inoculating beans with rhizobial inoculants enhanced growth even in water stressed environment. However, further studies on the mechanism involved warrants further studies. Furthermore, the interactive effects of varieties, *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* under water stressed environment shows the potential of these varieties to be used in drought tolerant studies. In a closely salt stress related study by Ndakidemi and Makoi, (2009) bean variety *JESCA* showed moderate tolerance to salinity, suggesting the potentiality of this variety in adverse environmental condition such as water stress.

## Conclusion

In conclusion, rhizobial inoculation significantly improved plant height (cm), number of leaves per plant, stem girth (mm), shoot and root dry weight ( $\text{g}^{-1}$  plant) as well as seed yields ( $\text{kg}^{-1}$  ha) as compared with un-inoculated treatments. Furthermore, water stress treatments imposed at vegetative and flowering stage significantly reduced plant height (cm), number of leaves, stem girth (mm), shoot and root dry weight ( $\text{g}^{-1}$  plant) as well as seed yields ( $\text{kg}^{-1}$  ha) as compared with plants supplied with water optimally. Varieties *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* recorded best bean yields as compared with *KAT B9* and *KAT B1*, hence indicating their genetic potential in performing in adverse water supply. The interactions between inoculations showed that *Rhizobium* inoculation in water stress treatment imposed at vegetative and flowering stage had greater positive effects on growth and yield as compared with un-inoculated treatments. These results suggest that inoculating beans with rhizobial inoculants enhanced growth even in water stressed environment. The interactive effects of varieties *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* by performing well under water stressed environment demonstrates their potential of being used in drought tolerance studies.

## Acknowledgement

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