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Root architecture and rhizobial inoculation in relation to drought stress response in common bean (*Phaseolus vulgaris* l.)

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Abstract: The present study was aimed at assessing the root traits and rhizobial inoculation in relation to drought in common bean, *Phaseolus vulgaris*. Drought caused the largest decrease in shoot biomass followed by plant height, while an increase was recorded inroot/shoot ratio. *Rhizobial* inoculation caused largest increase in shoot biomass followed by root volume and root biomass and smallest increase in rooting depth. WB-216 and WB-185 had better rooting depth in all treatments. However, WB-83 (92.67) had highest rooting depth under irrigated conditions and SR-1 had highest rooting depth under irrigated conditions treated with *rhizobium* (108.50). Similarly, WB-216 had highest root/shoot ratio under drought (2.693) followed by WB-185 (1.285) while lowest value was recorded for Arka Anoop (0.373). In *rhizobium* treated drought condition, WB-216 recorded highest root/shoot ratio (5.540) followed by SFB-1 (1.967). Under irrigated conditions (both with and without *rhizobium*), WB-185 recorded highest root/shoot ratio while lowest was recorded for SR-1 (0.166). The mean squares due to root depth, root biomass and root volume were significant whereas the mean squares due to water and *rhizobium* were non-significant. Among interactions the genotype x water regime was significant for rooting depth (5 % level), genotype x *rhizobia* was significant for rooting depth and root volume (1 % level) and the interaction of genotype x water regime x *rhizobium* was significant for rooting depth, root biomass and root volume (1 % level). The results reinforce the need to further analyse the potential of other soil microbes in common bean rhizosphere in amelioration of the effects of water stress.

Keywords: Common bean, Drought stress, Root traits, *Rhizobia*

INTRODUCTION

Water shortages are responsible for the greatest crop losses around the world and are expected to worsen, heightening international interest in crop drought tolerance. Within the U.S. alone, about 67% of crop losses over the last 50 years have been due to drought (Comas *et al*, 2013). Drought stress is a worldwide production constraint of common bean (Teran and Singh, 2002). Prolonged drought either as early season, or intermittent or terminal drought, which are generally enhanced by heat and low air relative moisture, are the most damaging for bean and cause an increased frequency of barren plants and incomplete seed setting. Globally about 60 % of beans are produced in areas with intermittent or terminal drought risk, making it the second largest contributor to yield reduction after disease (Rao, 2001). Water stress during the flowering and grain filling periods reduced seed yield and seed weight and accelerated maturity of dry bean (Singh, 1995). Abiotic stresses (such as drought and high temperature) are reported to cause greater yield reductions, given that they are widespread, often intense and occur almost every year (Wortman *et al.*, 1998).

Legumes have long been recognized as being sensitive to drought stress (Sprent, 1972). For a number of grain legumes, N_2 fixation has been shown to decline early during the soil dehydration cycle, preceding leaf gas exchange and all other measures of drought stress (Sinclair and Serraj, 1995). About two-thirds of common bean (*Phaseolus vulgaris* L.) production in the developing world occurs under conditions of significant drought stress (Graham and Ranalli, 1997). Because common bean is frequently grown on drought-prone soils, a high sensitivity to soil dehydration may constitute an important constraint on N accumulation and yield potential.

The crop-microbial interactions in pulses have been reported to enhance productivity, quality as well resilience to various biotic and abiotic stresses. Several limiting factors such as drought , salinity, and high temperatures, that can dehydrate the plant tissues and cause irreversible cellular damage and death. Symbiotic nitrogen-fixing bacteria such as *Rhizobium* and related genera have the capacity of synthesizing trehalose, a sugar whose accumulation has been detected in bacteroids as well as in nodules, and helps retain water in cells. The yield of bean plants inoculated with *R. etli* over expressing trehalose-6-phosphate synthase gene and grown with constant irrigation increased more than 50 %. (Suárez*et al*, 2008). Co-inoculation of bean with *Rhizobium* and both *Paenibacillus* strains resulted in increased plant growth, nitrogen content and nodulation compared to inoculation with *Rhizobium* alone. Drought stress triggers a change in phytohormonal balance, including an increase in leaf abscisic acid (ABA) content, a small decline in indole acetic acid (IAA) and gibberellic acid (GA3) and a sharp fall in zeatin content in bean leaves (Figueiredo *et al*, 2008). For legume crops depending on nitrogen-fixation, drought affects plant growth and metabolisms (Aydi *et al*., 2008). Moreover, it affects several aspects of nodule functioning including nitrogen fixation, metabolites synthesis (protein, malate and leghaemoglobin) and enzymatic activities (Mhadhbi *et al*., 2009). Drought can also limits nodulation through its effects on persistence and survival of *rhizobia* in the soil, root-hair colonization as well as infection by *rhizobia.*

The roots have been long recognized for defining the ability of the plants to access and acquire soil resources. Quantifying the architecture of root systems is important because crop productivity is almost always influenced by the availability and accessibility of water and nutrients in soil. Adaptation to drought stress encompasses morphological, physiological, and biochemical mechanisms (Rao, 2001), including a deeper root system.Water defines the limits of crop yields more than any other factor (Kramer and Boyer, 1997). One of the major challenges before plants under limited moisture situations is to acquire more water from deeper layers due to quick exhaustion by evaporative losses from both plants as well as top soil. Therefore, roots assume great significance in defining plants ability to survive and produce optimally under drought. However, in legumes such as common bean, roots are also intimately associated with rhizosphere microbes including *rhizobium* and the symbiotic association are known to confer fair degree of tolerance through various mechanisms including trehalose secretion (Suárez*et al*, 2008). In fact, the use of tolerant *rhizobia* to alleviate salt and drought stresses on legumes showed promising results (Mnasri *et al*., 2007 and Mhadhbi *et al*., 2009). However, N_2 fixation is more sensitive to moisture stress and constitutes an important constraint on nitrogen accumulation and the yield potential of legumes under drought stress. Even though the microbial partner is tolerant to stress as compared to plant partner, it will require further studies to establish the evidence that the tolerance is actually transferred to symbiosis relationship (Arrese-Igor *et al*, 2011). Studies on root architecture and soil microbes in relation to plant response to drought have been undertaken in isolation. The present study was undertaken to elucidate the relationship of root traits and rhizobial treatment on drought response in common bean.

MATERIALS AND METHODS

Plant material: The experimental material for present study comprised of six genotypes of common bean (*Phaseolus vulgaris* L.) viz., WB-185, WB-216, Wb-83, SFB-1, ARKA ANOOP and SR-1. All except WB-83 are determinate bush types while as WB-83 is an indeterminate pole type. SFB-1, SR-1 and ARKA ANOOP are released varieties by Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST-Kashmir) and Indian Institute of Horticulture Research (IIHR), Bangalore respectively.

Experimental set up

Laboratory experiment: For the measurement of traits viz., basal root whorl number, basal root number and basal root growth angle, seeds were germinated in transparent gel (2 % agar) filled plastic Petri plates (Christopher *et al*., 2012). Four seeds for each genotype were surface-sterilized with 0.5 % NaOCl for one minute, rinsed thoroughly with distilled water and were put in the petri plates containing moist filter paper. Two days after, the seeds germinated and the radi-

Table 1. Mean performance of root traits for six common bean (*Phaseolus vulgaris* L.) genotypes.

Genotype	Basal root angle (^{o})	Tap root length (cm)	Basal root number	Root length (cm)	Lateral root number
WB-185	44.623	13.833	12.333	59.633	38.667
WB-216	36.333	14.333	13.667	71.467	35.667
WB-83	44.667	12.000	8.333	35.300	26.333
SFB-1	51.983	13.667	10.000	32.167	24.667
Arka Anoop	46.333	12.333	12.333	34.700	20.667
SR-1	49.417	9.667	10.667	20.800	12.333
LSD (5%)	9.830	2.868	2.852	14.287	8.169

Table 2. Effects of drought on various root and shoot traits in common bean (*Phaseolus vulgaris* L.)

Treatment	Rooting depth (cm)	Root biomass (g)	Root volume (cm^3)	Plant height (cm)	Shoot biomass (g)	Root/shoot ratio
Drought	67.569	7.003	8.673	32.583	10.494	0.667
Irrigated	89.234	9.507	10.450	64.266	31.106	0.306
% increase or decrease	-24.278	-26.388	-17.000	-49.299	-66.263	+117.973
CD	8.330	1.332	1.634	11.124	4.840	0.134

cle emerged. The germinated seeds were transferred to the 15 x 15 cm square plastic Petri plates containing 2 % sterilized solid agar medium (2 % w/v) in darkness in germinator at 25 ^{o}C. The germinating seeds were placed from cut sides of the Petri plates with radicle inserted into the agar and kept for 7 days under darkness at room temperature and after 7 days data was recorded for basal root whorl number, number of basal roots and basal root growth angle. Basal root angle was measured as average of the angles of basal roots from the vertical plane.

Greenhouse experiment: The present study was conducted during 2015-16 at the controlled atmosphere greenhouse. Plants were maintained under optimum temperature (28°C) conditions from sowing to harvest. The seeds were sown in PVC columns of dimensions 120 cm height and 20 cm diameter. The columns were filled with equal quantity of a mixture of soil, sand and vermicompost in 2:2:1 ratio. The seeds were surface sterilised with NaOCl (10 %) for 5 minutes and rinsed with distilled water. The seeds in case of rhizobial inoculation treatment were treated with a locally isolated isolate of *Rhizobium phaseoli* liquid culture for 20 minutes following drying in shade before sowing. Initially four seeds were sown in each column at 4 cm depth and continuously irrigated till the crop reached the first trifoliate leaf stage, at which two competitive plant were retained per column. Drought stress was imposed by withholding water to drought treatments whereas irrigated treatment was regularly irrigated to field capacity. Three replications each were used for both drought and irrigated treatments in a factorial completely randomised design. Four weeks after the imposition of drought stress, the plants were harvested from columns. The roots were carefully harvested from columns and were carefully separated from the growing medium without any breakage in the root system. The shoot of each plant was separated by cutting at the base of the stem. After removing shoots, roots were laid on a flat surface and stretched to measure their length (from the base of the stem to the tip of the root system) as an estimate of rooting depth. Other parameters observed were root biomass, root volume, plant height and shoot biomass and root shoot ratio.

Table 3. Effects of rhizobium on various root and shoot traits in common bean (*Phaseolus vulgaris* L.).

Treatment	Rooting depth (cm)	Root biomass (g)	Root volume (cm^3)	Plant height (cm)	Shoot biomass (g)	Root/shoot ratio
Without rhizobium	74.222	6.0037	6.527	46.527	10.283	0.583
With rhizobium	82.291	10.485	12.520	45.500	29.250	0.358
% increase or decrease	+10.871	+74.640	+91.816	-2.014	+184.448	-38.603
CD	8.330	1.332	1.634	11.124	4.840	0.134

Table 4. Treatment wise values for root and shoot traits in six common bean (*Phaseolus vulgaris* L.) genotypes.

Genotype	Treatment	Rooting depth (cm)	Root biomass (g)	Root volume (cm^3)	Plant height (cm)	Shoot biomass (g)	Root/shoot ratio
WB-216	Drought	95.00	11.5	15.00	27.00	4.27	2.693
	Irrigated	78.25	5.00	5.00	35.00	10.67	0.468
	Drought + rhizobium	97.00	13.63	23.25	25.00	2.46	5.540
	Irrigated + rhizobium	96.75	13.75	15.00	36.75	26.75	0.514
WB-83	Drought	70.00	5.90	3.33	51.00	7.33	0.804
	Irrigated	92.67	8.25	10.00	122.33	12.95	0.637
	Drought + rhizobium	32.25	4.65	7.50	36.50	8.50	0.549
	Irrigated + rhizobium	95.75	9.56	13.33	118.33	18.22	0.520
WB-185	Drought	92.50	7.52	7.50	26.00	5.85	1.285
	Irrigated	85.25	10.17	7.50	42.00	13.57	0.749
	Drought + rhizobium	107.00	11.43	13.33	31.50	31.50	0.362
	Irrigated + rhizobium	97.75	17.50	13.47	46.00	15.57	1.123
SFB-1	Drought	43.25	2.27	5.00	27.00	3.12	0.727
	Irrigated	75.00	7.65	7.50	79.00	20.65	0.370
	Drought + rhizobium	35.33	6.10	6.67	27.00	3.10	1.967
	Irrigated + rhizobium	97.25	7.50	10.75	56.00	23.42	0.320
Arka Anoop	Drought	56.00	3.52	5.00	40.00	9.42	0.373
	Irrigated	72.50	4.62	5.00	42.00	17.30	0.267
	Drought + rhizobium	40.50	4.70	5.00	35.00	7.50	0.626
	Irrigated + rhizobium	92.67	10.00	11.30	54.00	38.55	0.259
SR-1	Drought	55.25	3.325	5.00	28.00	4.30	0.773
	Irrigated	75.00	2.32	2.50	39.00	13.97	0.166
	Drought + rhizobium	86.75	9.50	7.50	37.00	10.58	0.897
	Irrigated + rhizobium	108.50	17.50	23.15	44.00	36.75	0.476
CD		14.395	2.282	2.824	19.268	8.356	0.117

Table 5. Analysis of variation for root and shoot traits in common bean (*Phaseolus vulgaris* L.)

Source of Variation	d.f.	Root depth	Root biomass	Root volume	Plant height	Shoot biomass	Root shoot ratio
Genotype	5	1368.860**	85.435**	167.067**	243.335	414.567	511.56*
Water regime	1	110.510	0.844	6.000	219.010	12.042	334.66*
Rhizobium	5	49.594	0.260	0.011	61.760	7.742	9.41
Genotype x Water regime	1	977.260*	20.669	14.350	305.735	12.042	488.74*
Genotype x Rhizobium	5	1029.994**	7.735	21.950**	299.285	11.242	253.24*
Water regime x Rhizobium	1	319.010	10.010	1.500	78.844	1.500	200.72
Genotype x Water regime x Rhizobium	5	1611.260**	43.935**	56.200**	685.319	20.500	1096.98**
Error	69	416.390	10.483	16.023	742.448	140.521	118.12

RESULTS AND DISCUSSION

Lab experiment: The results pertaining to various root parameters and the treatment effects are presented in Table 1. Basal root growth angle was lowest in WB 216 (36.33) while as it was highest in Shalimar French Bean 1 (51.983). Tap root length was highest in WB 216 (14.333) while as it was lowest in SR1 (9.667). Similarly, the basal root number was highest in WB 216 (13.667) and lowest in case of WB 83 (8.33). The total root length was highest in WB216 (71.467) and lowest in SR-1 (20.00). The lateral root number was highest in WB 185 and lowest in SR 1 (12.333). Rehman *et al* (2015) reported similar results in common bean for root traits and response to PEG under laboratory conditions and concluded that the high throughput screening through laboratory based screening can be effectively used for large scale germplasm characterisation in common bean for response to water shortage. In fact, prolific root systems invariably confer the advantage in extracting water from deeper as well as shallower soil layers that is otherwise easily lost by evaporation. Drought tolerant genotypes have either a higher number of basal roots or the roots have a higher specific root length (SRL).

Greenhouse experiment: The main effects of water are presented in Table 2. Drought stress caused largest decrease in shoot biomass (66.263 %) followed by plant height (49.299 %), while as lowest decrease was recorded in root volume (17.000 %). The main effects of rhizobia are presented in Table 3. *Rhizobial* inoculation caused largest increase in shoot biomass (184.448 %) followed by root volume (91.816 %) and root biomass (74.640 %), whereas smallest increase was recorded for rooting depth (10.871%), however, a decrease of 38.593 % was recorded for root/shoot ratio. Based on CD values, all the increases or decreases caused by drought and rhizobium treatments were significant. Interestingly, the rhizobia caused a decrease in root/shoot ratio (-38.593 %) and plant height (-2.014 %), however the reduction was non-significant. *Rhizobium* has been reported to increase plant height, leaf area, photosynthetic rate and dry matter production under irrigated conditions (Thakur and Panwar, 1995). In the present study, the plant height recorded a decrease under rhizobial treatment, which was on account of decreased plant height under drought conditions as water stress significantly reduces rhizobial activity and N2 fixation (Pimratch *et al.*, 2008). In fact drought leads the plant and the bacteria to decrease their internal water potential to avoid desiccation (Tonon *et al.*, 2004). For legume crops depending on nitrogen-fixation, drought affects plant growth and metabolisms (Aydi *et al.*, 2008). Moreover, it affects several facets of nodule functioning including nitrogen fixation, metabolites synthesis (protein, malate and leghaemoglobin) and enzymatic activities (Marino *et al.*, 2007). Drought limits nodulation through its effects on persistence and survival of *rhizobia* in the soil, root-hair colonization and infection by *rhizobia* (Zahran, 1999).

The mean performance of genotypes for various traits under different treatments is present in Table 4. WB-216 and WB-185 had better rooting depth in all four cases viz., drought, irrigated, drought with *rhizobium* and irrigated without *rhizobium*. However, WB-83 had highest rooting depth under irrigated conditions (92.67) and SR-1 had highest rooting depth under irrigated conditions treated with *rhizobium* (108.50). Similar trend was recorded for root biomass and root volume. Plant height was highest in case of WB-83 under both irrigated and drought conditions, while as SR-1 had highest plant height under drought treated with *rhizobium*. This is understandably due to the fact that WB-83 is a pole type genotype whereas all others are bush types. The data for plant height did not correlate with shoot biomass and the lines having greater plant height did not have higher biomass. In case of root/shoot ratio WB-216 had highest ratio under drought followed by WB-185 while lowest value was recorded for Arka Anoop. In case of *rhizobium* treated drought condition WB-216 recorded highest root/shoot ratio followed by SFB-1 while lowest value was recorded for WB-185. Under irrigated conditions (both with and without *rhizobium*), WB-185 recorded highest value for root/shoot ratio while lowest was recorded for SR-1 and Arka Anoop respectively. Ahmed *et al* (2006) and Safapour *et al* (2011) also reported a significant effect on yield traits such as biological yield under rhizobial inoculation of common bean.

Analysis of variance for root and shoot traits (Table 5) revealed that among main effects the mean squares due to root depth, root biomass and root volume were significant whereas the mean squares due to water and *rhizobium* were non-significant. Among interactions the genotype x water regime was significant for rooting depth, genotype x rhizobia was significant for rooting depth and root volume and the second order interaction of genotype x water regime x *rhizobium* was significant for root depth, root biomass and root volume. The interaction component of water regime x rhizobia was non-significant for all the traits. Legumes and their symbiotic root nodule bacteria are extremely sensitive to drought stress (Sinclair *et al.*, 2001). The persistence of rhizobial strains, and their symbiotic performance is affected by various biotic and abiotic factors (Bordeleau *et al.*, 1994), with drought stress and nitrogen deprivation, being among the most significant. Other important factor is the root exudation ability, which it will determine plant microbe associations so that the survival and tolerance of rhizobia during water restriction. The fact that N2 fixation is more sensitive to decreasing soil water content relative to leaf gas exchange constitutes an important constraint on N accumulation and the yield potential of legumes subjected to soil drying (Serraj *et al.*, 2001). This sensitivity is particularly relevant in view of the facts that water is a major limiting factor in world agriculture, and that in general, most crop plants are highly sensitive to even mild dehydration stress (Mundree *et al.*, 2002).

Conclusion

The present study aimed at studying combined role of root traits and *rhizobium*, showed that rhizobial inoculation improves root parameters as well as overall growth of *P. Vulgaris* under drought stress. The rhizobial inoculation caused an increase of 184.448 % in shoot biomass, followed by root volume (91.816 %) and root biomass (74.640 %). Since the application of *rhizobium* is easy and does not require any wholesale changes in farming practices, it can be suitably included in crop management practices especially in case of rainfed pulses like common bean to enhance drought tolerance as well as favourably affect the plant growth. However, there is a need to explore the species diversity of native rhizobium strains that can be efficiently used for enhanced plant growth and productivity especially under stress conditions.

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