

Original Research Article

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## Mitigating Drought in Mungbean using *Rhizobium* Induced Alteration in Physiological Traits

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### ABSTRACT

#### Keywords

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A field experiment was conducted during *kharif* season at Crop Physiology Field Area, CCS Haryana Agricultural University, Hisar, India with the objectives to assess the mitigating effect of different *rhizobial* strains on physiological and biochemical traits in mungbean and to measure the association of these traits with crop performance under drought condition. Crop was raised under optimum conditions (irrigated) or drought stress without any post sowing irrigation (rainfed conditions). The experimental treatments consisting of (a) without inoculation (only RDF) and (b) with inoculation (RDF with combination of five *rhizobial* strains viz. *Vigna* 703 + PSB strain P-36, MR 63, MR 54, MB 17a and MH 8b2). The measurement of chlorophyll fluorescence (Fv/Fm), membrane stability index (MSI%), chlorophyll content, canopy temperature depression (CTD) were done at 50% flowering, which were found to be decreased by 16.3%, 17.7%, 2.9% and 88%, respectively under drought stress. The plants inoculated with *rhizobial* isolate MR63 and MB 17a showed greater Fv/Fm (18.7% and 15.9%), MSI% (19.4% and 17.9%), chlorophyll content (20.2% and 16.2%) and CTD (151.3% and 104.8%) respectively over RDF. Significant positive correlation was observed among seed yield and MSI (%); seed yield and chlorophyll fluorescence. CTD has a significant negative correlation with chlorophyll content and seed yield.

### Introduction

Mungbean is one of the most important food legumes grown in major part of the country. India alone accounts for more than half (54%) of the world production with production and productivity of 2.025 m ha and 428 kg/ha, respectively in *kharif* and 0.994 m ha and 640 kg/ha, respectively in summer season (Arneh, 2016). However, water stress has been found

to decrease productivity of most crops and in mungbean its effect is more pronounced at reproductive stage and yield is drastically reduced (Majeed *et al.*, 2016).

Drought tolerance is a highly complex phenomenon involving many tolerance mechanisms that are inter-related with each other. There are many traits like chlorophyll fluorescence (Fv/Fm), membrane stability

index (MSI%), chlorophyll content, canopy temperature depression (CTD) and yield related traits contributing towards drought stress tolerance (Talebi *et al.*, 2013). Stressful environments considerably hamper the process of photosynthesis in most plants by altering concentration of various pigments involved in this process. Light energy absorbed by Chlorophyll is transformed into Chlorophyll fluorescence (Maxwell and Johnson, 2000). Despite the fact that the extent of Chlorophyll fluorescence does not comprise more than 1–2% of total light absorbed by the Chlorophyll, its measurement is convenient and noninvasive (Ashraf and Harris, 2013). It gives a valuable insight into exploitation of the excitation energy by Photosystem II and indirectly by the other protein complexes of the thylakoid membranes (Roháček, 2002). The role of the intact cell membrane remains to be more critical for adaptation of plant in drought stress conditions. It has been reported that drought tolerant genotypes are superior to susceptible ones in maintaining membrane stability and lowering membrane injury under drought stress conditions (Pouresmael *et al.*, 2013).

Several studies have been focused on *rhizobium* mediated stress tolerance in legumes as the ability of symbiotic fixation offer an opportunity to improve nitrogen status of the soil and crop productivity under stress conditions. But to upgrade the existing *rhizobial* strains with more promising new *rhizobial* isolates is the demand of hour. So this work aimed to evaluate the responses of different promising *rhizobial* isolates for physiological traits and to correlate these traits with seed yield under drought condition

## Materials and Methods

The present investigation was carried out during *khari*f2016 at the drought plots of Crop

Physiology Area, Department of Agronomy, CCS Haryana Agricultural University, Hisar, India (29<sup>0</sup>10' N and 75<sup>0</sup>46' E). The soil of the experimental field was loamy sand, low in available Nitrogen (112.7 kg/ha) and medium in available Phosphorus (12.0 kg/ha). The mungbean variety MH-421 was used as the test crop. Sowing was done with hand ploughed and each treatment was sown in six rows of 2.0 m length, with row to row distance of 30 cm. There was distance of one meter between each rhizobial treatment to avoid mixing/ spread of rhizobia with each other. The experiment was laid out in randomized block design with three replications.

Crop was grown in two environments viz, drought condition (no post sowing irrigation with rainout shelter) and irrigated (two irrigations at pre-flowering and pod formation stage) with six treatment combinations of Recommended dose of fertilizer (RDF) and rhizobial strains i.e. RDF + *Rhizobium* sp.(*vigna*) 703 + PSB strain P-36, RDF + MR 63, RDF + MR 54, RDF + MB 17a and RDF + MH 8b2. The *rhizobial* isolates were procured from Department of Microbiology, CCS Haryana Agricultural University, Hisar and the seed inoculation was done 2-3 h before sowing. All the recommended agronomic practices were followed for raising the crop. Three plants per treatment were taken as one replication to recording the data. All the physiological parameters were recorded at flowering stage on third or fourth leaf (youngest fully expanded) from the apex on the main shoot.

Canopy temperature Depression (°C) was measured using hand held Infrared Thermometer (Model AG-42, Tele temp Corp. Fullerton) between 13:00 to 14:00 hours on cloudless, bright days while Chlorophyll fluorescence (Fv/Fm) was recorded in intact plants using chlorophyll

fluorometer (OS-30p, Opti-Science, Inc., Hudson, USA) between 9:00 to 10:00 AM. For chlorophyll estimation, leaf discs (0.05 g) were washed, blotted dry and dipped in test tubes containing 5 ml of dimethyl sulfoxide (DMSO) overnight as described by Sawhney and Singh (2002). The extracted chlorophyll in DMSO was estimated by recording its absorbance at 663 and 645 nm. The membrane stability was estimated by the procedure of Dionisio-Sese and Tobita (1998). 100 mg of leaf tissue was taken separately in 20 ml test tubes containing 10 ml of de-ionized water at 25°C. After 4 h, the electrical conductivity (ECa) of the solution was measured. Then the samples were kept in boiling water bath for 1 h to achieve total killing of the tissue. After cooling, the ECb of the solution was measured. Membrane stability index (MSI) was calculated as follows:

$$\text{MSI (\%)} = 1 - \frac{\text{ECa}}{\text{ECa} + \text{ECb}} \times 100$$

The total number of branches and pods per plant were counted at physiological maturity. Biological and seed yield was recorded for each plots and expressed in kg per hectare. During The results of both the years were pooled and analyzed using online statistical tool OPSTAT software of the Computer Centre, Department of Mathematics and Statistics, CCS Haryana Agricultural University, Hisar. Treatments, environments and interaction between treatments and environments were compared using critical difference (CD) at 5% level of significance. Correlation and regression were also calculated.

## Results and Discussion

Canopy temperature depression (CTD) as measured by thermal imaging is the difference in temperature between the canopy surface and the surrounding air. The crop

canopy was warmer in drought as compared to irrigated at flowering stage. Canopy temperature depression increased (0.75 to 1.42°C) significantly under water stress as shown in Table 1. Canopy temperature increased under drought might be due to decreased transpiration resulted from stomatal closure. As the rate of transpiration decreased, the amount of heat that can be dissipated contributed to higher canopy temperature under water stress as reported by Moradi *et al.*, (2008) and Rao *et al.*, (2015). Among the *rhizobial* treatments, RDF alone maintained warmer canopy with canopy temperature depression value of 1.50 °C and the coolest canopy was maintained in RDF + MR 63 (0.60°C) followed by RDF + MB 17a (0.73°C) irrespective of the irrigation environment. The interaction of irrigation environment and *rhizobial* strain was found significant. The photosynthetic efficiency (Fv/Fm) significantly declined from 0.703 to 0.588 under drought condition (Table 1). The highest value of quantum yield was observed in plants treated with *rhizobial* strain MR 63 followed by MB 17a (0.699 and 0.675 respectively), whereas, the quantum yield in RDF was observed to be lowest (0.568). Similar effect of drought stress on quantum yield was also observed by Summy *et al.*, (2015) in chickpea. Total chlorophyll content decreased (2.9%) under water stress over irrigated. Such decrease in chlorophyll content in the leaves of plants may be attributed to the high rate of chlorophyll degradation more than its biosynthesis under water stress conditions (Raina *et al.*, 2016). Application of *rhizobial* isolates increased the total chlorophyll content and the increase was more in RDF + MR 63 (4.91 mg/g FW) followed by RDF + MB 17a (4.70 mg/g FW) over RDF (3.91 mg/g FW). The increased chlorophyll in *rhizobial* inoculated treatments may be due to improved plant water status resulted in reduced chlorophyll degradation. Similar increase in chlorophyll content with

application of *rhizobial* treatments was reported by Tairo *et al.*, (2017).

The reduction in number of pods (13.17 to 9.51) and number of branches (3.67 to 3.33) per plant was observed under drought (Table 2). This reduction in the yield attributing characters might be due to

adversely affected physiological processes under drought (Table 1). The yield attributes contributing toward the seed yield were the development of pods and number of branches per plant. The drought stress adversely affected both of these parameters (Praharaj *et al.*, 2016 and Davari, 2017).

**Table.1** Effect of soil moisture and rhizobial isolates on Physio-chemical parameters of mungbean

Treatment	Canopy temperature depression (°C)			Chlorophyll fluorescence (Fv/Fm)			Membrane stability index (%)			Chlorophyll content (mg/g FW)		
	IR	D	Mean	IR	D	Mean	IR	D	Mean	IR	D	Mean
RDF (N and P)	1.14	1.85	1.50	0.613	0.523	0.568	13.04	10.82	11.93	3.93	3.88	3.91
RDF + Vigna703+P-36	1.03	1.63	1.33	0.680	0.560	0.620	15.81	12.16	13.99	4.22	4.14	4.18
RDF + MR 63	0.21	0.98	0.60	0.777	0.620	0.699	20.91	17.84	19.38	5.00	4.82	4.91
RDF + MR 54	0.92	1.50	1.21	0.707	0.600	0.654	17.32	14.35	15.84	4.31	4.17	4.24
RDF + MB 17a	0.37	1.09	0.73	0.733	0.617	0.675	19.25	16.57	17.91	4.75	4.64	4.70
RDF + MH 8b2	0.84	1.47	1.16	0.707	0.610	0.659	17.93	14.72	16.33	4.34	4.14	4.24
Mean	0.75	1.42		0.703	0.588		17.38	14.41		4.43	4.30	
CD (P=0.05)	E=0.18; T=0.32; E×T= 0.42			E= 0.034 T=0.059 E×T= 0.071			E= 1.19; T=2.04; E×T= 2.71			E= 0.10; T= 0.22;E×T=0.37		

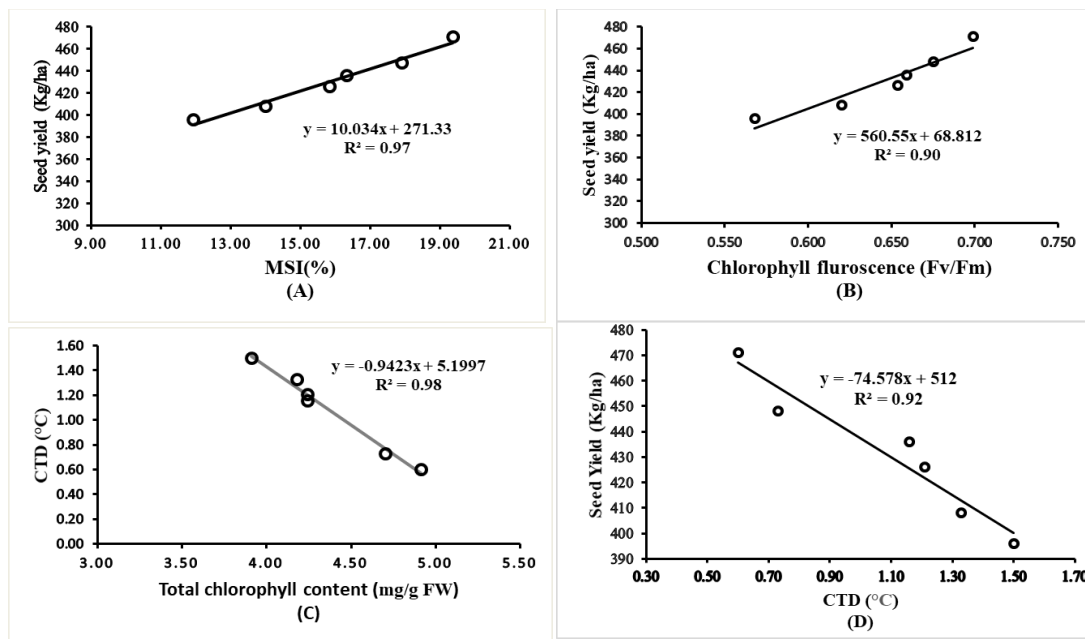
\* E=Environment, T= Treatment, IR= Irrigated, D=Drought

**Table.2** Effect of soil moisture and rhizobial isolates on yield attributes and yield of mungbean

Treatment	# branches plant <sup>-1</sup>			# pods plant <sup>-1</sup>			Biological yield (kg ha <sup>-1</sup> )			Seed yield (kg ha <sup>-1</sup> )		
	IR	D	Mean	IR	D	Mean	IR	D	Mean	IR	D	Mean
RDF (N and P)	3.37	3.00	3.19	11.33	7.67	9.50	1852	1452	1652	469	322	396
RDF + Vigna703+P-36	3.57	3.13	3.35	12.33	8.67	10.50	1907	1533	1720	485	331	408
RDF + MR 63	4.03	3.77	3.90	15.00	11.33	13.17	2130	1730	1930	542	400	471
RDF + MR 54	3.53	3.27	3.40	13.00	9.27	11.14	1963	1582	1773	501	351	426
RDF + MB 17a	3.83	3.50	3.67	14.00	10.43	12.22	2037	1656	1847	521	375	448
RDF + MH 8b2	3.70	3.33	3.52	13.33	9.67	11.50	1982	1563	1773	514	357	436
Mean	3.67	3.33		13.17	9.51		1979	1586		505	356	
CD (P=0.05)	E=0.21; T=0.43; E×T=0.53			E=0.56; T=1.04; E×T= 1.67			E=127; T= 88; E×T= 186			E=52; T= 32; E×T= 76		

\* E=Environment, T= Treatment, IR= Irrigated, D=Drought

**Fig.1** Relationship between Seed yield and (A) Membrane stability index (MSI %), (B) Chlorophyll fluorescence & between Canopy temperature depression (CTD) and (C) Chlorophyll content and (D) Seed yield



Maximum number of branches (3.90 and 3.67) was observed in plants inoculated with RDF + MR 63 and RDF + MB 17a, respectively while, the minimum was observed in RDF (3.19) followed by RDF + *Vigna* 703+P 36(3.35) irrespective of the environments. Among the *rhizobial* treatments, maximum number of pods were observed in MR 63 (13.17) and MB 17a (12.22), while the minimum was noticed in control (RDF) followed by RDF + *Vigna* 703+P 36 (10.50) which was statistically at par with RDF. Similar response was observed for number of branches with *rhizobial* isolates. The interaction between *rhizobial* treatments with the environments was found to be significant.

Crop inoculated with MR 63 and MB 17a *rhizobial* strains showed maximum increase in seed yield in both the irrigated and drought conditions over RDF. Thus, the response of applied *rhizobial* inoculation in terms of yield and its attributes was relatively more as

compared to non-inoculated plants. Similar response of *rhizobial* inoculation induced yield improvement was reported by Kumari *et al.*, (2015), Raof *et al.*, (2016) and Tena *et al.*, (2016).

The CTD was significantly correlated with the chlorophyll content and seed yield (Fig. 1A and B). The higher the CTD, the lower were the chlorophyll content and seed yield and vice-versa. Seed yield was positively and significantly correlated with MSI (%) and Fv/Fm (Fig. 1C and D).

On the basis of this study, it is concluded that *rhizobial* inoculation significantly improved physiological traits that had direct association with yield formation via yield attributes. The measurements of CTD, MSI, Fv/Fm and chlorophyll content could be used as an easy and rapid tool to evaluate the relative performance of crop under drought conditions. Plants inoculated with *rhizobial* isolate MR63 and MB 17a were more

promising with better photosynthetic efficiency, low membrane injury, cooler canopy temperature and higher seed yield.

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