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Impact of Different *Rhizobial* Strains on Physiological Responses and Seed Yield of Mungbean [*Vigna radiata* (L.) Wilczek] under Field Conditions

Sapna, K.D. Sharma

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ABSTRACT

Background: Water availability is a main controlling factor for agricultural productivity. Erratic monsoon and ever-growing population limit the water accessibility to mungbean and thus reduce its yield. *Rhizobia*–legume interactions are environmental friendly and are very well known for boosting the production potential of mungbean. But, the screening of new and better *rhizobial* isolates is essential to improve mungbean yield in sub-optimal conditions. So this study was conducted with the objectives to evaluate the *rhizobial* isolates for the physiological traits in mungbean and to measure the association of these traits with yield under moisture stress.

Methods: In this field experiment during *kharif* 2016, five *rhizobial* strains of mungbean *viz.* V*igna* 703 + PSB strain P-36, MR 63, MR 54, MB 17a and MH 8b2 were tested with respect and in combination with RDF at two water levels *viz*. control and drought. Moisture stress was created by withholding irrigations at flowering and pod formation stages.

Result: Growth and physiological traits were altered under moisture stress. *Rhizobial* isolates MR 63 and MB 17a sustained higher yield over other *rhizobial* strains under both moisture regimes. These strains maintained higher RWC, leaf water potential and photosynthesis during stress and appear promising for drought tolerance.

Key words: Drought, Mungbean, Rhizobial Inoculation, Yield.

INTRODUCTION

The mungbean (*Vigna radiata*), alternatively known as the green gram or mung is a plant species in the *Fabaceae* or *Leguminosae* family. It is an important pulse crop having high nutritive value. Its seed contains 24.2% protein content, 1.3% fat and 60.4% carbohydrates; calcium (Ca) is 118 and phosphorus (P) is 340 mg per 100 g of seed, respectively (Imran *et al*. 2016). In India, green gram is grown ~3.38 Mha with an annual production of ~1.61 Mt with a productivity of ~474 kg ha^{-1} (Annonymous, 2016). But the majority of the pulse production area is under rain fed environment frequently prone to drought (Kumar and Sharma 2009). Drought stress is the major environmental factor that affects plant establishment in the field, osmotic behavior and photosynthetic ability of cells (Majeed *et al*. 2016). Besides this, moisture deficit inhibits many metabolic processes and consecutively slows down the development of plants with loss of yield and productivity around the world (Baroowa *et al*. 2016). Depending upon the geographical region, nearly 40-100% yield losses due to various environmental stresses have been observed in mungbean. It has been speculated that this yield reduction will further worsen with the dramatic climate changes expected in future.

There is a great possibility to increase production potential of legume plants by exploiting better colonization of their root and rhizosphere through *rhizobial* inoculation. Legumes, has the ability to fix atmospheric nitrogen through symbiotic association with *rhizobia*. This ability of symbiotic fixation may offer an opportunity to improve nitrogen status of the soil (Sulochana and Gadgi 2017). Also the bacteria lodging around the plant roots (*rhizobacteria*) are more versatile in transforming, mobilizing and solubilizing the nutrients compared to those from bulk soils (Hayat *et al*. 2010). Therefore, the *rhizobacteria* are the dominant deriving forces in recycling the soil nutrients and consequently, they are crucial for soil fertility and crop productivity (Glick, 2012). Many researchers carried out experiments on *rhizobium* inoculation with and without fertilizers on mungbean crop (Assefa *et al.* 2017) and found increased nitrogen contents of seed, number of nodules, yield and yield components.

Yield is a complex trait resulting from the expression and association of different components. Almost all agree that screening for difficult and complex traits such as drought tolerance could be simplified by identifying physiological characters that are closely linked to yield in water-limited environments. This study was conducted primarily to determine whether the drought imposed at flowering and pod formation stages in mungbean affects leaf water status

Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar-125 004, Haryana, India.

Corresponding Author: Sapna, Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar-125 004, Haryana, India. Email: sapnayadav173@gmail.com

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and photosynthetic activity, influencing dry matter partitioning to different plant parts. Secondly, to determine which, if any, of the measured parameters could be useful for evaluating *rhizobial* isolates for drought tolerance in mungbean.

MATERIALS AND METHODS

The experiment was laid out in factorial RBD (Randomized Block Design) during *Kharif* season of 2016 in concrete drought plots (6 m x 45 m) of Crop Physiology field area, Department of Agronomy, CCS Haryana Agricultural University, Hisar. The plot size for each treatment was 2.5 × 1.8 m (six rows of 2.5 m length with 30 cm spacing). The soil of the experimental field was sandy loam in texture having EC (0.10 d Sm^{-1}), pH 8.6, low in available nitrogen (112.7 kg/ha), medium in available phosphorus (12.0 kg/ha).

The six treatments were selected with three replicates consisted of a control RDF (Recommended Dose of Fertilizer) and five rhizobial isolates with RDF *i.e*. RDF + *Rhizobium* sp. (*Vigna*) 703 + PSB strain P-36, RDF + MR 63, RDF + MR 54, RDF + MB 17a and RDF + MH 8b2. Mungbean *rhizobial* isolates were obtained from Department of Microbiology, CCS Haryana Agricultural University, Hisar and the seed inoculation was done 2-3 h before sowing. The crop was grown under two environments, i.e., control (normal irrigated) and stressed (withholding irrigations at flowering and pod formation stages. Recommended dose of fertilizers (20 kg N and 40 kg P_2O_5) and crop protection measures were adopted as per package and practices given by CCS Haryana Agriculture University.

Plant water relation parameters were measured at flowering stage during 09:30 to 10.30 AM. Water potential of leaf (LWP) was measured with the help of Pressure Chamber (Model PMS- 3005, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA) and the calculations was done using formula = - 10 bars = - 1Mpa. Measurement of relative water content (RWC) was done by the method of Barrs and Weatherley, (1962). RWC was calculated by the following formula =

$$RWC\,(\%) = \frac{\text{Fresh weight-Dry weight}}{\text{Turgid weight-Dry weight}} \times 100$$

The photosynthetic rate was measured on flag leaf using Infra-Red Gas Analyzer (IRGA, LCA-Analytical Development Company, Hoddeson, England). Transpiration rate and stomatal conductance were also determined by IRGA. The observations were taken of leaf exposed directly to sunlight on three plants randomly in each plot.

For dry matter partitioning, nine plants were harvested from three plots to make three replications at 30, 45 and 60 DAS. Roots were washed clean and each plant was separated into roots, leaves, stems and pods. Dry weights of each plant part were recorded separately after drying at 70°C for 24 h. The number of pods per plant was recorded after the final harvest at physiological maturity (60 DAS). The

Table 1: Effect of drought stress on physiological parameters of mungbean.

Treatment	Leaf water potential (Mpa)			Relative water content (%)			Photosynthetic rate (µM $m^{-2}s^{-1}$)			Transpiration rate (mM $m^{-2}s^{-1}$)			Stomatal conductance (mM $m^{-2}s^{-1}$)		
	IR	D	Mean	IR	D	Mean	IR	D	Mean	IR	D	Mean	IR	D	Mean
RDF (N and P)	-0.99	-1.23	-1.11	74.12	64.21	69.17	10.27	6.74	8.51	3.77	1.65	2.71	0.30	0.18	0.24
RDF+Vigna703+P-36	-0.84	-1.16	-1.00	76.51	66.64	71.58	11.67	7.59	9.63	3.89	1.89	2.89	0.35	0.19	0.27
RDF + MR 63	-0.68	-0.88	-0.78	81.37	70.01	75.69	14.48	10.10	12.29	5.47	2.57	4.02	0.58	0.23	0.41
RDF + MR 54	-0.76	-1.10	-0.93	77.01	68.88	72.95	12.91	9.07	10.99	4.16	2.06	3.11	0.41	0.19	0.30
RDF + MB 17a	-0.70	-0.94	-0.82	80.29	69.40	74.85	13.15	9.84	11.50	5.03	2.29	3.66	0.51	0.20	0.36
RDF + MH 8b2	-0.78	-1.03	-0.91	77.68	67.32	72.50	12.70	9.17	10.94	4.38	2.15	3.27	0.39	0.18	0.29
Mean	-0.79	-1.06		77.83	67.74		12.53	8.75		4.45	2.10		0.42	0.20	
CD (P=0.05)	E=0.17; T=0.23; E×T= 0.31			E= 1.69; T=2.42; E×T= 3.10			E= 0.70; T=1.21; E×T= 1.65			E= 0.45; T=0.78; E×T=1.02			E= 0.04; T=0.07; E×T= 0.10		

*E= Environment, T= Treatment, NS= Non significant, IR= Irrigated, D= Droughted.

remaining plot was harvested at 68 DAS and the plants were sun dried for 5 days to obtain total biomass and seed yield.

Data was analyzed using online statistical analysis package (OPSTAT, Computer Section, CCS Haryana Agricultural University, Hisar, India) with level of significance at P= 0.05. Correlation and regression were also calculated.

RESULTS AND DISCUSSION

The partitioning of dry matter to leaves was significantly higher than stems and roots except to stems at 30 DAS and roots at 45 DAS for the treatment RDF as shown in Chart 1. Afterwards, the dry matter partitioning to leaves and roots declined significantly whereas no such decline was observed for stems. Pods and nodules were not present at 30 DAS. The dry matter partitioning to nodules declined from 45 DAS to 60 DAS, while the partitioning of dry matter to pods was significantly higher at 60 DAS as compared to 45 DAS. Among the treatments, MR 63 and MH 17a received significantly higher dry matter in pods at 45 DAS while at 60 DAS, MR 54 and MH 8b2 showed higher dry matter in pods.

Droughted plants diverted significantly higher dry matter to roots and stems, while well watered plants to pods and grains. The dry matter mobilization to leaves was similar in both the environmental conditions *i.e*. irrigated and stressed. Partitioning of dry matter to nodules was noticed highest in plants treated with *rhizobial* strain MR63 at both the stages of observation (data not shown).

Leaf water potential decreased significantly under soil moisture stress as compared to irrigated control (-0.79 to -1.60 MPa). Decreased leaf water potential (ψw) under stress may be due to loss of gradient (ψw) between the soil and roots, which is the guiding principle for water movement and decline in transpiration pull (Table 1). Our results are in line with the findings of Baroowa *et al*. (2015) who reported similar trends of water potential in mungbean under water deficit. Among the *rhizobial* treatments, mean maximum leaf water potential was observed in MR 63 (-0.78 MPa) followed by MB 17a (-0.82 MPa) and it was minimum in RDF (-1.11 MPa). It can be inferred that maintenance of higher leaf water potential by the *rhizobial* isolate MR 63 might be due to its better osmotic adjustment ability to maintain active

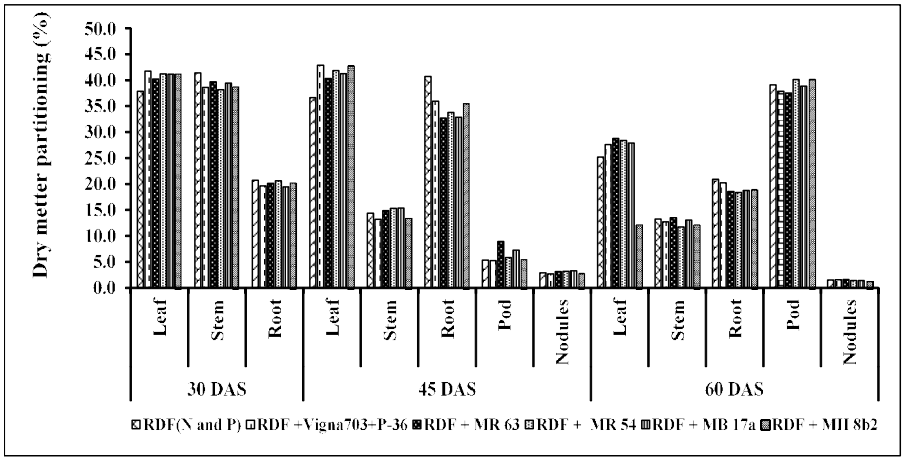

Chart 1: Dry matter partitioning in mungbean at 30, 45 and 60 DAS in irrigated conditions.
*DAS=Days after sowing.

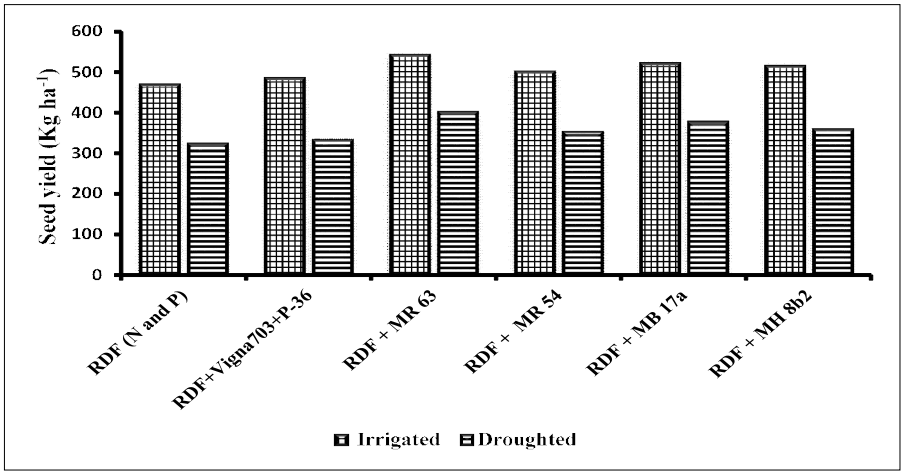

Chart 2: Effect of soil moisture stress on seed yield of mungbean.

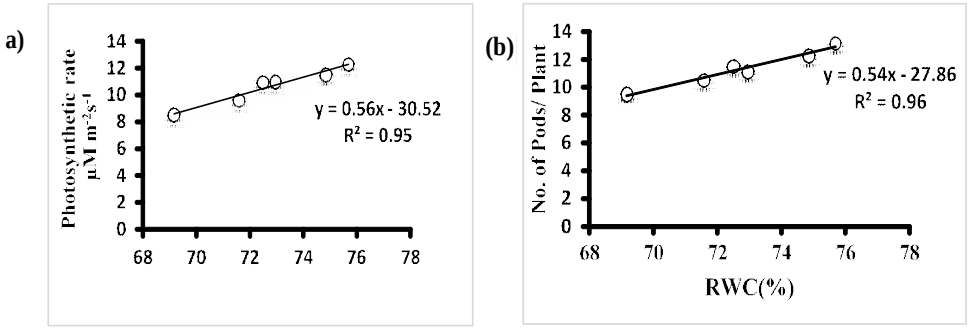

Fig 1: Relationship between leaf water content (RWC) and (a) No. of pods/plant (b) Photosynthetic rate.

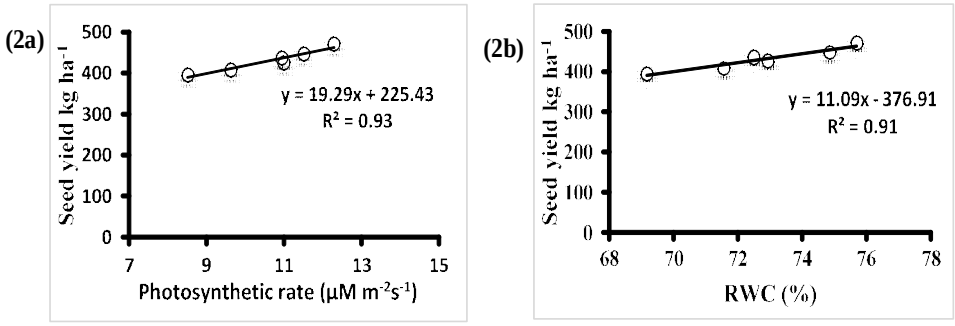

Fig 2: Relationship between seed yield and (a) Photosynthetic rate (b) RWC.

photosynthesis and transpiration even under water stress conditions.

RWC is a reliable and widely used parameter of plant tolerance to dehydration. The results of relative water content showed reduction in RWC when exposed to drought stress (77.83 to 67.74%) irrespective of the *rhizobial* treatments (Table 1). This might be due to decline in stomatal conductance and transpiration rate under drought stress. Such decline under drought stress coincides with the earlier findings in *Phaseolus vulgaris* (Martinez *et al.* 2007) and *Medicago truncatula* **(**Nunes *et al*. 2008). Plants treated with RDF + MR 63 showed higher RWC (75.7%) followed by RDF + MB 17a (74.9%) over the RDF control (69.2%) irrespective to the environment. The genotypic variations among rhizobial strains have shown varied behaviour under similar water conditions.

Photosynthetic rate was reduced significantly under the water stressed condition as compared to normal irrigated (12.53 to 8.75 µM $m^{-2}s^{-1}$). Similarly, transpiration rate and stomatal conductance also decreased significantly under the drought stress (4.45 to 2.10 mM $m^{-2}s^{-1}$ and 0.42 to 0.20 mM $m^{-2}s^{-1}$) respectively when compared on mean basis irrespective of the *rhizobial* treatments (Table 1). Reduction in gas exchange parameters may be due to decreased leaf water potential and RWC under water stress which led to loss of leaf turgor and ultimately decreased stomatal conductance (Table 1). Similar result was reported in chickpea (*Cicer arietinum* L.) by Khadraji *et al*. (2017) and, in Common bean (*P. vulgaris* L.) by Kıymaz *et al*. (2019). As evident in Table 1, treatment RDF had lowest values of photosynthetic rate (8.51 µM $m^{-2}s^{-1}$) and transpiration rate (2.71 (8.51 mM $m^{-2}s^{-1}$). The gas exchange parameters get enhanced with the application of *rhizobial* isolate MR 63 which has higher capability to combat drought than rest of the *rhizobial* isolates. Therefore, this isolate showed maximum photosynthetic rate transpiration rate and stomatal conductance under stressed as well as under normal irrigated condition.

Drought caused a significant reduction in the yield (chart 2). Drought stress reduced the source strength by reducing photosynthesis and it may be due to adverse growing environment reflected in lower plant water status (Table 1). These findings were in line with the results of Sharma and Dhanda (2014) and Praharaj *et al*. (2016) who reported that seed yield of mungbean was affected by the irrigation amount and supplemental irrigation, particularly at the pod filling stage improve plant water status resulting increased yields. The present study revealed that the *rhizobial* strain MR 63 produced the maximum yield under both the drought and irrigated environment (Chart 2). Similar response of *rhizobial* inoculation induced yield improvement was reported by Choudhary *et al.* (2019) and Tena *et al*. (2016).

Association of seed yield with physiological traits

It is interesting to note that RWC showed strong relationships with photosynthetic rates, number of pods per plant and seed yield (Fig 1a, 1b and 2b).The higher the RWC, the higher were the rates of photosynthesis and number of pods per plant and vice-versa. Traits that showed good correlation with yield were rates of photosynthesis and RWC (Fig 2a and 2b).

CONCLUSION

The results of this study showed that drought stress affect

the dry matter partitioning and plant water relation parameters that had direct bearing on yield formation via gaseous exchange parameters. Therefore, the measurement of RWC, LWP, photosynthesis, transpiration and stomatal conductance which are simple and rapid could be exploited in mungbean for crop improvement programmes of drought tolerance. Treatment of *rhizobial* isolates mitigated the effects of drought stress. In water shortage situation, *rhizobial* isolates MR 63 and MB 17a were more promising with better plant water status and higher seed yield.

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