



## Impact of phosphate solubilizing bacteria (*Bacillus polymixa*) on drought tolerance of green gram [*Vigna radiata* (L.) Wilczek]

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**Abstract:** The main focus of the present study is to evaluate the effect of priming of green gram (*Vigna radiata*) with phosphate solubilising bacteria (PSB) during drought stress. Drought is the major abiotic stress factor which diminishing the growth and development of agricultural in Kerala. So immediate steps need to be taken to overcome the adverse effect of drought stress for the development of agriculture. Phosphate Solubilizing Bacteria are one of the best microorganisms found to be simultaneously increasing the insoluble soil Phosphorus uptake by the plant and crop yield. In the study, the seeds of *Vigna radiata* were subjected to priming treatment with 0.5 % and 1% phosphate solubilising bacteria. Physiological and biochemical parameters like germination percentage, root and shoot length, relative water content (RWC), amount of chlorophyll, protein, proline and yield were studied. Inoculation with phosphate solubilising bacteria showed remarkable variation in both physiological and biochemical parameters of green gram plants. Among the two concentrations tested, 1% phosphate solubilising bacteria was found to be effective in mitigating the effect of water stress, stimulating early flowering and also in increasing yield.

**Key words:** Germination; Relative Water Content; Chlorophyll; Protein; Proline.

### Introduction

Drought stress is a major abiotic problem, widely distributed worldwide over 1.2 billion ha in rainfed agricultural land. The drought environment has been reported as key factor that limit plant growth and development prior to the loss of productivity, especially of crop species (Neumann, 2008; Shao *et al.*, 2008). Water stress leads to substantial variation in morphology, anatomy, physiology and biochemistry of plants which ultimately reflect on the yield potentials (Kramer, 1969). It is one of the major environmental constraints on plant growth and combined with high temperature and soil physico-chemical deficiencies, restricts crop production all over the world (Ceccarelli, 1984; Agarwal *et al.*, 1999). Drought causes a variety of biochemical, physiological and metabolic changes (Xiong and Zhu, 2002), which may result in oxidative stress and effect plant metabolism, performance and yield (Shafi *et al.*, 2009). Priming of seeds with various chemicals or even with water can be enabled the plants to improve the health and hence such plants may become resistant to water stress (Chivasa *et al.*, 2000; Harris *et al.*, 2004; Shintu and Jayaram 2015). Both short and prolonged period of water stress affect nutrient characteristics, photosynthesis and other metabolic activities of plants and ultimately the growth and productivity of such plants are adversely affected. Phosphate Solubilising Bacteria (PSB) is a group of beneficial bacteria capable of hydrolysing organic and inorganic phosphorus from insoluble compounds.

Phosphobacterium is one among the soil micro organism, which plays an important role in improving the chemical and physical nature of soil,

adding organic matter, solubilising the insoluble phosphate, increasing availability and utilization of growth and yield (Ravikumar *et al.* 2010).

Phosphorus is one of the major essential elements involved in numerous functions in plant growth and development. Only 0.1% of the total phosphorus in the soil is available to the plants because of its low solubility and fixation in soil (Gaur, 1990; Chhonkar, 1998). In this context, phosphate solubilising micro organisms efficiently take part in the utilization of unavailable native phosphates as well as phosphates (Lagreid *et al.*, 1999). Considering these facts the authors made an attempt to study the effect of priming of green gram seeds with phosphorus solubilising bacteria that represents an important ecological adaptation to resist the plants from water stress.

### Materials and Methods

For the present study, the seeds of green gram [*Vigna radiata* (L.) Wilczek] variety CO6 were procured from the Tamil Nadu Agricultural University, Coimbatore. Healthy seeds were manually selected from the seed lot and were divided into 3 sets. First set of seeds was not inoculated with phosphobacterium (PSB) and was considered as untreated control. The second and third sets were inoculated with 0.5% and 1% PSB (*Bacillus polymixa*) procured from Agrobiotech Research Centre, Kottayam, Kerala. All the treated as well as untreated control seeds were sown in garden pots filled with garden mixture. After 21 days of vegetative growth both the PSB treated and control plants were divided into two sets each of which one each was subjected to water stress

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treatment for 3 days and the other sets were regularly irrigated. After 3 days water stress treatment the plants were irrigated regularly as in the earlier case.

The following parameters were studied by using standard procedures: Germination percentage, root length, shoot length, relative water content (RWC) (Bars & Weatherly 1962), total chlorophyll (Arnon, 1949), total protein (Lowry *et al.*, 1951), proline (Bates *et al.*, 1973) and yield per plant. All the data were collected as detailed below: on the previous day of commencement of water stress treatment (0<sup>th</sup> day), 1<sup>st</sup> day (24 hrs after water stress), 2<sup>nd</sup> day (48 hrs after water stress), 3<sup>rd</sup> day (72 hrs after water stress) and 24 hrs after re-irrigation (1<sup>st</sup> day of recovery) and 48 hrs after re-irrigation (2<sup>nd</sup> day of recovery).

## Results

There were significant changes in both physiological and biochemical parameters caused by phosphobacterium and water deficit, which was more pronounced in plants without bacterium inoculation.

### Germination percentage

Significant differences were observed in germination percentage among treatments and

control seeds of green gram (Table 1). Seeds treated with 1% PSB had higher germination percentage compared to other treatment and control.

**Table 1:** Effect of PSB on germination (%) of green gram [*Vigna radiata* (L.) Wilczek] seeds.

Treatment (%)	Germination % on 1 <sup>st</sup> day	Germination % on 2 <sup>nd</sup> day	Germination % on 3 <sup>rd</sup> day
0	50.5	61.1	73.3
0.5	95.3	96.5	97.2
1	96.8	97.7	100

### Root length and shoot length

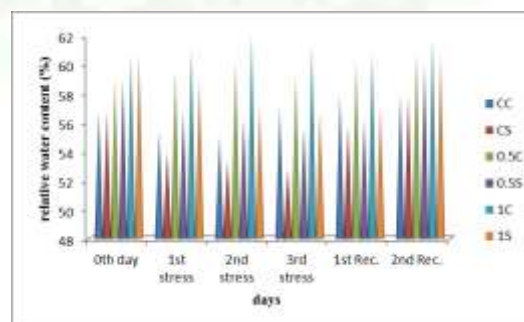
The root length and shoot length of green gram plants increased gradually and significantly throughout the period of study (Table 2). This increase was negligible in control plants as compared to 0.5% and 1% PSB treatment. Among treatments, 1% PSB treated plants showed a rapid increase in both root and shoot length. However, the plants treated with 0.5% PSB showed a gradual increase but not as much as 1% PSB. Whereas, stressed plants showed a considerable decrease in root and shoot length. Among this stress conditions, plants treated with 1% PSB showed a significant increase in root and shoot length. It was amazing to note that during re-irrigation the treated plants showed high rate of recovery.

**Table 2:** Effect of PSB on root length and shoot length (cm) of green gram [*Vigna radiata* (L.) Wilczek].

Treatment & plant part		0 <sup>th</sup> day	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	1 <sup>st</sup> rec.	2 <sup>nd</sup> rec.
CC	Root	5.9±0.21	6.8±0.06	8.4±0.11	9.1±0.12	9.6±0.12	9.9±0.17
	Shoot	20.00±0.30	22.10±0.12	22.6±0.22	23.2±0.09	23.6±0.16	23.8±0.22
CS	Root	5.9±0.21	6.1±0.03	7.3±0.12	8.3±0.21	9.3±0.25	9.8±0.24
	Shoot	20.00±0.30	22.00±0.61	22.4±0.50	22.8±0.33	23.5±0.41	23.90±0.40
0.5C	Root	6.7±0.19	7.5±0.26	8.2±0.28	8.9±0.29	9.4±0.31	10.9±0.34
	Shoot	19.60±0.15	21.70±0.44	22.80±0.46	23.9±0.28	24.50±0.31	25.70±0.40
0.5S	Root	6.7±0.19	7.2±0.19	8.4±0.28	9.1±0.31	9.8±0.22	10.8±0.33
	Shoot	19.60±0.15	22.50±0.20	22.90±0.35	23.70±0.27	24.80±0.14	25.90±0.19
1C	Root	9.3±0.67	9.8±0.33	10.4±0.39	11.4±0.31	11.6±0.34	11.8±0.38
	Shoot	19.90±0.18	22.40±0.50	23.10±0.31	23.60±0.46	24.90±0.30	25.60±0.40
1S	Root	9.3±0.67	9.7±0.25	10.6±0.33	10.9±0.34	11.5±0.35	12.1±0.32
	Shoot	19.90±0.18	22.50±0.36	23.00±0.41	23.30±0.52	24.80±0.40	25.40±0.49

### Relative water content (RWC)

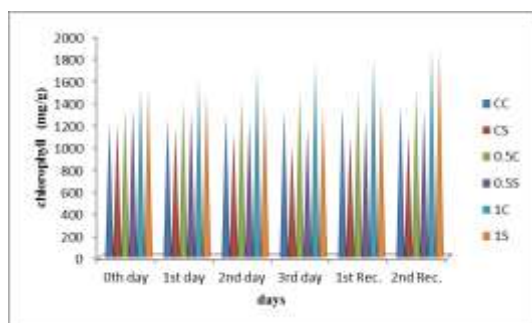
The relative water content of leaves was more or less same during the period of study in the untreated control plants (Fig. 1). The plants under water stress showed a decrease in RWC during the 1<sup>st</sup> day of stress and the value was more or less same up to 3<sup>rd</sup> day, but during re-watering the RWC was increased significantly. The plants treated with 0.5 % PSB showed a slight increase in RWC and more or less same value was maintained throughout the period of study; these plants when exposed to water stress exhibited a negligible decrease in RWC even during re-watering. Similar pattern of results was obtained in plants treated with 1% PSB and the plants exposed to water stress.



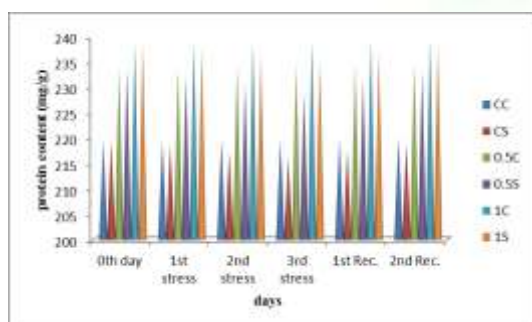
**Figure 1:** Effect of PSB on relative water content (%) of leaves of green gram [*Vigna radiata* (L.) Wilczek].

**Total Chlorophyll**

The amount of total chlorophyll was less in untreated control green gram plants, which increased gradually throughout the period of study (Fig 3). But it was found that the decrease in total chlorophyll during water stress was higher in untreated control plants, and it was increased during re-irrigation. Plants treated with 1 % PSB showed elevation in total chlorophyll under normal irrigation, but a slight decrease was observed during water stress. A gradual and significant increase in chlorophyll content was recorded during re-irrigation. The recovery rate was higher in 1% PSB treated plants than 0.5 % PSB treated plants.



**Figure 2:** Effect of PSB on chlorophyll content (mg/g) of green gram [*Vigna radiata* (L.) Wilczek].

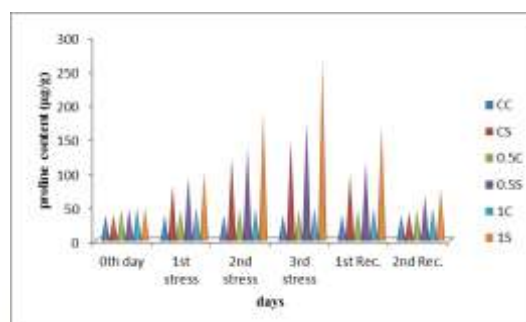


**Figure 3:** Effect of PSB on protein content (mg/g) of green gram [*Vigna radiata* (L.) Wilczek].

**Total Protein**

High rate of total protein content was observed in 1% PSB treated plants compared to 0.5% PSB treated and control plants (Fig 4). A low rate of protein content was noticed in water stressed

untreated control plants but was increased during the re-irrigation period. Whereas the water stressed plants of both 0.5% and 1% PSB treatment showed a negligible loss of protein content.



**Figure 4:** Effect of PSB on proline content (µg/g) of green gram [*Vigna radiata* (L.) Wilczek] [Control (CC), stress in control (CS), control of 0.5% treatment (0.5C), stress of 0.5% treatment (0.5S), control of 1% treatment (1C), stress of 1% treatment (1S)] [0<sup>th</sup> day- without stress, 1<sup>st</sup> day- 1<sup>st</sup> day of stress, 2<sup>nd</sup> day-2<sup>nd</sup> day of stress, 3<sup>rd</sup> day- 3<sup>rd</sup> day of stress, 1<sup>st</sup> rec.- 1<sup>st</sup> day of recovery, 2<sup>nd</sup> rec.- 2<sup>nd</sup> day of recovery]

**Proline**

It was amazing to note that both the concentrations of PSB treated plants showed high rate of proline content during the water stress as compared to the un-treated control plants of which 1% PSB treated plants exhibited highest rate (Fig 5).

**Yield**

The grain yield of green gram plants was found to be influenced by PSB treatment, and significantly higher yield was recorded in plants treated with 1 % PSB under normal irrigation in comparison with untreated control and other treatment (Table 3). The various yield parameters such as number and fresh weight of pods per plant, number and fresh weight of seeds per plant and number and fresh weight of seeds per pod were found to be higher in 10 % PSB treated plants under normal irrigation compared to 0.5 % PSB treated plants. Minimum number and fresh weight of pod per plant, number and fresh weight of seeds per pod was produced by untreated control green gram plants.

**Table 3:** Effect of PSB on yield and various yield components of green gram [*Vigna radiata* (L.) Wilczek].

Treatment	Number of pods/plant	Fresh weight of pods/plant(g)	Number of Seeds/plant	Fresh weight of seeds/plant(g)	Number of seeds/pod	Fresh weight of seeds/pod(g)
CC	8.5±0.2	3.00±0.11	56.2±1.87	1.7±0.19	8.8±0.41	0.42±0.15
CS	7.8±0.73	2.62±0.28	53±0.96	1.5±0.13	8.5±0.50	0.36±0.18
0.5C	9.5±0.44	3.95±0.41	87±2.02	2.93±0.62	10.83±0.83	0.53±0.26
0.5S	8.3±0.51	3.08±0.19	68.3±1.01	2.73±0.17	9.16±0.33	0.40±0.08
1C	10.33±1.1	4.83±0.88	105.3±2.4	3.53±0.73	10.6±0.56	0.52±0.07
1S	9.8±0.82	3.9±0.30	81.16±1.23	2.83±0.90	9.8±0.60	0.45±0.22

## Discussion

From the present investigation it is evident that the seed germination percentage was higher in plants treated with 1 % PSB than plants treated with 0.5 % PSB and untreated plants (Table 1). Similar results were obtained in *Cicer arietinum* Sharma *et al.* (2007) and according to those authors, treatment with phosphate solubilizing bacteria was beneficial to increase seed germination as well as radical and plumule length. Kadkhodaie and Bagheri (2011) suggested that hydro priming leads to quick water absorption, and cell division will be done with more speed and this will increase the germination percentage, length of plumule and radicle. In green gram, the seeds were primed with two different concentrations of PSB and higher percentage of germination and radicle emergence was obtained in 1% PSB treated plants. This indicates that priming of green gram seeds with PSB enhanced its water absorption capacity and this leads to higher germination percentage.

An increased root and shoot length were observed in green gram plants treated with 1% PSB (Table 2). Similar pattern of results was obtained in *Sorghum bicolor* plants, which were inoculated with *Azospirillum* (Basaglia *et al.*, 2003). According to Dobbeleere *et al.* (2001) the superiority of bio-priming and combination of all priming methods was due to perturbations in the auxin / cytokinin balance that can lead to increased root length and shoot length due to inoculation with microbes. It was found that the increase in plant height may be due to the presence of sufficient amount of phosphorus which plays a major role towards cell division and elongation in the meristematic regions (Bahadur and Singh, 1990). Studies with the application of PSB revealed an increased plant height in Maize (Nanjundappa *et al.*, 2000) which is also in tune with the results obtained in the present investigation.

Priming of green gram seeds with PSB showed an increase in the relative water content (RWC), which was very prominent in 1% PSB treated plants (Fig 1). Yordanov *et al.* (2003) suggested that the mild drought induced plants to regulate water loss and uptake allowing maintenance of their leaf water content within the limits. There are evidences which support this view. According to Valentovic *et al.* (2006) the RWC in the leaves of maize plants at low water potential decreased significantly compared to its control. In the present study the RWC, in leaves of drought affected green gram plant was significantly lower than the control. This result is in tune with the observations made in fenugreek plant (Singh and Singh, 2010). These authors found that the plants inoculated with PSB exhibited highest level of RWC in comparison to non inoculated plants under controlled conditions. Jiang and Huang

(2002) also reported that RWC was decreased under water deficit condition. All these observations revealed that RWC in leaves of drought affected plants were significantly reduced as a result of reduced water uptake and storage by the plants. So it can be presumed from the present study that the comparatively high RWC in the leaves of PSB treated plants may be due to the higher ability of water uptake and restoration by the plants which were treated with PSB. Thus PSB can be considered as a bio- priming agent in order to tolerate drought.

High rate of total chlorophyll content was noticed in PSB treated green gram plants than the control plants (Fig 3). Similar results were obtained (Singh and Singh, 2010) in fenugreek and the authors observed highest amount of total chlorophyll in plants inoculated with *B. polymyxa* than control, which was slightly decreased under stress. That was due to the impaired metabolic activities due to stress and leads to the inhibition of photosynthetic rate as suggested by Duan *et al.* (2006) and Zgalli *et al.* (2006). The photosynthetic pigments were reduced under stress, while high availability of phosphorus supplied by PSB inoculated plants, caused betterment in the amount of pigments. This might be due to the positive role of phosphorus which helped in the inhibition of degradation of pigments under stress (Singh and Singh, 2010). The results of enhanced chlorophyll content in the present study may be due to the high availability of phosphorus which was supplied by the PSB as proposed by Singh and Singh (2010); Ambreen *et al.* (2012) and Shintu and Jayaram (2015).

Total protein in the PSB treated plant was found higher compared to non-inoculated control plants (Fig.4). PSB may cause to enhance the availability of insoluble phosphorus which ultimately intensifies the accumulation of protein. Evidence is increasing in favour of a relationship between the accumulation of drought induced proteins and physiological adaptations to water limitation (Riccardi *et al.* 1998). Radin (1984) suggested that high phosphorus caused stomatal opening and facilitate plant to accumulate more protein in inoculated plants compared to non-inoculated one. This may be the reason of high rate of protein content in the PSB treated green gram plants.

High level of accumulation of proline content was observed in PSB inoculated plants than non-inoculated plants under drought stress (Fig 5). Hanson and Hitz (1982) suggested that proline accumulation was a consequence of stress induced damage to cells and protein synthesized during water deficit may be served as an organic nitrogen reserve that can be utilized during recovery. Similar observations were made by Sankar *et al.* (2007). According to those authors increased proline in

the stressed plants may be an adaptation, the purpose of which is to overcome the stress condition and the reduced proline oxidase may be the reason for increasing protein accumulation. The enhanced drought resistance in green gram plants treated with *Pseudomonas fluorescens* and *Bacillus subtilis* were reported by Saravanakumar *et al.* (2011). The authors observed high proline accumulation in *Pseudomonas* treated plants, but low level of proline accumulation was recorded in untreated plants. The authors further suggested that accumulation of proline might be due to the growth promoting activity of beneficial microbes. So it can be concluded that the increase in proline content during drought may be helpful to the plants and can be utilized for its recovery. It can also be presumed that the accumulation of proline during stress may be beneficial to the plants by enhancing root growth which also helps to absorb more water. The PSB treatment enhanced the production of proline during stress and this may be more beneficial to root growth as well as water uptake.

In addition to the favourable effect on growth of crop plants, bio-priming is also known to increase the yield during drought (Casanovas *et al.*, 2003). In the present study also, maximum yield was occurred in 1% PSB treated and water stressed plants (Table 3). So it is presumed that the increase in grain yield in PSB treated plants exposed to water stress may be due to the positive impact of PSB on the other physiological and biochemical parameters studied. So it can be concluded that phosphate solubilizing bacterium helped green gram plants to improve its water status, and thereby tolerate water stress to a certain extend.

## Conclusion

From the results, it is clear that the application of PSB reduced the effect of drought stress in PSB treated plants compared to untreated control plants. The plants raised from 1% PSB treatment showed remarkable results in physiological and biochemical parameters, which were followed by 0.5% PSB treatment, compared to control plants. So, it can be concluded that priming of green gram seeds with 1% PSB can be recommended for the farming community as a means to fight against drought stress.

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