Tropical Plant Research
An International Journal
© Society for Tropical Plant Research

ISSN (E): 2349 – 1183
ISSN (P): 2349 – 9265
2(1): 17–22, 2015

Research article

# Phosphate solubilising bacteria (*Bacillus polymyxa*) - An effective approach to mitigate drought in tomato (*Lycopersicon esculentum* Mill.)

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**Abstract:** The main aim of the present investigation is to evaluate the effect of priming of seeds of tomato (*Lycopersicon esculentum*) with phosphate solubilising bacteria (PSB) during drought stress condition. The use of phosphate solubilising bacteria as inoculants is found to be simultaneously increasing the Phosphorus uptake by the plant and crop yield. As the farmers in the state of Kerala are severely fed up with the water stress condition prevailing in the summer season, the present attempt may become miniature step to stretch a helping hand to them. In the study, the seeds of tomato (*L. esculentum*) cv. Anakha were subjected to priming treatment with 0.5 % and 1% phosphate solubilising bacteria. The parameters like germination percentage, root length, shoot length, relative water content, amount of chlorophyll, protein, proline and yield were studied. Inoculation with phosphate solubilising bacteria showed remarkable variation in both physiological and biochemical parameters of tomato plants. Among the two concentrations tested, 0.5% phosphate solubilising bacteria was found to be effective in mitigating the effect of water stress, stimulating early flowering and also in increasing yield.
**Keywords:** *Bacillus polymyxa* - Germination - Chlorophyll - Protein - Proline.

[**Cite as:** Shintu PV & Jayaram KM (2015) Phosphate solubilising bacteria (*Bacillus polymyxa*) - An effective approach to mitigate drought in tomato (*Lycopersicon esculentum* Mill.). *Tropical Plant Research* 2(1): 17–22]

## INTRODUCTION

Water limitation is one of the most important factors to reduce agricultural crop production, which is related to global climate changes; especially drought and heat stress (Ciais *et al*. 2005). Drought (water stress) is the major problem in agriculture and the ability to withstand such stress is of immense economic importance. Water stress leads to substantial variation in morphology, anatomy, physiology and biochemistry of plants, which ultimately reflected on yield potentials (Kramer 1969). These physiological and biochemical changes are appears to be the result of accumulation of compatible solutes and specific proteins that can be rapidly induced by osmotic stress (Shao *et al.* 2005). Water stress either short or prolonged, adversely affect photosynthesis and other metabolic activities of plants and ultimately the growth and productivity of plants.

Phosphobacterium is one among the soil microorganism, 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). Most of the Indian soils are deficient in available form of phosphorus and its requirement is met by the addition of phosphatic fertilizers but the use efficiency of applied phosphorus rarely exceeds 30% due to its fixation as Fe and Al phosphates in acid soil and Ca and Mg phosphates in alkaline soils. In this context, phosphate solubilising microorganisms efficiently take part in the utilization of unavailable native phosphates as well as phosphates (Lagreid *et al*. 1999). Various studies showed that priming of seeds with various chemicals or even water can enable the plants to improve the health and hence such plants may become resistant to water stress (Chivasa *et al*. 2000, Harris *et al*. 2004). Considering these facts the authors made an attempt to study the effect of priming tomato seeds with phosphorus solubilising bacteria that represents an important ecological adaptation to resist the plants from water stress.

## MATERIALS AND METHODS


Received: 03 December 2014 Published online: 28 February 2015

For the present study, seeds of tomato (*Lycopersicon esculentum* Mill.) cv. Anakha were procured from the Regional Agricultural Research Station, Palakkad, Kerala. Healthy seeds were selected and were divided in to 2 sets. First set of seeds was non-inoculated (unprimed) and considered as control and the other set was inoculated/primed with 0.5% and 1% phosphate solubilising bacterium (*Bacillus polymyxa*) 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 experimental and control plants were divided into two sets each of which one each was subjected to water stress treatment for 3 days and the other sets were regularly irrigated. After 3days water stress treatment the plants were irrigated regularly as in the other case.

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

## RESULTS

There was 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*

Seeds treated with 0.5% PSB showed highest rate of germination percentage (93.3%), compared to untreated control seeds (81.11%), (Fig 1).

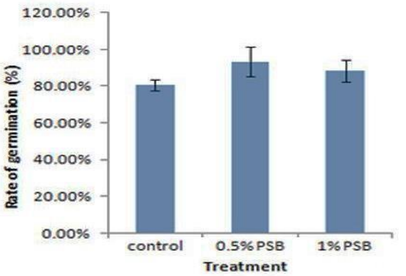


**Figure 1.** Effect of PSB on germination percentage in Tomato (*Lycopersicon esculentum* Mill.).

*Root length and shoot length*

The root length and shoot length of untreated control plants increased gradually during the study (Table 1). But this increase was negligible as compared to the 0.5% and 1% PSB treated unstressed plants. Among treatments, 0.5% PSB treated plants showed a rapid increase in both root and shoot length. However the plants treated with 1% PSB showed a gradual increase but not as much as 0.5% PSB. Whereas, stressed plants showed a considerable decrease in root and shoot length. Among this stress conditions, plants treated with 0.5% 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 1.** Effect of PSB on root length and shoot length (cm) of Tomato (*Lycopersicon esculentum* Mill.)

| Treatment & Plant part | | $0^{th}$ day | $1^{st}$ day | $2^{nd}$ day | $3^{rd}$ day | $1^{st}$rec. | $2^{nd}$rec. |
|---|---|---|---|---|---|---|---|
| CC | Root | 6.3±0.51 | 7.4±0.92 | 7.9±0.28 | 9.6±0.80 | 9.7±0.58 | 10.8±0.69 |
| | Shoot | 18.5±0.29 | 22.5±0.52 | 23.9±0.69 | 24.4±0.11 | 24.6±0.40 | 27.0±0.34 |
| CS | Root | 6.3±0.51 | 6.3±0.46 | 7.1±0.51 | 7.5±0.34 | 7.8±0.20 | 9.3±0.56 |
| | Shoot | 18.5±0.29 | 18.7±0.23 | 19.1±0.87 | 21.1±0.46 | 22.0±0.52 | 24.4±0.17 |
| 0.5C | Root | 7.4±0.81 | 8.6±0.79 | 9.6±0.40 | 10.9±0.11 | 11.1±0.75 | 13.7±0.23 |
| | Shoot | 20.4±0.62 | 22.6±0.40 | 27.1±0.34 | 28.8±0.75 | 31.1±0.29 | 31.3±0.98 |
| 0.5S | Root | 7.4±0.81 | 6.8±0.40 | 6.8±0.69 | 7.6±0.59 | 9.5±0.69 | 10.2±0.46 |
| | Shoot | 20.4±0.62 | 21.0±0.75 | 21.9±0.52 | 24.2±0.92 | 24.6±0.58 | 28.1±0.40 |
| 1C | Root | 6.23±0.23 | 6.8±0.40 | 8.6±0.46 | 9.9±0.98 | 10.4±0.92 | 12.1±0.52 |
| | Shoot | 18.9±0.69 | 22.2±0.44 | 26.0±0.46 | 27.1±0.17 | 28.1±0.12 | 26.3±0.64 |
| 1S | Root | 6.23±0.23 | 6.6±0.39 | 7.2±0.87 | 7.3±0.75 | 8.9±0.64 | 9.4±0.35 |
| | Shoot | 18.9±0.69 | 19.1±0.52 | 19.6±0.23 | 22.6±0.69 | 23.9±0.92 | 26.2±0.81 |

**Note:** 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).
$0^{th}$ day- Without stress; $1^{st}$ day- $1^{st}$ day of stress; $2^{nd}$ day- $2^{nd}$ day of stress; $3^{rd}$ day- $3^{rd}$ day of stress; $1^{st}$rec.- $1^{st}$ day of recovery; $2^{nd}$rec- $2^{nd}$ day of recovery.

*Relative water content (RWC)*

The plants under water stress showed a decrease in RWC during 1st and 2nd day of stress and the values remain unchanged on the 3rd day of stress but in the untreated control plants it was more or less same (Fig. 2A). Plants treated with 0.5% PSB showed a slight increase in RWC and more or less same level was retained throughout the period of study; these plants when exposed to water stress exhibited a negligible decrease in RWC. Identical pattern of results were obtained in plants treated with 1% PSB that exposed to water stress.

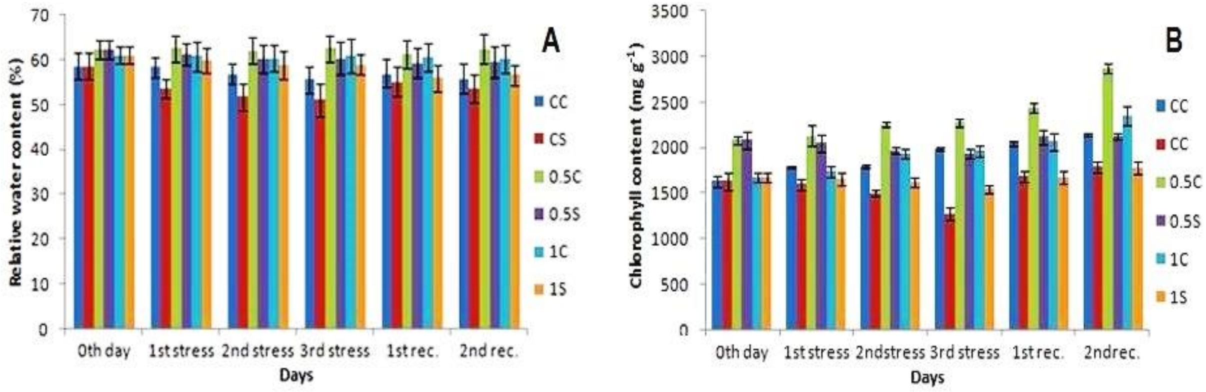


**Figure 2.** Effect of PSB on Tomato (*Lycopersicon esculentum*) leaves: **A**, Relative water content (%); **B**, chlorophyll content (mg $g^{-1}$). [CC- Control; CS- Stress in control; 0.5C- Control of 0.5% treatment; 0.5S- Stress in 0.5% treatment; 1C- Control of 1% treatment;1S- Stress in 1% treatment]

*Chlorophyll*

PSB treated unstressed plants exhibited high rate of chlorophyll content compared to control plants (Fig. 2B) and it was prominent in 0.5% PSB treatment. Whereas all the stressed plants showed a decreased level of chlorophyll content in both PSB treated and untreated conditions. During re-irrigation the chlorophyll content was found increased in PSB treated plants compared to unstressed control plants.

*Protein*

High rate of protein content was observed in 1% PSB treated plants compared to 0.5% PSB treated and control plants (Fig. 3A). A low rate of protein content was noticed in stressed 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.

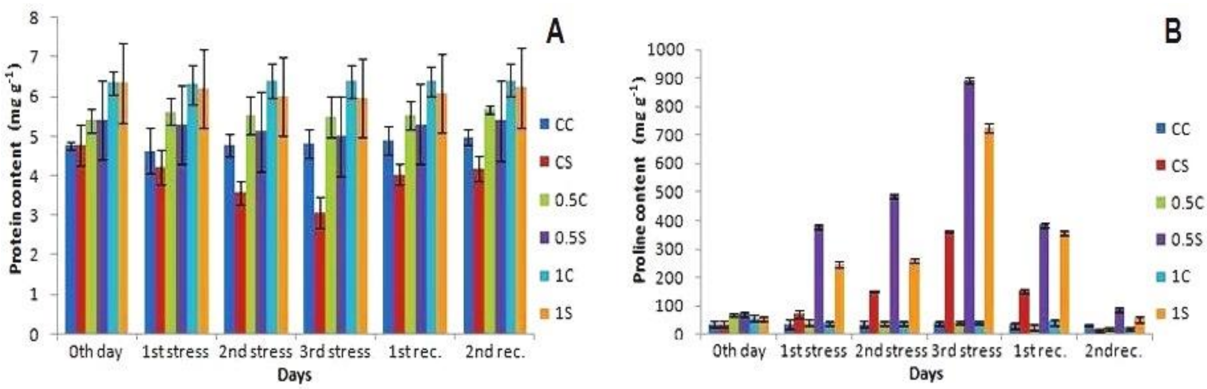


**Figure 3.** Effect of PSB on Tomato (*Lycopersicon esculentum* Mill.) leaves: **A**, Protein content (mg $g^{-1}$); **B**, Proline content (µg $g^{-1}$). [CC- Control; CS- Stress in control; 0.5C- Control of 0.5% treatment; 0.5S- Stress in 0.5% treatment; 1C- Control of 1% treatment;1S- Stress in 1% treatment]

*Proline*

It was interesting to note that both PSB treated plants showed high rate of proline content during the stress as compared to the untreated control of which 0.5% PSB treated plants exhibited highest rate. The PSB treated water stressed tomato plants and untreated control plants exhibited a decrease in the rate of increase in proline content during re-irrigation (Fig. 3B).

*Yield*

Significant variation in the total number of fruits was found in the study. Tomato seeds treated with PSB along with water stress showed maximum number of fruits compared to their respective control plants. But the fresh weight of fruits was lesser in stressed plants (Fig. 4). Among the treatments, the seeds primed with 0.5% PSB showed maximum yield compared to the other treatment and control.

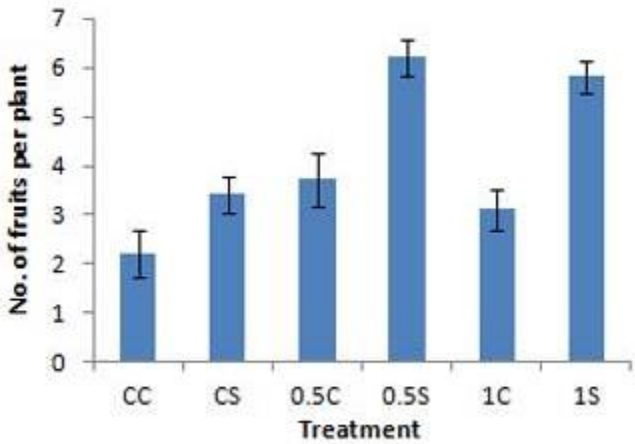


**Figure 4.** Effect of PSB on yield of Tomato (*Lycopersicon esculentum* Mill.). [CC- Control; CS- Stress in control; 0.5C- Control of 0.5% treatment; 0.5S- Stress in 0.5% treatment; 1C- Control of 1% treatment;1S- Stress in 1% treatment]

## DISCUSSION

Tomato seeds primed or inoculated with phosphobacterium showed an increased percentage of germination (Fig. 1). Studies conducted by Demir & Mavi (2004) observed a delay in the emergence of radical of unprimed water melon seeds by 4 days compared to primed seeds. Similar type of results was obtained in osmopriming of lentil seeds (Ghassemi-Golezani *et al*. 2008). According to those authors priming was helpful in reducing the risk of poor stand establishment under drought and permit more uniform growth under drought on saline soils.

Studies conducted by Marulanda *et al.* (2007) revealed that inoculation of lavender plants with native beneficial microorganisms might increase drought tolerance of plants growing in arid or semiarid areas. These micro-organisms seem to have advanced mechanisms to cope up with drought stress. During priming, seeds are partially hydrated so that pre-germinative metabolic activities proceed, while radicle protrusion is prevented, then are dried back to the original moisture level (McDonald 2000). Kaya *et al.* (2006) worked on germination of sunflower under drought and salt stress reported that hydro-priming improved both rate of germination and mean germination time. In the present study also, the germination percentage was shown to be maximum in PSB inoculated seeds and it is presumed that phosphobacterium has a pivotal role in inducing germination by improved pre-germinative metabolic activities.

The root and shoot length of tomato plants were high in PSB inoculated plants as compared to the control (Table 1). Similar results were obtained in other crops where seeds treated with *Pseudomonas fluorescens* have increased the growth of host plants (Barka *et al*. 2000, Niranjan *et al*. 2003). It was also reported that seed priming with *Pseudomonas* affected root length, root and shoot dry weight and plant height, significantly (Jalal *et al.* 2014). From this we can presume that PSB also have some role in promoting the plant growth.

A gradual decrease in RWC in response to water deficit was observed in the present study (Fig. 2A), which is in corroboration with the observations of Jing & Huang (2002). According to those authors the inoculated plants under stress exhibited less decrease in RWC compared to non-inoculated plants under stress condition. This can be explained by the fact that phosphorous may help in root elongation and the roots of PSB primed plants may absorb more soil phosphorus which could be in a non-available form during water stress.

It was surprising to note that the plants treated with PSB and subjected to water stress showed high rate of chlorophyll content than the untreated control plants (Fig. 2B). Similar results were obtained in Fenugreek plants in which highest amount of total chlorophyll were recorded in PSB treated ones (Singh & Singh 2010). The higher rate of persistence of chlorophyll content in plants under stress and treated with PSB may be attributed to decreased chlorophyll degradation and increased chlorophyll synthesis, as reported by Jayakumar & Thangaraj (1998). According to them, the application of plant growth regulators to groundnut resulted in high chlorophyll content without the modification of leaf anatomy and delayed chlorophyll degradation. From our present study, it is evident that the effect of PSB is beneficial to the non-degradation of chlorophyll pigment and that may be the reason of high chlorophyll content in PSB treated plants. The increase in the photosynthetic rate obviously elevate the plant growth and there by the productivity of plants.

In the present study also PSB may cause to enhance the availability of insoluble phosphorus which ultimately intensifies the accumulation of protein (Fig. 3A). Evidence is increasing in favor of a relationship between the accumulation of drought induced proteins and physiological adaptations to water limitation (Riccardi *et al.* 1998). Radian (1984) suggested that high phosphorus caused stomatal opening and facilitate plant to accumulate more protein in inoculated plants compared to non-inoculated one.

Since the first report on proline accumulation in wilting perennial rye grass (Kemble & Mac Pherson 1954), numerous studies have shown that the proline content in higher plants increases under different environmental stresses. The primary response of drought stress is osmotic adjustment through proline accumulation was well established in many plants (Raymond & Smirnoff 2002). From our study, it was clear that PSB treated plant under stress showed excessive proline content to cope up with the drought condition. The rise in the proline content in the present study may be due to the positive response of PSB on water stress.

In addition to the favorable 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 observed in 0.5% PSB treated and water stressed plants (Fig. 4). 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 solubilising bacterium helped tomato plants to improve its water status, and thereby tolerate water stress to a certain extend.

## CONCLUSION

The present study revealed that PSB have an important role in increasing the yield as well as in counteracting the effect of drought stress. The plants raised from 0.5% PSB treatment showed remarkable results in physiological and biochemical parameters, which were followed by 1% PSB treatment, compared to control plants. So, it can be concluded that priming of tomato seeds with 0.5% PSB can be recommended for the farming community cultivating tomato plants, as a means to fight against drought stress.

## ACKNOWLEDGEMENTS

The authors are gratefully acknowledged to the Head, Department of Botany, University of Calicut, for providing necessary facilities in order to complete the work in time.

## REFERENCES

Arnon D T (1949) Copper enzymes in isolated chloroplasts poly phenol oxidase in *Beta vulgaris*. *Plant Physiology* 24: 1–15.

Barka E A, Belarbi A, Hachet C, Nowak J & Audran J C (2000) Enhancement of *in vitro* growth and resistance to gray mold of *Vitis vinifera* co-cultured with plant growth-promoting rhizobacteria. *FEMS Microbiology Letters* 186: 91–95.

Bars H D & Weatherly P E (1962) A re-examination of relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Science* 15: 413–428.

Bates L, Waldren R P & Teare I D ( 1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39: 205–207.

Casanovas E M, Barassi C A, Andrade F H & Sueldo R J (2003) *Azospirillum* inoculated maize plants response to irrigation resistance imposed during flowering. *Cereal Research Communication* 31: 395–402.

Chivasa W, Harris D, Chiduza C, Mashingaidze A B & Nyamudeza P (2000) Determination of optimum on farm seed priming time for maize (*Zea mays* L.) and sorghum (*Sorghum bicolor*) for use to improve stand establishment in semi arid agriculture**.** *Tanzanian Journal of Agricultural Science* 3: 103–112.

Ciais P M, Reichstein N, Viovy A & Granier (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 472: 529–533.

Demir I & Mavi K (2004) The effect of priming on seedling emergence of differentially matured watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) seeds. *Scientia Horticulturae* 102: 467–473.

Ghassemi-Golezani K, Aliloo A A, Valizadeh M & Moghaddam M (2008) Effects of hydro and osmo-priming on seed germination and field emergence of lentil (*Lens culinaris* Medik.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 36 (1): 29–33.

Harris D, Rashid A, Ali S & Hollington P A (2004) 'On from' seed priming with maize in Pakistan. Proceedings of 8$^{th}$ Asian regional maize workshop: New technologies for the new millennium held at Bangkok, Thailand, Cimmyt, Mexico, pp. 316–321.

Jalal Jalilian1 Razieh Khalilzadeh & Edris Khanpaye (2014) Improving of barley seedling growth by seed priming under water deficit stress. *Journal of Stress Physiology & Biochemistry* 10: 125–134.

Jayakumar P & Thangaraj M (1998) Physiological and biochemical effects of Mepiquat chloride in ground nut (Arachis hypogea). *Madras Agricultural J*ournal 85: 23–26.

Jiang Y & Hung B (2002) Protein alterations in tall fescue in response to drought stress and abscissic acid. *Crop Science* 42: 202–207.

Kaya M D, Okcu G, Atak M, Cikili Y & Kolsarici O (2006) Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *Europian Journal of Agronomy* 24: 291–295.

Kemble A R & Mac Pherson H T (1954) Liberation of amino acids in perennial ray grass during wilting. *Biochemical Journal* 58: 46–59.

Kramer P J (1969) *Plant and soil water relationships- A modern synthesis*, Mc Graw Hill Book Co. Newyork, pp. 482.

Lagreid M, Bockman O C & Kaarstad O (1999) *Agricultural fertilizers and the environment*. CABI Publishing. Oxon, UK, pp: 294

Lowry O H, Rosenbrough R J, Farr A L & Randall R J (1951) Protein measurement with Folin phenol reagent**.** *The Journal of Biological Chemistry*193: 265–275.

Marulanda A, Porcel R, Barea JM & Azco´n R (2007) Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought-sensitive Glomus species. *Microbial Ecology* 54: 543–552.

McDonald M B, Black M & Bewley J D (2000) *Seed Technology and Its Biological Basis.* Sheffield Academic Press. Sheffield. UK, pp: 287–325.

Niranjan Raj S, Chaluvaraju G, Amruthesh K N, Shetty H S, Reddy M S & Kloepper J W (2003) Induction of growth promotion and resistance against downy mildew on pearl millet (*Pennisetum glaucum*) by rhizobacteria. *Plant Disease* 87: 380–384.

Radian J W (1984) Stomatal response to water stress and abscissic acid in phosphorus-deficient cotton plants. *Plant physiol*ogy 76: 392–394.

Ravikumar S, Shanthy S, Kalairasi A & Sumaya S (2010) Effect of halophytic phosphobacteria on *Avicenia officinalis* seedlings. *Annual Biology Research* 1: 254–260.

Raymond M J & Smirnoff N (2002) Proline metabolism and transport in maize seedlings at low water potential. *Annual Botany* 89: 813–823.

Riccardi F, Gazeau P & Vienne D (1998) Protein changes in response to progressive water deficit in maize, quantitative variation and polypeptide identification. *Plant Physiology* 117: 1253–1263.

Shao H B, Liang Z S, Shao M A, Sun S M & Hu Z M (2005) Investigation on dynamic changes of photosynthetic charecteristics of 10 wheat (*Triticum aestivum*) genotypes during two vegetative-growth stages at water deficits. *Biointerfaces* 47:132–139.

Singh, D & Singh N B (2010) Water stress tolerance in Fenugreek (*Trigonella foenum-graecum* L.) inoculated with *Bacillus polymyxa*, a phosphate solubilizing bacterium. *Journal of Indian Botanical Society* 89: 86–91.