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Research article

Priming of *Abelmoschus esculentus* (L.) Moench (okra) seeds with liquid phosphobacterium: An approach to mitigate drought stress

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Abstract: The present investigation aimed to evaluate the effect of priming of *Abelmoschus esculentus* (okra) seeds with liquid phosphobaterium (LPB) on water stress. The phosphobacterium is known for its use as inoculants to increase the uptake of soil phosphorus as well as crop yield. The availability of rain fall is getting decreased every year causing severe drought and that may adversely affect the agricultural crops in the state of Kerala. So the aim of the study is to provide a helping hand to the farming community to fight against drought stress. In the present study the seeds of okra cv. Arka anamika were subjected to priming treatment with 5% and 10% liquid phosphobaterium, and the parameters like biomass, relative water content, chlorophyll content, total protein and yield were studied. Priming with liquid phosphobaterium showed considerable variation in both the physiological and biochemical parameters. Among the concentrations of liquid phosphobaterium tested seeds primed with 10% liquid phosphobaterium were found to effective in mitigating the effect of water stress, stimulating early flowering and also increase in yield.

Keywords: Drought - Re-irrigation - Biomass - Chlorophyll - Protein.

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INTRODUCTION

Agricultural crop productions have been determined by the availability of soil water and which in turn related to global climate changes (Cias *et al*. 2005). Drought is one of the major causes in the field of agriculture all over the world. Depending upon the period of exposure to drought (water stress) and growth stage of plants, water scarcity experienced plants responded differently. Drought stress leads to the commendable variation in the morphological, anatomical, physiological and biochemical parameters of plants which is finally reflected on yield potential (Kramer 1969, Shintu & Jayaram 2015). Drought, irrespective of the length of exposure of the plant and severity, adversely affected photosynthesis and other metabolic activities of plants and ultimately the growth productivity of such plants.

Phosphobacteria (Phosphate solublizing bacteria- PSB) is one of the most useful plant soil microorganisms, widely used as bio-fertilizer. PSB plays an important role in enhancement of plant growth by improving texture of soil by adding organic matter to the soil, solublizing the insoluble phosphorous in soil (Bhattacharya & Jain 2000, Ravikumar *et al*. 2010). Inorder to compensate phosphorus deficiency in soil phosphate fertilizer are being used widely. However the increased use of chemical fertilizers, cause soil contamination. In such condition PSB efficiently take part in the utilization of unavailable native phosphates (Lagreid *et al*. 1999). The studies conducted by various authors revealed that priming or pre-sowing treatment of seeds with various chemicals or even with water can enable the plants to improve the health and such plant may become resist water stress (Chivasa *et al.* 2000, Harris *et al*. 2004, Shintu & Jayaram 2015). During drought stress these microorganisms help to accumulate large amount of compatible solutes and accelerate the production of antioxidant enzymes in plants and reduce the adverse effect of drought (Mayak *et al*. 2004). Considering all these facts the authors made an attempt to study the effect of priming of *Abelmoschus esculentus* (L.) Moench

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(okra) seeds with liquid phosphobacterium (LPB), a cheapest method that adapt to overcome the adverse effect of water stress.

MATERIALS AND METHODS

For the present study, seeds of *Abelmoschus esculentus* (L.) Moench cv. Arka anamika (okra) were procured from the Regional Agricultural Research Station, Mele Pattambi, Palakkad District, Kerala State. Healthy seeds were manually selected from the seed lot and were divided to 3 sets. First set was not inoculated with Liquid phosphobacterium (LPB) and considered as control, the second and third sets were inoculated with different concentrations of LPB such as 5% and 10% respectively. The pre weighed seeds were surface sterilized with teepol and 0.1% mercuric chloride solutions and were kept in respective concentrations of LPB solutions for six hours with continuous shaking. Thereafter the seeds were air dried until the weight became equal to the initial weight. All the seeds were sown in garden pots filled with potting mixture. The experimental setup was maintained in the open field of the Department of Botany, University of Calicut (35±5°C temperature). After 17 days of vegetative growth the control and LPB treated plants were again divided in to two sets, one set kept as irrigated regularly and the other set as non-irrigated for 3 days. After 3 days of water stress the plants were re-irrigated as in the other case and was continued until taking yield.

The following parameters were studied by using standard procedures: fresh and dry weight of plants (biomass), relative water content (RWC) (Bars & Weatherly1962), total chlorophyll (Arnon 1949),total protein (Lowry *et al*.1951) and yield parameters like fruit length, fruit fresh and dry weight, number of fruits per plant and seed per pod. All the data were collected as detailed below: on the previous day of commencement of water stress treatment (0thday), 1stday (24hrs after water stress), 2ndday (48 hrs after water stress), 3rdday (72hrs after water stress), 24 and 48 hrs after re-irrigation (1stand 2ndday of recovery respectively).

Analysis of variance was performed using SPSS software 16. Means were compared using the Duncan test at 5% probability level. The data is an average of three independent experiments each with three replicates (n=9). The data represent Mean±Standard Error (SE).

RESULTS

Biomass

The plants showed significant reduction in biomass under water stressed condition and which was prominent in untreated (control) water stressed plants than LPB treated plants subjected to drought stress (Fig. 1). Plants treated with 10% LPB exhibited lesser biomass reduction compared to other treatment and control. During re-irrigation LPB treated plants showed fastest recovery, compared to untreated plants given drought.

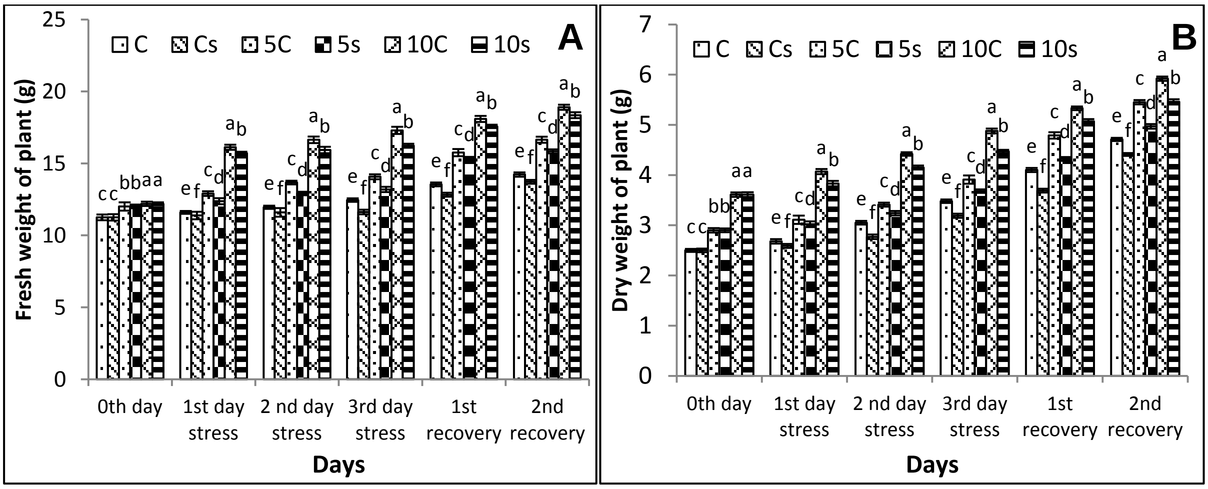

Figure 1. Effect of liquid phosphobacterium on *Abelmoschus esculentus* (L.) Moench (Okra): **A,** Fresh weight; **B,** Dry weight. (C- Control; Cs- Control with drought stress; 5C- 5% LPB treated plants; 5Cs- 5% LPB treated plants with drought stress; 10C- 10% LPB treated plants; 10Cs- 10% LPB treated plants with drought stress)

Relative water content (RWC)

RWC was observed high in all the irrigated set of plants and among the plants subjected to water stress treatment, LPB treated plants exhibited high rate of RWC of which highest rate was observed in 10% LPB treated plants (Fig. 2). The same pattern of increase in RWC was noticed during re-irrigation also.

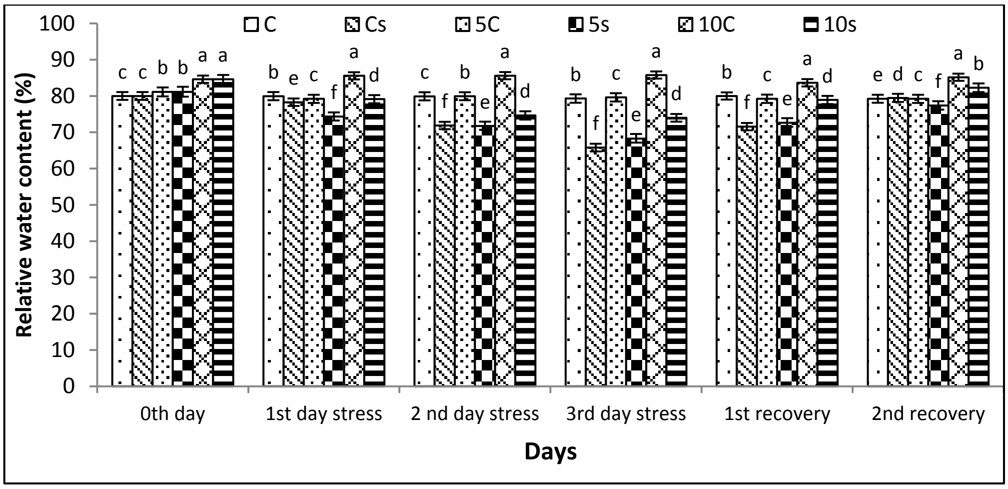

Figure 2. Effect of liquid phosphobacterium on relative water content of *Abelmoschus esculentus* (L.) Moench (Okra). (C- Control; Cs- Control with drought stress; 5C- 5% LPB treated plants; 5Cs- 5% LPB treated plants with drought stress; 10C- 10% LPB treated plants; 10Cs- 10% LPB treated plants with drought stress)

Total Chlorophyll

The total chlorophyll content of untreated water stressed plants was found decreased more significantly throughout the period of water stress treatment compared to LPB treated water stressed plants (Fig. 3A). Among the LPB treated plants highest rate of chlorophyll content was observed in 10% LPB treated plants. During re-irrigation LPB treated plants showed fastest recovery, compared to untreated plants.

Total protein

The okra plants treated with LPB (5% and 10%) exhibited higher rate of accumulation of protein content compared to control plants (Fig. 3B). But the plants exposed to drought stress the decrease of protein content was greater in untreated water stressed plants compared to LPB treated plants. The recovery of protein content was found faster in LPB treated water stressed plants compared to untreated water stressed plants.

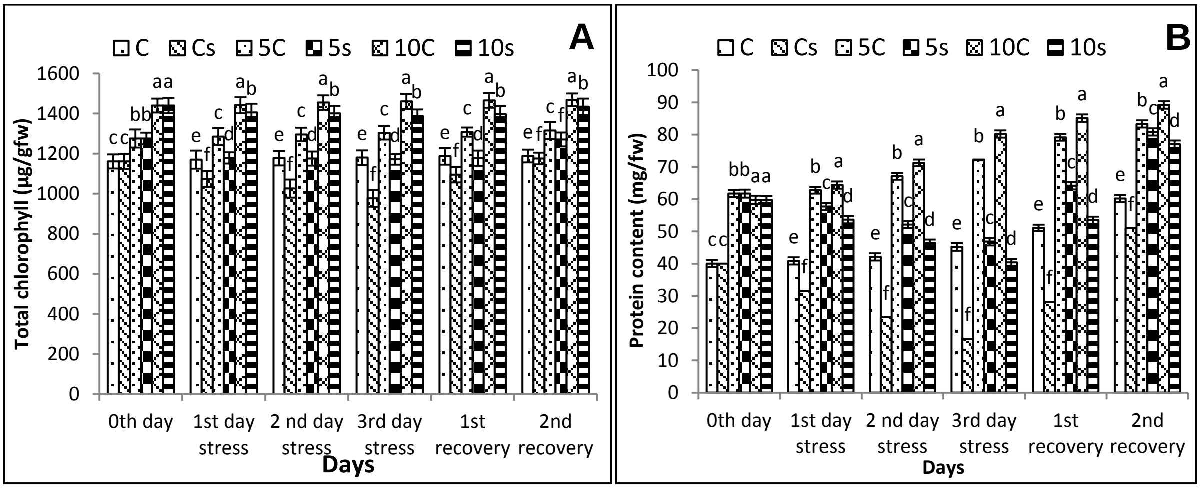

Figure 3. Effect of liquid phosphobacteriumon *Abelmoschus esculentus* (L.) Moench (Okra): **A,** Chlorophyll; **B,** Protein. (C- Control; Cs- Control with drought stress; 5C- 5% LPB treated plants; 5Cs- 5% LPB treated plants with drought stress; 10C- 10% LPB treated plants; 10Cs- 10% LPB treated plants with drought stress)

Yield

Drought stress exhibited a reduction of yield in okra, which was measured by using parameters like length of fruit, fresh weight of fruit, dry weight of fruit, number of fruit per plant and number of seed per fruit but the reduction was more prominent in untreated plants than LPB treated plants (Fig. 4). The plants treated with 10% LPB showed significant increase in yield parameters when compared to 5% LPB treated plants and control plants.

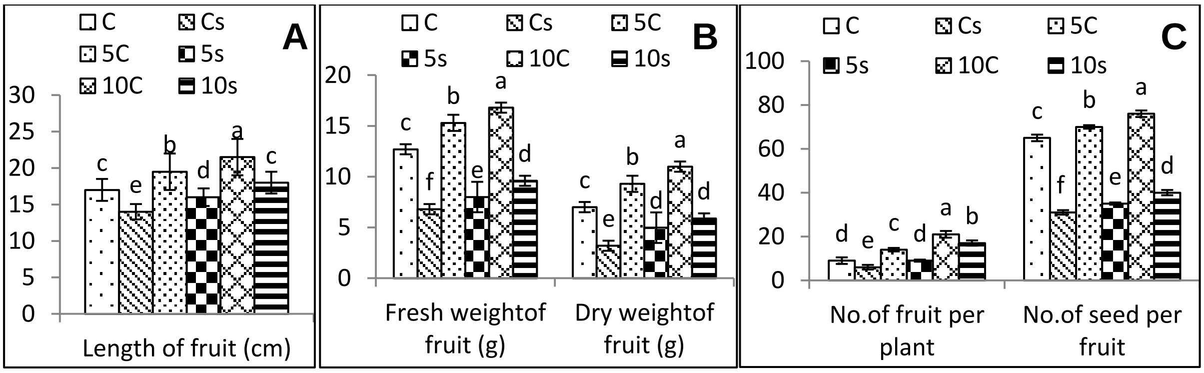

Figure 4. Effect of liquid phosphobacterium on yield parameters of *Abelmoschus esculentus* (L.) Moench (Okra): **A,** Length of fruit; **B,** Fresh and dry weight of fruit; **C,** Number of fruit per plant and seed per fruit. (C- Control; Cs- Control with drought stress; 5C- 5% LPB treated plants; 5Cs- 5% LPB treated plants with drought stress; 10C- 10% LPB treated plants; 10Cs- 10% LPB treated plants with drought stress)

DISCUSSION AND CONCLUSION

The present study showed that the LPB inoculated plants exhibited an increased rate of fresh and dry weight of which 10% LPB inoculation exhibited higher rate of fresh and dry weight (Fig. 1 A–B). The studies conducted by Singh & Singh (2010) also revealed higher dry matter content in PSB treated fenugreek plants, which reflected the tolerance of the plants due to bacterial inoculums. Similar pattern of dry matter increase was observed in finger millet treated with agrochemicals like $CaCl_2$ (Maitra *et al*. 1998), rice plants treated with *Rhizobacterium* (Raj *et al*. 2012) and black gram treated with bio-fertilizers (Selvakumar *et al*. 2012). All these results are in tune with the results obtained in the present study, which may lead to the conclusion that priming has an important role in nutrient uptake and growth, that may resulted in the high rate of biomass in plants treated with LPB.

Priming of okra seeds with LPB showed an increase in RWC which was very prominent in 10% LPB treated plants (Fig. 2). Studies conducted by Yordanov *et al*. (2003) observed that mild drought helped plants to regulate water loss and uptake, allowing maintenance of their leaf water content within the limits. According to Velentovic *et al*. (2006) RWC in the leaves of maize plants at low water potential decreased significantly compared to control. Similarly the RWC in drought affected leaves of okra was significantly lower than the continuously irrigated control plants. These are in confirmation with the results obtained in fenugreek (Singh & Singh 2010) and tomato (Shintu & Jayaram 2015) and the authors found that the plants inoculated with PSB exhibited highest level of RWC as compared to non-inoculated plants under controlled condition. All these observations revealed that the RWC in leaves of drought affected plants were significantly reduced as a result of relative water uptake and storage by the plants. So it can be presumed that comparatively high RWC in the leaves of LPB treated plants may be due to the higher activity of water uptake and restoration by these plants. Thus LPB can be considered as a bio-priming agent in order to tolerate drought stress.

Total chlorophyll content of LPB treated plants was found decreased but the decrease was not lesser than untreated plants (Fig. 3A). Drought stress produced changes in the total chlorophyll content (Farooq *et al*. 2009). The results obtained in the present study showed a high rate of chlorophyll pigment in plants raised from 10% LPB treated seeds. Estill *et al*. (1991) and Ashraf *et al*. (1994) observed same pattern of changes in chlorophyll content in stressed alfalfa and wheat respectively. Similarly fenugreek plants treated with PSB also showed identical results (Singh & Singh 2010). Moreover the studies conducted by Rupa (2007) also revealed that, under moisture stress conditions there will be degradation in pigment composition which ultimately induced a decrease in the chlorophyll content. In the present study total chlorophyll content of okra leaves decreased with increased moisture stress, but it was found increased during the recovery period. Higher persistence of chlorophyll content in plants under stress due to LPB may be attributed to decreased chlorophyll degradation and increased chlorophyll synthesis, as suggested by Jayakumar & Thangaraj (1998). So in the present study also higher level of photosynthetic pigment was obtained in LPB treated drought affected plants that may be due to non-degradation of chlorophyll. The LPB's regulatory effect on phosphate solubilisation may be beneficial to the non-degradation of chlorophyll pigments and that may be the reason for getting high chlorophyll content in the LPB treated drought affected plants.

The leaves of LPB treated okra plants exhibited an increase in total protein content which was more prominent in 10% LPB treatment (Fig. 3B). Similar results were obtained in fenugreek plants treated with phosphobacterium (Singh & Singh 2010). According to those authors the non-inoculated plants exhibited a lesser amount of protein than inoculated plants. Selvakumar *et al*. (2012) opined that double inoculation of *Rhizobium* with Phosphobacteria yielded more protein content than single and non-inoculated in black gram. *Rhizobium* increased protein content in sunflower by increasing nitrogen uptake (Shehata & EL-Khawas 2003). Radin (1984) suggested that high phosphorous caused stomatal opening, and facilitate plants to accumulate more protein in inoculated plants compared to non-inoculated plants. Accumulation of ABA caused by deficiency of phosphorus is directly proportional to the degree of water stress and thus resulted in stomatal closure and low photosynthetic rate (Singh & Singh 2010). So in the present study it can be presumed that LPB may cause to enhance the uptake of insoluble soil phosphorous and thus resulted in increased stomatal opening and ultimately enhanced the accumulation of protein in okra plants.

In addition to the beneficial effect on growth of plants, bio-priming is also known to increase the yield by its significant effect during drought on yield parameters of crop under study (Casanovas *et al*. 2003). In the present study, maximum yield was obtained in 10% LPB treated and water stressed okra plants which were followed by its control plants (Fig. 4). These results revealed that the LPB has an important role in increasing the yield and also to reduce the adverse effect on drought stress. According to Prabhakar & Saraf (1991) the interaction effect of limited irrigation regimes and phosphorous fertilizers was significant on sorghum grain yield. Chauhan *et al*. (1995) observed that inoculation of *Azospirillum* as a bio-fertilizer markedly increased pod number and seed yield of *Brassica napus* plants over the non-inoculated plants. Zodape (2001) suggested that, the increase in yield with bio-fertilizer application was due to micro-element and plant growth regulator contained in the fertilizer. So it can be presumed that the increase in yield of LPB treated plants exposed to water stress may be due to the positive effect of priming of okra seeds with LPB. Priming with LPB also enhanced the potential of the plant to drought stress by influencing on various physiological as well as biochemical parameters studied. So it can be concluded that LPB may beneficially affected plants to improve water status of okra plants that may help them to tolerate water deficit condition to a certain extend and to give high productivity. So, liquid phosphobacterium can be recommended to the farming community as a priming agent to their vegetable crops, in order to fight against mild drought stress.

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