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Effect of osmolytes and plant growth regulating chemicals in overcoming water stress in Bhendi during seed germination

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Abstract
Water stress is a serious threat to bhendi as it inhibits seed germination and seedling growth. Seed treatment with osmolytes and plant growth regulating chemicals found to be effective in mitigating water stress. In order to understand the response of bhendi to osmolytes and plant growth regulating chemicals in terms of seed germination and seedling growth, a laboratory experiment was conducted in petriplates using Bhendi CO4 hybrid seeds. Various components such as 50mM Proline, 50mM Trehalose, 50mM Glycine betaine, 100ppm Ascorbic acid, 1% KCl and 100ppm Salicylic acid were used. The water stress was induced by using PEG 6000 at a standardized concentration of -1.5 Bars. In this study, germination percentage was found to be maximum (78.78%) for the seeds treated with 50mM glycine betaine and the seeds treated with 50mM proline recorded the maximum shoot length (7.90 cm), root length (2.23 cm) and total dry matter production (0.42 g) while the seeds treated with 50mM glycine betaine recorded the maximum vigour index (745.86) as well as stress tolerance index (STI) (65.88). Results suggest that the pre-soaking of bhendi seeds in glycine betaine (50mM) under water stress condition can mitigate the effect of water stress, thereby achieve higher germination percentage with high vigour index and STI.

Keywords: Bhendi, water stress, seed germination, osmolytes, plant growth regulating chemicals

Introduction
Bhendi (*Abelmoschus esculentus* (L.) Moench, popularly known as lady's finger or okra, is an annual vegetable grown in tropical and subtropical regions of the world. It is the only vegetable crop of significance in the Malvaceae family. India is the largest producer of okra in the world. The edible part is a pod. The plant may have single or branched stem. Flowers are borne singly at every node or an alternate node. It is a seed propagated crop sensitive to frost, low temperature (below 15 °C), water-logging and drought conditions.
In 2009-2010, the total world area and the production under bhendi cultivation was 0.43 million hectares and 4.54 million tons respectively. In India production of okra was 5784 thousand tonnes and productivity 11.1 tonnes/hectare. (Indian Horticulture database, Ministry of Agriculture, Government of India, 2011) Okra is grown for its immature fruits. Okra is a good source of vitamins, calcium, potassium and other minerals. Okra is an important source of both soluble and insoluble fibre. Soluble fibre helps to lower serum cholesterol and reduces the risk of heart diseases. Insoluble fibre helps to keep the intestinal tract healthy and decreases certain forms of cancer. Per 100 g okra fruits have 89.6 g moisture, 6.4 g carbohydrates, 1.9 g protein, 0.2 g fat, 0.07 g thiamine, 0.1 g riboflavin, 13 mg vitamin C, 66 mg calcium and 1.5 mg iron.
Water stress is primarily caused by the water deficit, *i.e.* drought or high soil salinity. Water stress inhibits cell enlargement more than cell division. It reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters. (Jaleel *et al.*, 2009) [7]. Seed germination is first critical and the most sensitive stage in the life cycle of plants and the seeds exposed to unfavourable environmental conditions like water stress may have to compromise the seedlings establishment.
High-molecular-weight organic solutes such as poly ethylene glycol are not taken up by cells and can be used at high external concentrations as osmotica to induce water deficiency (water stress) in plants. Polyethylene glycol molecules with a molecular weight of 6000 are inert, non-ionic and virtually impermeable chain used to induce water stress and maintain a uniform water potential (Rohamare *et al.*, 2013).

Osmotic adjustment (OA) is referred as a net increase in solute concentration, may be perceived as important survival mechanism to drought stress. As water is being removed from the plant cells, its osmotic potential is reduced due to the effect of solute concentration. However, during the course of cellular water loss, solutes are actively accumulated. This reduces the out flow of water from cell, thereby reducing loss of turgor and allows stomatal opening and expansion growth to continue progressively at lower water potentials.

Osmolytes are low molecular weight and soluble compounds. Many studies indicated that the accumulation of compatible solutes in plants causes resistance to various stresses such as drought, high temperature and high salinity. The primary function of compatible solutes is to prevent water loss to maintain cell turgor and to maintain the gradient for water uptake into the cell, protect and stabilize 3D structure of proteins and photosynthetic apparatus (Papageorgiou & Murata, 1995) [11], regulate cellular osmotic adjustment and detoxify reactive oxygen stresses. Upon relief from stress these solutes are metabolized and are considered as an important energy source (Hare *et al.*, 1998) [5]. Compatible solutes are divided into three major groups - amino acids (e.g. proline), polyamins and quaternary amines (e.g. glycinebetaine), polyol (e.g. mannitol, trehalose) and sugars like sucrose and oligosaccharides (Hare *et al.*, 1998) [5]. Seed treatment with osmolytes and plant growth regulating chemicals can help the seeds to overcome water stress. It enhances the rate of seed germination and seedling growth. With this background, a laboratory study was carried to find out the effect of plant growth regulating chemicals and osmolytes in mitigating water stress induced by PEG 6000 during seed germination in bhendi.

Materials and methods

An attempt was made to alleviate the water stress effect by soaking the bhendi seeds in osmolytes and plant growth regulating chemicals. The experiment was carried out as laboratory study at Crop Physiology Unit, Department of Seed Science and Technology, AC & RI, Madurai. The experiment was laid out under completely randomized block design with eight treatments and three replications. The seeds of bhendi CO4 hybrid were placed in petriplates. The petridishes for the experiment were sterilized using 0.01 per cent $HgCl_2$ and 70 per cent ethanol and finally repeated washing with distilled water. The water stress was created by using PEG 6000 in different concentrations *viz*., -0.5 Bars, -1.0 Bars, -1.5 Bars and -2.0 Bars. Among the concentrations of PEG 6000 used, only 50 per cent of the seeds were germinated in -1.5 Bars concentration of PEG 6000 compared with control. Hence, -1.5 Bars was selected to carry out the experiment.

Seeds were soaked in osmolytes and plant growth regulating chemicals as per treatment schedule *viz.*, T1: Absolute control, T2: Control (-1.5 Bars), T3: Proline @ 50mM, T4: Ascorbic Acid @ 100ppm, T5: Glycinebetaine @ 50mM, T6: Trehalose @ 50mM, T7: KCl @ 1%, T8: Salicylic acid @ 100ppm solutions for 12 hours. After the seeds were shade dried for 4 hrs and placed on germination paper in each petridish separately. The petridishes were kept in laboratory under room temperature. The seeds were allowed to germinate by pouring the -1.5 Bars solution of 10 ml each once in three days. Distilled water was used for maintaining the absolute control. The growth parameters were measured on 15 days after placing the seeds in petriplates. The observations recorded were germination percentage, Shoot length, root length, total dry matter production. The shoot length of individual plants were expressed from the first cotyledonary node to the tip of the main stem and expressed in centimeters (cm). The root length of each plant was measured from the cotyledonary node to the root tip and expressed in cm. The vigour index was calculated using the formula proposed by Abdul-Baki and Anderson (1973) and the stress tolerance index was calculated using the formula proposed by Dhopte and Livera (1989) and expressed as per cent. The data were analyzed statistically by the methods outlined by Gomez and Gomez (1984).

Results and Discussion

Water stress caused a significant reduction in seed germination rate and seedling growth. Experimental results showed that the seed treatment with osmolytes and plant growth regulating chemicals has showed a significant increase in germination percentage, shoot and root length, total dry matter production. Among the osmolytes and plant growth regulating chemicals, the maximum germination percentage was achieved seed treatment with 50mM Glycine betaine (78.78%) followed by 50mM Proline (70.08%) and the lowest germination percentage was achieved in the control of -1.5bars PEG 6000 (48.33%) (Table 1). Similar results were observed in presowing seed treatment of glycine betaine in mitigating water stress and act as a osmoprotectant in wheat (Mahmood *et al.*, 2009) [10]. However, glycine betaine as a foliar spray found to be effective than seed treatment in sunflower (Iqbal *et al.*, 2005) [6] and aromatic rice (Farooq *et al.*, 2008) [4].

The shoot length was maximum for seeds treated with 50mM Proline (7.90 cm) followed by 50mM Glycine betaine (7.37 cm) and minimum for control of -1.5bars PEG 6000 (4.83 cm) (Table 1). The root length was maximum seed treated with 50mM Proline (2.23 cm) followed by 50mM Glycine betaine (2.10cm) and minimum for control of -1.5bars PEG 6000 (1.63 cm) (Table 1). The total dry matter production was observed to be maximum for seed treatment with 50mM Proline (0.42g) followed by 50mM Trehalose (0.40g) and minimum for control of -1.5bars PEG 6000 (0.29g) (Table 1). Similar results were observed with pre-sowing seed treatment with proline in alleviating the effect of water stress in spring wheat **(**Kamran *et al.*, 2009) [8] and faba bean plants (Taie *et al.*, 2013) [13]. Since, proline as a proteinogenic amino acid act as an osmoprotectant, maintains protein structure and cell membrane from damage (Dawood. 2016) [2].

Table 1: Effect of osmolytes and plant growth regulating chemicals on germination %, shoot length, root length, total dry matter production (TDMP) and STI

Treatment	Germination Percentage (%)	Shoot length (cm)	Root length (cm)	TDMP(g)	STI
T_1: Absolute control	91.67	8.63	3.73	0.51	-
T_2: Control (-1.5bars)	48.33	4.83	1.63	0.29	27.60
T_3: Proline (50mM)	70.08	7.90	2.23	0.42	62.73
T_4: Ascorbic acid (100 ppm)	66.92	6.80	2.03	0.39	52.27
T_5: Glycine betaine (50mM)	78.78	7.37	2.10	0.35	65.88
T_6:Trehalose (50mM)	65.17	6.53	2.00	0.40	49.11
T_7: KCl (1%)	62.10	5.90	1.97	0.37	43.09
T_8: Salicylic acid (100ppm)	64.82	5.73	2.03	0.39	43.97
Mean	68.48	6.71	2.22	0.39	49.24
SEd	1.58	0.22	0.04	0.02	1.46
CD (P=0.05)	3.34	0.46	0.09	0.05	3.14

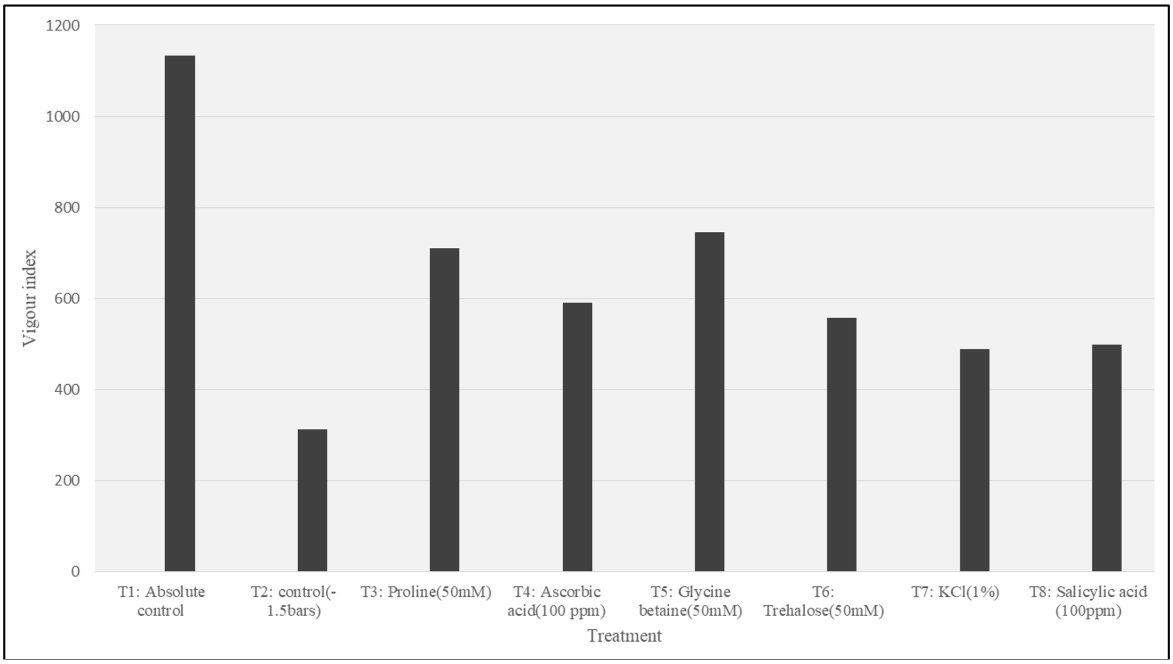

Fig 1: Effect of osmolytes and pl-ant growth regulating chemicals on Vigour index

The vigour index shows the level of performance of seed or seed lot during germination and seedling emergence which is found to be maximum the seeds treated with 50mM Glycine betaine (745.86) followed by 50mM Proline (710.16) and minimum for control of -1.5bars PEG 6000 (312.29) (Figure 1). The stress tolerance index shows the ability of the plant to withstand water stress and it was maximum for seed treatment with 50mM Glycine betaine (65.88) followed by 50mM Proline (62.73) and minimum for control of -1.5bars PEG 6000 (27.60) (Table 1). Similar results were observed in presowing seed treatment of glycine betaine in mitigating water stress and act as a osmoprotectant in sunflower (Iqbal *et al.*, 2005) [6]. Since glycine betaine, quaternary ammonium compound, act as a osmoprotectant for plants and protects cell components from stress conditions by regulating the water potential equilibrium in the cell, thereby, maintaining the turgor pressure and protecting cell from dehydration during water deficit conditions (Kaya *et al.*, 2013) [9]. Osmolytes and plant growth regulating chemicals maintains cellular osmotic balance and protects plants from water stress. Hence pre-soaking seed treatment with these osmolytes can help the plants in mitigating water stress conditions.

Among the osmolytes and plant growth regulating chemicals used the germination percentage, vigour index and stress tolerance index were found to be maximum for the seeds treated with 50mM Glycine betaine. Hence, the glycine betaine at a concentration of 50mM as seed treatment under water stress condition will be effective in mitigating water stress.

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