



RESEARCH ARTICLE

Mitigating moisture stress in bhendi through osmolyte application

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Abstract

The purpose of this study was to identify the best osmolyte for foliar application to overcome moisture stress under field conditions in Bhendi (*Abelmoschus esculentus* L. Moench). Foliar applications of the osmolytes, viz., proline (50 mM), glycine betaine (50 mM), ascorbic acid (100 ppm), salicylic acid (100 ppm) and KCl (1 %) were applied twice, at 25 and 45 days after sowing (DAS), to mitigate moisture stress imposed by withholding irrigation during the vegetative and reproductive phases of crop growth. Data on yield parameters and yield were recorded. The results revealed that, in terms of the yield attributes, foliar sprays of proline (50 mM) resulted in increased pod length (12.2 cm) and higher pod yield per plant (335.1 g) in Bhendi. However, the highest benefit cost ratio of 1.52 and 1.23 was obtained by applying KCl (1%) followed by salicylic acid (100 ppm) during moisture stress at the reproductive stage. From the study, it is concluded that foliar sprays of proline (50 mM) and salicylic acid (100 ppm) were effective in mitigating moisture stress under field conditions in Bhendi.

Keywords

bhendi; moisture stress; osmolyte; yield; B:C ratio

Introduction

Drought is one of the most detrimental factors affecting plant growth and productivity and is considered a serious threat to sustainable crop production in the context of climate change. Drought affects different physiological processes in plants, including ion uptake and translocation, photosynthesis, respiration and carbohydrate and nutrient metabolism (1). Drought stress reduces growth rate, which in turn affects the transpiration rate, thereby lowering biomass yield (2). Bhendi (*Abelmoschus esculentus* L. Moench) is popularly known as lady's finger or okra. It is the only vegetable crop of significance in the Malvaceae family. The genus *Abelmoschus* originated in India (3) which is also the largest producer (67.1%) of bhendi, followed by Nigeria (15.4%) and Sudan (9.3 %). Bhendi is an annual, often cross-pollinated crop usually propagated through seeds and is cultivated for its immature pods or green non-fibrous fruits containing round seeds and eaten as a vegetable. The roots and stem of bhendi are used for clarification of sugarcane juice during the preparation of gur or brown sugar (4). The oil content of bhendi seed is about 20-40 percent. Various reports suggest that the seed oil of okra can be used as an alternate source of edible oil. Due to its high fibre and mucilage content, bhendi can be used to cure gastric problems and treat digestive issues (5). Bhendi is highly sensitive to

drought, low temperature and flood conditions. Bhendi is grown in the tropics, sub-tropics and in some warmer areas of temperate regions. Its cultivation is also spread out to dry areas, where drought limits the plant growth and pod yield (optimum yields of 2-3 tonnes ha⁻¹) of okra (6). Drought stress is a major constraint in hampering plant productivity by cell elongation, enlargement, division at the cellular level, reducing leaf size, limiting stem extension, restricting the root proliferation, ion uptake, translocation of photo-assimilates, photosynthesis, respiration, carbohydrates and nutrient metabolism.

Various strategies have been proposed to sustain crop production under drought conditions, in particular the development of new crop varieties with enhanced drought tolerance (7). Genetic improvement of crop plants for drought tolerance is a long-term endeavour, which requires, among other things, the availability of genetic sources of tolerance, knowledge of the physiological mechanisms and genetic controls of tolerance traits at different developmental stages and employment of suitable germplasm and screening and breeding protocols. An alternative and quicker strategy to promote plant drought tolerance is an exogenous application of various compounds, including organic solutes (organic osmolytes and plant growth regulators) and mineral nutrients. Recently, this strategy has gained considerable attention because of its efficiency, feasibility and cost- and labour-effectiveness. Organic osmolytes can be externally applied through three different means: as a pre-sowing seed treatment, through the rooting medium, or as a foliar spray. The application of osmolytes through the soil (growth medium) does not seem to be feasible because supplemented osmolytes are prone to degradation by soil microorganisms (8). Further, the large-scale use of osmolytes as soil additives to ensure adequate supply to plants is very costly. Thus, the application of osmolytes as foliar application is an effective method to mitigate moisture stress in crops. To reduce the negative impacts of drought stress, a quick strategy would be the foliar application of plant growth regulating chemicals including osmolytes. Osmoregulation is the most common physiological adaptation, which reduces cellular water potential via the accumulation of a variety of organic and inorganic solutes in the cell. As a consequence, such plants are capable of taking up water from a low water potential medium to sustain normal or near normal physiological processes necessary for growth and development. Exogenous proline application is known to increase abiotic stress tolerance in plants. Proline protects protein structures and cell membranes from damage, thereby reducing enzyme denaturation. When barley roots were subjected to salt stress, exogenous proline application in low concentrations stimulated the uptake of potassium (9). During stress, potassium acts as a primary osmoticum. Potassium fertilization increases nitrate uptake and increases dry matter production during drought (10). Exogenous potassium application can alleviate drought-induced negative effects on plants by regulating protein synthesis and osmoregulation. Potassium (K) is one of the essential macronutrients required by plants. It serves as a primary osmoticum under stressful environments. Potassium fertilization increased nitrate assimilation and dry matter production under drought (11).

Exogenous application of potassium can alleviate drought-induced negative effects on plants by regulating protein synthesis and osmoregulation (12). Potassium application enhances cotton plants potential for sustaining high nitrogen-metabolizing enzyme activities and related components to supplement osmotic adjustment under soil drought conditions (13). Glycine betaine, a quaternary ammonium compound, is an effective osmo protectant against abiotic stress. Salicylic acid plays an important role in enhancing abiotic stress tolerance in plants. Optimized concentrations of salicylic acid lead to significant improvements in both the growth and quality of tomatoes, especially during challenging summer months. Among different concentrations of salicylic acid tested, foliar treatments with 250 mg L⁻¹ showed the most promising results, enhancing various morpho-physiological parameters crucial for plant development as well as significantly increasing the activity of antioxidant enzymes and the expression of antioxidant genes (14). With this background, the present study was planned to examine the effect of different osmolytes on the yield of bhendi subjected to moisture stress.

Materials and Methods

A field experiment was conducted in 2019 and 2020 to investigate the effect of osmolytes on Bhendi hybrid CO 4 subjected to moisture stress. The experiment was conducted in the "C" block of Agricultural College and Research Institute, Madurai (Plate 1). The farm is located at 9°54' N latitude and 78°54' E longitude at an elevation of 147 m above MSL. The maximum and minimum temperatures during the study period were 34.83°C and 19.33°C, respectively and the relative humidity ranged between 78% and 92%. The soil type is sandy clay loam with a soil pH of 7.05 and organic matter content of 0.74 percent. The experiment was laid out in a split-plot design with three replications. Ridges and furrows were formed using a ridge former and a spacing of 45 cm × 30 cm was adopted. The irrigation channels were formed at 90 cm in width. The plots were separated by 0.3 meters, and the blocks were separated by 1.0 m. Sowing, gap filling, applying pre-emergence herbicides and fertilizers, weeding, irrigation and pod picking were carried out as per schedule. During vegetative and reproductive stages, water stress was imposed by withholding irrigation. Water-stressed plots were irrigated at 50 percent depletion of available soil moisture (15).

Before irrigation, the available soil moisture was measured gravimetrically by pooling samples taken from 0-15 cm and 15-30 cm soil layers. The gravimetric method for measuring soil moisture consists of a series of systematic steps designed to accurately assess the water content in soil samples. First, representative soil samples are collected from the field using a soil auger or core sampler, taking care to minimize disturbance and moisture loss. These samples are then transferred to airtight containers to prevent evaporation during transport to the laboratory. Once in the lab, the initial mass of the wet soil is recorded by weighing the containers. Next, the samples are dried in a forced-air oven at a consistent temperature of 105°C for 24 hours to ensure complete moisture removal. After drying, the containers are



Plate 1. Field view of bhendi

allowed to cool in a desiccator, which prevents any moisture reabsorption from the atmosphere. Finally, the dried samples are weighed again to obtain their final mass (16).

The moisture content is then calculated using the following formula:

$$\text{Gravimetric Water Content (GWC) (\%)} = \frac{(\text{Wet Soil Mass} - \text{Dry Soil Mass})}{\text{Dry Soil Mass}} \times 100$$

Irrigation was done sufficiently to restore soil moisture to field capacity in the top 0-30 cm layer. Moisture-stressed plants were given foliar treatments at 25 and 45 DAS with proline (50 mM), KCl (1%), glycine betaine (50 mM), ascorbic acid (100 ppm) and salicylic acid (100 ppm) to study their effect on mitigating water stress in plants. The treatments were named M0 - No stress (control), M1 - Moisture stress at the vegetative stage, M2 - moisture stress at the reproductive stage, Subplot: S1 - Control, S2 - Proline (50 mM) S3 - KCl (1%), S4 - Glycine betaine (50 mM), S5 - Ascorbic acid (100 ppm) and S6 - Salicylic acid (100 ppm). Data on the number of pods, pod length, individual pod weight and pod yield per plant were recorded from three representative samples collected randomly from each replication and the benefit cost ratio was estimated. The data on various parameters were analyzed statistically as per the procedure suggested by (17).

Results and Discussion

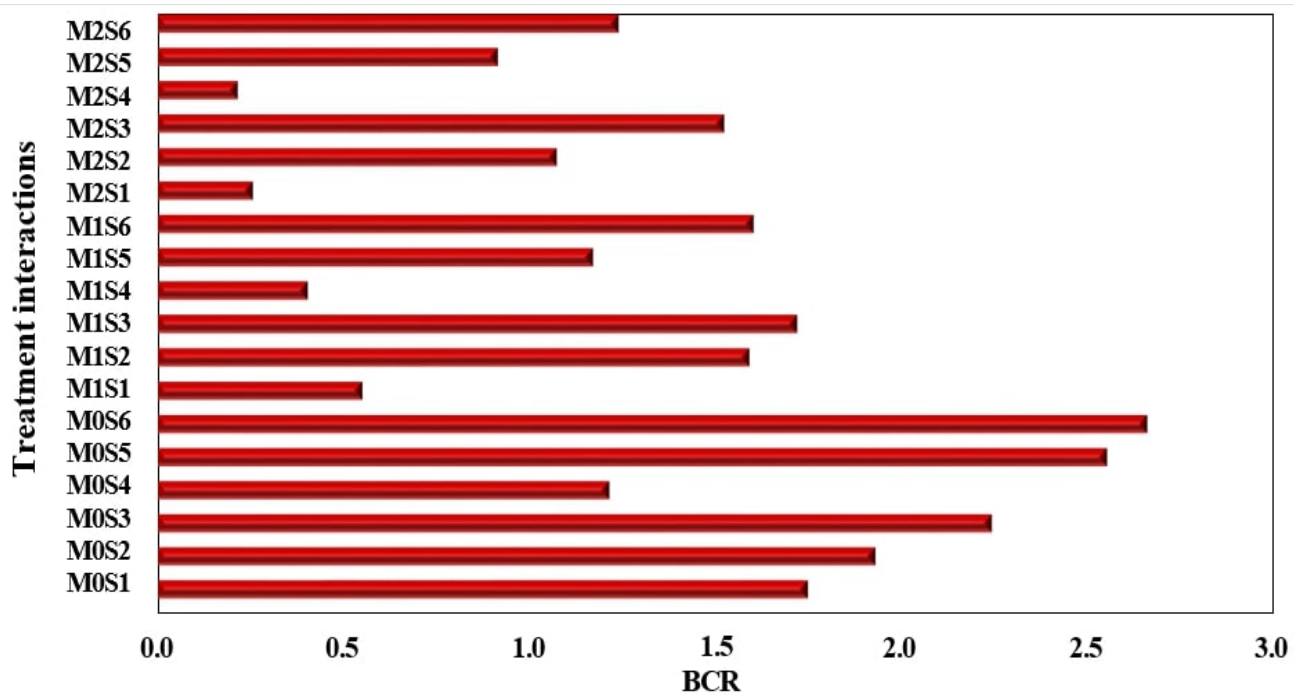
A foliar spray of osmolytes resulted in significant improvement in yield parameters of Bhendi subjected to moisture stress. The effect of osmolytes on pod length and pod number is given in Table 1. In the present study, the maximum pod length of 13.4 cm was observed in the no moisture stress control plot. Among the various osmolyte treatments, foliar sprays of glycine betaine (50 mM) recorded a maximum pod length of 12.2 cm and a minimum pod length

of 11.5 cm was observed in ascorbic acid (100 ppm) treatment. Application of glycine betaine (50 mM) during moisture stress at the vegetative stage resulted in the highest pod length of 12.6 cm, followed by ascorbic acid (100 ppm) application during moisture stress at the vegetative stage (12.5 cm). Moisture stress at the reproductive stage resulted in a reduction in pod length. Moisture stress at the reproductive stage recorded the lowest pod length (8.1 cm) which is the most sensitive stage to moisture stress and was on par with the treatment combination of ascorbic acid (100 ppm) application under moisture stress at the reproductive stage. Application of proline (100 mM) resulted in maximum pod length during reproductive stage moisture stress (Fig.1). Proline, a proteinogenic amino acid acts as an osmoprotectant, maintains cell turgor as a signalling molecule (18), promotes the uptake of mineral nutrients from moisture deficit soil (19), enhances gaseous exchange, prevents electrolyte leakage and lower down the formation of reactive oxygen species (20). The pod length and the number of pods per plant were reduced with the imposition of drought. The number of bolls in cotton was reduced by 5.9% during the drought (21). Under a limited water supply, the number of pods per plant is reduced either due to flower shedding or flower abortion. Water stress at flowering generally results in barrenness.

The number of pods per plant was affected by moisture stress (Table 1). As expected, a higher number of pods per plant (18.2) was observed in the no moisture stress plot compared to those subjected to moisture stress at vegetative and reproductive stages. Application of proline (50 mM) recorded the maximum pods per plant (18.7) under irrigated conditions. In the interaction studies, the highest number of pods per plant (19.0) was observed under moisture stress at the reproductive stage with the application of glycine betaine (50 mM), followed by the proline (50 mM) with 18 pods per plant. Water deficit at critical stages of crop growth, such as flowering, affects the plant more severely than at the vegetative stage (22). This has been demonstrated through several experiments in okra (23), where the drought

Table 1. Effect of osmolytes on pod length and pod number in Bhendi Hybrid CO 4 under moisture stress conditions

Treatment	Pod length (cm)				Number of pods per plant			
	M0	M1	M2	Mean	M0	M1	M2	Mean
S1	12.7	9.1	8.1	10.0	17.4	13.3	13.3	14.7
S2	13.2	11.8	10.2	12.2	19.2	18.3	18.5	18.7
S3	13.6	12.0	9.4	11.7	18.5	17.4	17.6	17.9
S4	14.0	12.6	9.9	11.7	18.3	17.3	19.0	18.2
S5	13.5	12.5	8.6	11.5	17.5	16.2	16.7	16.8
S6	13.7	11.9	8.9	11.5	18.0	17.7	17.6	17.8
Mean	13.4	11.6	9.2		18.2	16.7	17.1	
	SEd			CD (P:0.05)	SEd			CD (P:0.05)
M	0.18			0.51	0.19			0.54
S	0.30			0.62	0.39			0.79
M × S	0.51			1.10	0.64			1.36

Main plotM₀- No stressM₁- Moisture stress at vegetative stageM₂- Moisture stress at reproductive stage**Sub plot**S₁- ControlS₂- Proline (50mM)S₃- KCl (1%)S₄- Glycine betaine (50mM)S₅- Ascorbic acid (100ppm)S₆- Salicylic acid (100ppm)**Fig 1.** Effect of plant osmolytes on benefit cost ratio of bhendi hybrid CO4 under moisture stress

significantly affected growth and yield. Drought-induced reduction in yield components is associated with the closure of stomata under low moisture levels, which leads to a decrease in CO₂ entry through the stomata, subsequently resulting in a decreased assimilate production. In the present study, the foliar application of proline (50mM) recorded the maximum number of pods per plant under irrigated as well as under drought conditions. This might be because proline is regarded as a source of energy, carbon and nitrogen under moisture stress when both starch and protein synthesis are inhibited. Proline acts as a protective agent and stabilizes the functional proteins as well as enzymes in the cell sap. Similar results were observed in cowpea plants, where an increased number of pods and higher grain filling percentage under moisture stress condition (24).

Moisture stress significantly reduced the yield of wheat to a maximum level and this reduced yield in wheat was due to water stress at or before flowering and spike emergence which reduced the number of spikelets per spike. The flowering stage is considered the most sensitive phase of crop growth and any stress during that phase results in reduced yield (25). Application of osmolytes increased individual pod weight both under non-stressed and water-stressed conditions (Table 2). Proline (50mM) application resulted in the maximum value for individual pod weight both under water-stressed and non-stressed conditions; followed by KCl (%) treatment under water-stressed conditions. Moisture stress at the reproductive stage resulted in a significant reduction in individual pod weight compared to moisture stress at the vegetative stage indicating the reproductive stage to be very sensitive to moisture stress.

A considerable difference in the total pod yield in bhendi was observed depending on the level of moisture tension by application of osmolytes. The highest pod yield of 350.7 g per plant was recorded in no moisture stress control, and the minimum yield of 215.4 g plant⁻¹ was observed due to moisture stress at the reproductive stage (Table 2). A maximum yield of 335.1g per plant was observed in foliar sprays of proline (50 mM) (Plate 3) and a minimum yield per plant of 253.4 g was observed in ascorbic acid (100 ppm). The combination of proline (50 mM) application and moisture stress at the vegetative stage produced the highest pod yield per plant of 328.5 g, followed by KCl (1%) and moisture stress at the vegetative stage (268.7 g per plant). Moisture stress at the reproductive stage without osmoprotectants resulted in a lower yield of 123.2 g per plant indicating the reproductive stage to be more sensitive to moisture stress than the vegetative stage in Bhendi. Reduced crop yields due to moisture stress during the flowering phase have been reported earlier (26).

Yield declines in okra due to a decrease in yield attributes induced by water deficit have been observed (27). Under water deficit stress, limited extraction of water and nutrients by roots shortens the life cycle, accelerates physiological senescence and decreases photosynthetic translocations, resulting in reduced growth and fruit yields in vegetable crops (28). In the present study, a foliar spray of proline (50 mM) increased the yield of bhendi under both normal and stress conditions. Previous studies have also shown that foliar application of proline under drought improved the yield of wheat (29, 30). Proline application is well known to provide an energy source for plant growth and to protect enzymes and proteins. Likewise, the achene yield of sunflower under stress conditions was improved through proline application (31).

A notable difference in the gross return of bhendi due to moisture stress and foliar application of plant growth regulators was observed. Among the various moisture stress treatments, the highest BCR of 2.05 was observed in the No

Table 2. Effect osmolytes on pod weight and pod yield in Bhendi Hybrid CO 4 under moisture stress conditions

Treatment	Individual pod weight (g per pod)				Pod yield per plant (g)			
	M0	M1	M2	Mean	M0	M1	M2	Mean
S1	15.7	11.5	9.3	12.2	273.8	152.8	123.2	183.3
S2	21.6	18.0	14.2	17.9	415.1	328.5	261.7	335.1
S3	17.5	15.4	14.1	15.7	323.4	268.7	248.2	280.1
S4	19.5	14.3	12.6	15.5	355.5	248.5	240.3	281.4
S5	20.4	13.3	11.3	15.0	356.1	215.2	188.9	253.4
S6	21.1	15.1	13.1	16.4	380.6	267.1	230.1	292.6
Mean	19.3	14.6	12.4		350.7	246.8	215.4	
	SEd			CD (P:0.05)	SEd			CD (P:0.05)
M	0.94			1.21	10.40			28.88
S	0.46			0.94	8.20			16.74
M x S	0.85			1.89	16.62			38.72

Main plot

M₀- No stress

M₁- Moisture stress at vegetative stage

M₂- Moisture stress at reproductive stage

Sub plot

S₁- Control

S₂- Proline (50mM)

S₃- KCl (1%)

S₄- Glycine betaine (50mM)

S₅- Ascorbic acid (100ppm)

S₆- Salicylic acid (100ppm)

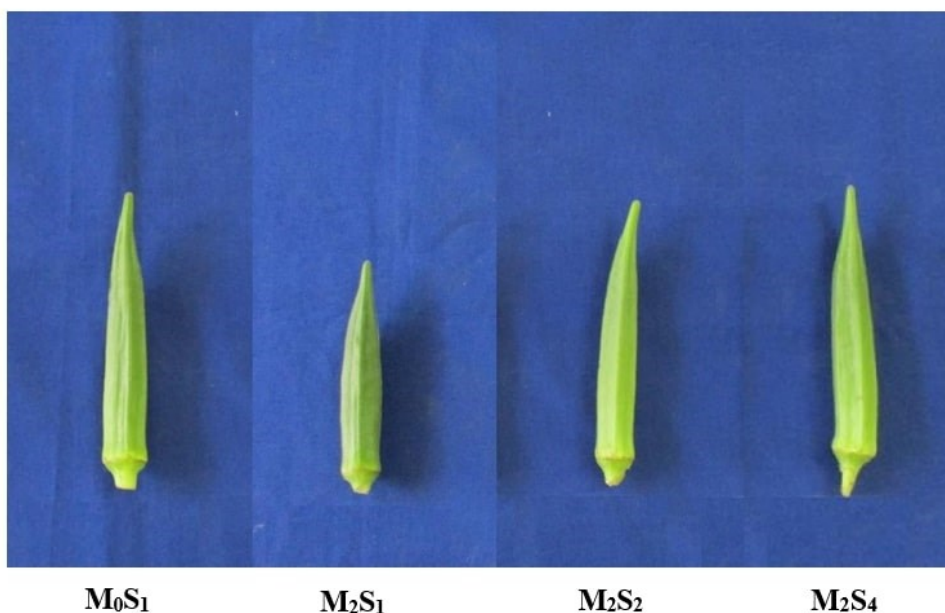


Plate 2. Variation in pod length in Bhendi Hybrid CO 4 under different treatments

stress (control) applied plot (Fig. 2). On the other hand, moisture stress at the reproductive stage registered the lowest BCR of 0.87. The highest benefit-cost ratio was found in the treatment interaction of moisture stress at the reproductive stage and salicylic acid (100 ppm) followed by KCl (1%) (Fig. 2). These results align with earlier reports where the application of salicylic acid yielded the maximum benefit cost ratio compared to other treatments (32). This might be attributed to the low cost incurred on chemicals.

Osmolytes can regulate many complex mechanisms and key physiological processes under abiotic stress environments. These include plant water relations, photosynthetic rate, proline metabolism, nitrogen metabolism and the antioxidant defense system. By modulating gene expressions related to reactive oxygen species, osmotic adjustment, metabolite biosynthesis, hormone upregulations and maintaining redox potential, osmolytes help to regulate source-sink homeostasis and improve plant resilience (33). Osmolytes aid in the efficient utilization of metabolites in specific physiological processes, which increase chlorophyll protein synthesis, vigour growth, and subsequently active translocation of photoassimilates from source to sink tissues, thus improving physical quality attributes and pod yields (34). These results can be primarily attributed to foliar spraying with osmolytes at various growth stages which encourages plant photosynthesis, maintenance of cell water for a longer duration, plant regeneration through stimulation of cell growth, and potential mechanisms of translocation of photoassimilates, which eventually led to improved dry weight accumulation and other quality attributes under water deficit conditions (35, 36).

Conclusion

Uncertainties in climatic conditions, particularly drought, subject plants to various abiotic stresses, including moisture stress, which interferes with normal physiological and biochemical processes. Reduced water availability directly correlates with reduced biomass production, ultimately lowering crop yield. In this study, under field conditions, the foliar spray of proline (50 mM) and salicylic acid (100 ppm) positively responded to mitigating moisture stress. But while considering economics, the foliar spray of KCl (1%) and salicylic acid (100 ppm) was effective in overcoming moisture stress compared to other treatments. The findings suggest that the strategic use of osmolytes like proline and KCl could serve as an effective mitigation strategy for moisture stress in Bendi. Future studies could focus on optimizing concentrations, exploring combinations with other stress-relief agents and assessing long-term impacts on plant health and resilience across abiotic stress conditions.

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Authors' contributions

RA carried out the field experiments and did the statistical analysis. CP conceived and designed the experiment. RG, MPK, GS, PG, MM, NS & NA revised and finalised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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