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Influence of plant growth regulating chemicals on physiology of bhendi under moisture stress

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Abstract

Drought or moisture stress reduces growth and yield of various crops by decreasing chlorophyll pigments, photosynthetic rate, stomatal conductance as well as transpiration rates. Under such water limited conditions, the productivity of the crop can be improved by foliar spray of plant growth regulating chemicals. In the present study, split plot design was employed with moisture stress as main plot (M_0 – No stress, M_1 – Moisture stress at vegetative stage, M_2 – Moisture stress at reproductive stage) and foliar spray of plant growth regulating chemicals as sub plot (S_1 – Control, S_2 – Proline (50 mM), S_3 – KCl (1%), S_4 – Glycine betaine (50 mM), S_5 – Ascorbic acid (100 ppm), S_6 – Salicylic acid (100 ppm)) in three replicates. Among the physiological parameters observed, the maximum Relative Water Content (RWC) (89.8%) was observed with glycine betaine (50 mM) treatment at 40 Days. While the highest Chlorophyll Stability Index (CSI) (88.2%) was observed in proline (50 mM) treatment at 60 Days. Similarly, the Membrane Stability Index (MSI) (82.1%) was maximum with proline (50 mM) treatment at 40 Days. With respect to biochemical parameters, the higher leaf proline content of 315.9 μg g^{-1} was observed in proline (50 mM) treatment at 80 Days. The maximum leaf soluble protein content (17.1 mg g^{-1}). While, the catalase activity was maximum (7.9 μg H_2O_2 g^{-1} min^{-1}) with proline (50 mM) as well as ascorbic acid (100 ppm) at 60 Days.

Keywords: *Moisture stress, Plant growth regulating chemicals, Glycine betaine, Proline*

1. Introduction

Bhendi (*Abelmoschus esculentus* L.) Moench, 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 (Masters *et al.*, 1875). India being largest producer (67.1%) in Bhendi, followed by Nigeria (15.4%) and Sudan (9.3%). It is an annual, often cross pollinated crop usually propagated through seeds. It is cultivated for its immature pods or green non-fibrous fruits containing round seeds and eaten as vegetable. In the preparation of gur or brown sugar, the roots and stem of bhendi is used for clarification of sugarcane juice (Chauhan, 1972). The oil content of bhendi seed is about 20-40%. Various scientists reported that seed oil of okra can be used as an alternate source of edible oil. Due to its high fiber and mucilage content, bhendi can be used to cure gastric problems and treat digestive issues (Gemede *et al.*, 2015).

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Bhendi is highly sensitive to drought, low temperature and flood conditions. Among various stresses, drought is one among the utmost adverse factors affecting plant growth and productivity and considered as serious threat for sustainable crop production in the circumstance on altering climate (Anjum *et al*., 2011). Under water limited conditions, the productivity of the crop can be improved by adopting suitable management practices especially at critical stages of crop growth. One such management practice is the use of certain chemicals which facilitate maintenance of internal water balance at an optimal level (Kramer and Boyer, 1995).

Exogenous application of proline is known to induce abiotic stress tolerance in plants, since, proline protect protein structure and cell membranes from damage, and thereby reduce enzyme denaturation. Maggio *et al.* (2002) reported that proline accumulation under stress can also act as a regulatory and signaling molecule in activating a variety of responses.

Glycine betaine, an important quaternary ammonium compound is considered to be one of the most effective osmoprotectant against abiotic stress (Dawood, 2018). It is reported that, glycine betaine is the only solute accumulated in higher plants subjected to osmotic stress, satisfies other solute requirements (Gorham *et al*., 2000).

Ascorbic acid, a non-enzymatic complex, plays important role like protection against Reactive Oxygen Species (ROS) that are formed from photosynthetic and respiratory processes (Naz *et al*., 2016). Foliar spray of ascorbic acid can alleviate oxidative damage occurred due to drought in okra (Amin *et al*., 2009).

Salicylic acid (SA) plays an important role in plants in abiotic stress tolerance (Raskin, 1992). It acts as an important signaling molecule in plants and has different effect on tolerance to abiotic stress.

Potassium (K) is one of the essential macronutrient required by the plants. It serves as a primary osmoticum under stressful environments. Potassium fertilization increased nitrate assimilation and dry matter production under drought (Zhang *et al*., 2014). Exogenous application of potassium can alleviate drought induced negative effects on plant by regulating protein synthesis and osmoregulation (Maathuis and Sanders, 1996).

2. Materials and methods

The experiment was conducted in the "C" block of Agricultural College and Research Institute, Madurai. The farm is located at 9°54" N latitude and 78°54" E longitude at an elevation of 147 m above Mean Sea Level (MSL). The soil type is sandy clay loam soil with available soil moisture at field capacity and permanent wilting point was 23.1% and 9.6% respectively. Split plot design was used with moisture stress as main plot (M_0 – No stress, M_1 – Moisture stress at vegetative stage, M_2 – Moisture stress at reproductive stage) and foliar spray of plant growth regulating chemicals as sub plot (S_1 – Control, S_2 – Proline (50 mM), S_3 – KCl (1%), S_4 – Glycine betaine (50 mM), S_5 – Ascorbic acid (100 ppm), S_6 – Salicylic acid (100 ppm)) in three replicates. Water stress was imposed by withholding irrigation at vegetative and reproductive stages. Water stress plots were irrigated at 50% depletion of available soil moisture. The available soil moisture was estimated gravimetrically before each irrigation by taking and pooling samples at 0-15 cm and 15-30 cm soil layers (Dastane, 1967). The amount of water at each irrigation was sufficient to raise the soil moisture content of top 0-30 cm layer back to field capacity. The following observations were taken on 40, 60 and 80 days after sowing.

2.1. Relative water content (RWC)

The RWC was estimated according to Barrs and Weatherley (1962) and calculated by using following formula and expressed as percent.

$$RWC = [(Fresh\ weight - Dry\ weight) / (Turgid\ weight - Dry\ weight)] \times 100$$

2.2. Chlorophyll stability index (CSI)

CSI was determined by utilizing following protocol by adopting the method of (Murphy, 1962) and expressed in percentage.

2.3. Membrane stability index (MSI)

MSI was determined by utilizing following protocol by adopting the method of Premachandra *et al.* (1990) as modified by Sairam (1994) and expressed in percentage.

2.4. Leaf soluble protein

Soluble protein content in the leaf was estimated by the procedure described by (Lowry *et al*., 1951) using Folin Ciocalteau reagent and expressed as mg g^{-1} fresh weight.

2.5. ***Leaf proline***

The proline content of the leaf was estimated through the protocol given by Bates *et al.* (1973) and expressed in mg g^{-1}.

2.6. Catalase activity

Catalase activity was determined by following titration method using potassium permanganate (Gopalachari, 1963) expressed as μg H_2O_2 g^{-1} min^{-1}.

3. Results and discussion

With regard to RWC, the interaction effect of moisture stress and plant growth regulating chemicals responded significantly. Among different treatment combinations, the treatment combination of M_2 – Moisture stress at reproductive stage with S_4 – Glycine betaine (50 mM) showed highest RWC of 93.2% at 40 Days (Table 1). Since, glycine betaine offers osmoprotection, thereby protecting cell organelles from dehydration through regulating water potential equilibrium and maintaining turgor pressure in the cell (Kaya *et al.*, 2013). This report was proved by the use of glycine betaine in wheat cultivar where the RWC improved under drought conditions (Salama *et al.*, 2015).

Table 1: Effect of plant growth regulating chemicals on relative water content (%) in bhendi hybrid CO_4 under moisture stress conditions

Treat-ment	Relative water content (%)											
	40 Days				60 Days				80 Days			
	M_0	M_1	M_2	Mean	M_0	M_1	M_2	Mean	M_0	M_1	M_2	Mean
S_1	90.3	73.2	89.9	**84.5**	81.3	65.9	68.6	**71.9**	77.2	62.6	65.2	**68.3**
S_2	93.2	81.4	92.6	**89.0**	83.8	73.2	81.0	**79.4**	79.6	69.6	77.0	**75.4**
S_3	92.7	80.5	91.7	**88.3**	83.4	72.5	79.1	**78.3**	79.2	68.8	75.2	**74.4**
S_4	94.3	81.9	93.2	**89.8**	84.9	74.0	82.1	**80.3**	80.6	70.3	78.0	**76.3**
S_5	90.9	78.2	90.8	**86.6**	81.8	70.3	77.6	**76.6**	**77.7**	**66.8**	**73.7**	**72.7**
S_6	92.6	80.5	91.2	**88.1**	83.3	72.5	80.5	**78.8**	79.1	68.8	76.5	**74.8**
Mean	**92.3**	**79.3**	**91.6**		**83.1**	**71.4**	**78.2**		**78.9**	**67.8**	**74.3**	

	40 Days SEd	40 Days CD (P:0.05)	60 Days SEd	60 Days CD (P:0.05)	80 Days SEd	80 Days CD (P:0.05)
M	**0.42**	**1.18**	**0.78**	**2.17**	**0.31**	**0.85**
S	**0.69**	**1.43**	**0.81**	**1.66**	**0.99**	**2.04**
M x S	**1.18**	**2.53**	**1.51**	**3.37**	**1.61**	**3.33**

The values of CSI was found to be increasing up to 60 Days, after that started declining at both moisture stress and plant growth regulating chemicals. Among different treatment combinations, the treatment combination of M_2 – Moisture stress at reproductive stage with S_2 – Proline (50 mM) showed highest CSI of 87.5% at 60 Days (Table 2). This findings were supported by foliar application of osmolytes like proline in safflower in stabilizing the chlorophyll content (Janmohammadi *et al.*, 2017).

The MSI differs for each treatments and get decreased with increment in age of bhendi. Among different treatments with plant growth regulating chemicals, foliar spray of S_2 – Proline (50 mM) recorded maximum MSI of 82.1, 70.2 and 55.6% at 40, 60 and 80 Days respectively (Table 3). Membrane stability will be lost or started to decline with increase in stress level, which is due to electrolyte leakage from damaged tissues (Farooq *et al.*, 2008). This electrolyte leakage is due to increased lipid peroxidation of biomembrane. Under

Table 2: Effect of plant growth regulating chemicals on chlorophyll stability index (%) in bhendi hybrid CO_4 under moisture stress conditions

Treat-ment	Chlorophyll stability index (%)											
	40 Days				60 Days				80 Days			
	M_0	M_1	M_2	Mean	M_0	M_1	M_2	Mean	M_0	M_1	M_2	Mean
S_1	83.2	58.8	82.9	**75.0**	90.2	64.6	75.1	**76.6**	77.8	54.9	63.8	**65.5**
S_2	86.3	76.7	85.3	**82.7**	92.9	84.3	87.5	**88.2**	79.0	71.7	74.4	**75.0**
S_3	85.7	71.3	85.0	**80.7**	92.2	78.5	87.2	**86.0**	78.4	66.7	74.1	**73.1**
S_4	84.7	70.5	84.2	**79.8**	91.2	77.6	86.4	**85.1**	77.5	65.9	73.5	**72.3**
S_5	83.9	65.5	82.7	**77.4**	90.9	72.1	84.8	**82.6**	76.7	61.3	72.1	**70.0**
S_6	84.7	72.6	84.7	**80.7**	91.1	79.9	87.0	**86.0**	77.5	67.9	73.9	**73.1**
Mean	**84.7**	**69.2**	**84.1**		**91.4**	**76.2**	**84.7**		**77.8**	**64.7**	**72.0**	

	SEd	CD (P:0.05)	SEd	CD (P:0.05)	SEd	CD (P:0.05)
M	**0.88**	**2.43**	**1.12**	**3.11**	**0.74**	**2.06**
S	**1.48**	**3.02**	**1.48**	**3.03**	**1.00**	**2.05**
M x S	**2.49**	**5.33**	**2.60**	**5.66**	**1.76**	**3.81**

Table 3: Effect of plant growth regulating chemicals on membrane stability index (%) in bhendi hybrid CO_4 under moisture stress conditions

Treat-ment	Membrane stability index (%)											
	40 Days				60 Days				80 Days			
	M_0	M_1	M_2	Mean	M_0	M_1	M_2	Mean	M_0	M_1	M_2	Mean
S_1	78.9	68.5	76.4	**74.6**	72.6	65.4	58.1	**65.4**	54.7	49.2	48.3	**50.7**
S_2	86.0	77.4	83.0	**82.1**	78.6	70.7	62.9	**70.7**	59.1	53.2	54.4	**55.6**
S_3	81.8	73.6	81.7	**79.0**	76.8	69.1	61.4	**69.1**	58.3	52.4	51.3	**54.0**
S_4	80.5	72.5	78.1	**77.0**	76.9	69.2	61.5	**69.2**	57.8	52.0	51.0	**53.6**
S_5	76.2	71.0	76.2	**74.5**	74.4	67.0	59.6	**67.0**	56.0	50.4	49.2	**51.9**
S_6	85.0	76.5	85.2	**82.2**	77.4	69.7	61.9	**69.7**	57.9	52.1	53.2	**54.4**
Mean	**81.4**	**73.3**	**80.1**		**76.1**	**68.5**	**60.9**		**57.3**	**51.6**	**51.2**	

	SEd	CD (P:0.05)	SEd	CD (P:0.05)	SEd	CD (P:0.05)
M	**0.94**	**2.62**	**0.56**	**1.54**	**0.41**	**1.14**
S	**0.95**	**1.94**	**0.85**	**1.74**	**0.56**	**1.15**
M x S	**NS**		**NS**		**NS**	

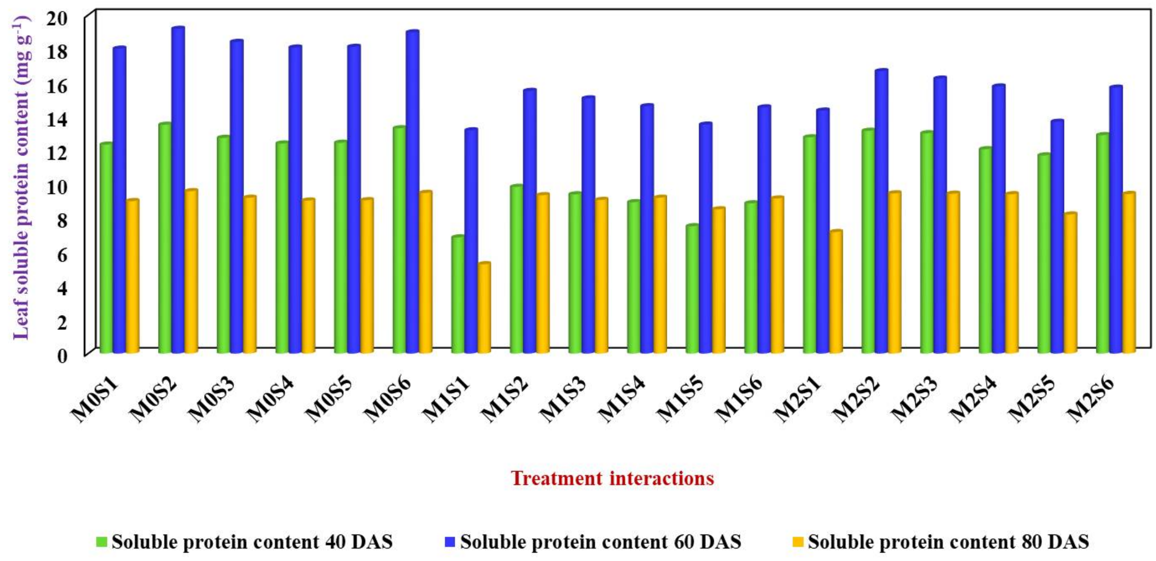

Figure 1: Effect of plant growth regulating chemicals on leaf soluble protein content (mg g^{-1}) of bhendi hybrid CO_4 under moisture stress

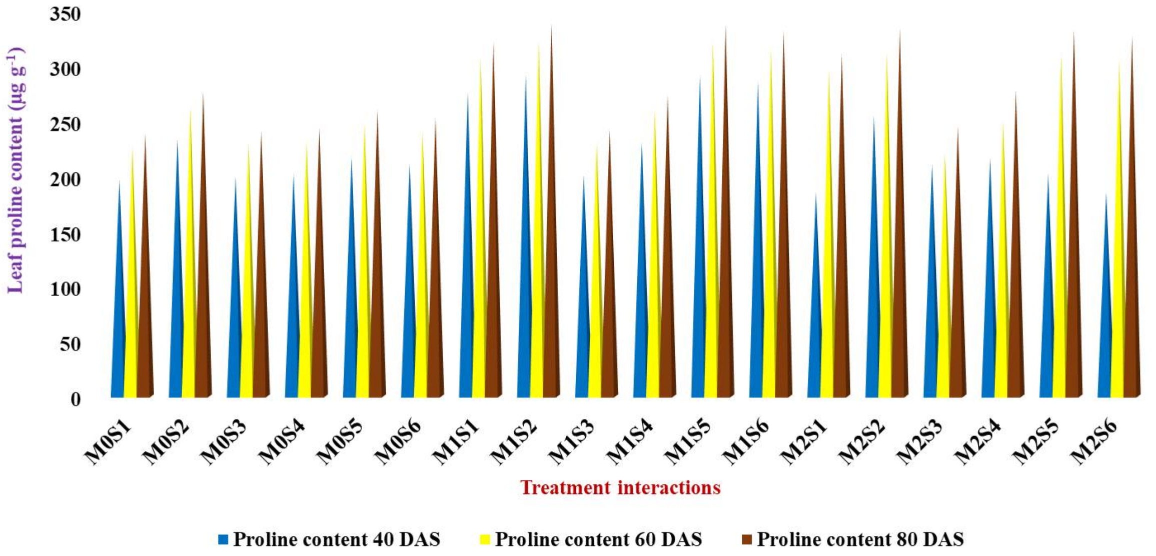

Figure 2: Effect of plant growth regulating chemicals on leaf proline content (µg g^{-1}) of bhendi hybrid CO_4 under moisture stress

present study the foliar spray of proline (50 mM) responded positively to moisture stress, where it maintained the stability of the membrane through reduced electrolyte leakage. Lower the leakage percentage indicates higher membrane stability.

The leaf soluble protein content mostly represents the RuBisCO activity in plants. The protein content reduced to nearly 56% under drought which might be due to either protein degradation or inhibition of synthesis. In okra, drought reduced soluble protein to nearly 22.4% (Raza *et al.*, 2013). Among different treatment combinations, the treatment combination of M_2 – Moisture stress at reproductive stage with S_2 – Proline (50 mM) showed highest leaf soluble protein content of 16.7 mg g^{-1} at 60 Days (Figure 1). The positive role of proline in maintaining soluble protein content in leaf tissue of some plants like maize where exogenous proline application maintained homeostasis under stress condition (De Freitas *et al.*, 2018).

Proline accumulated during stress in higher plants. The amount of proline increased upon ageing of bhendi and found to be maximum at 80 Days. Among different treatment combinations, the treatment

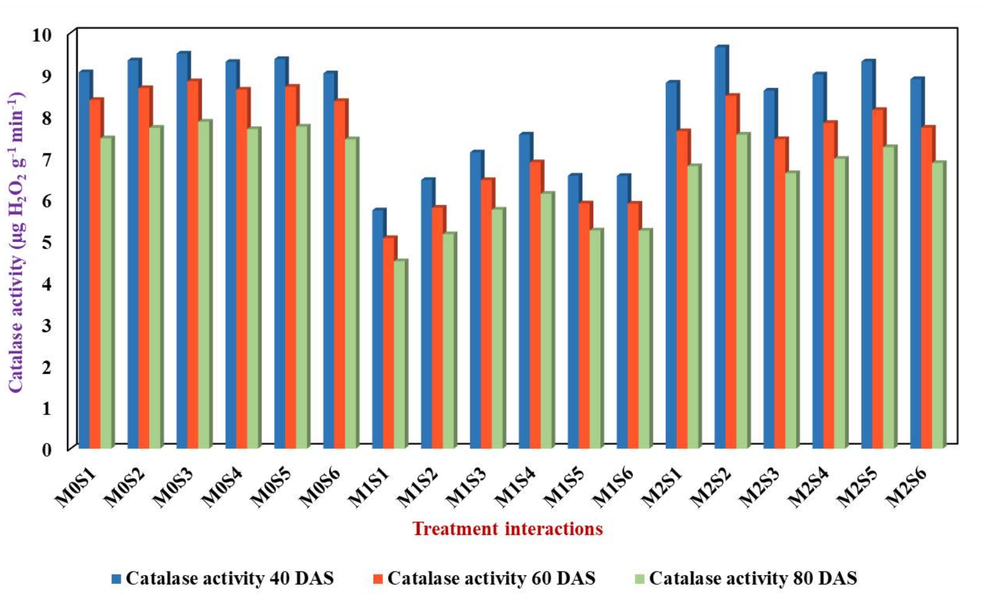

Figure 3: Effect of plant growth regulating chemicals on catalase activity (µg H2O2 g-1 min-1) of Bhendi Hybrid CO_4 under moisture stress

combination of M_1 – Moisture stress at vegetative stage with S_2 – Proline (50 mM) recorded highest leaf proline content of 291.8, 321.6 and 337.9 μg g^{-1} at 40, 60 and 80 Days respectively (Figure 2). This results was supported by Merwad *et al.* (2018), where there was increased accumulation of proline was observed with exogenous proline application which served as a resistance index. Moreover, it enhanced the growth and uptake of mineral ions from water limited soil, maintained water status, enhanced activity of antioxidant enzymes like catalase, peroxidase and superoxide dismutase. Similar results were observed in faba bean treated with proline (Taie *et al.*, 2013a). Foliar spray of proline mitigated drought stress through decreasing the rate photoinhibition and increased proline content scavenging the ROS production in leaves of *Arabidopsis thaliana* (Moustakas *et al.*, 2011). However, some reports provided by Taie *et al.* (2013), where proline spray combined with sea water had no significant effect in enhancement of endogenous proline.

Plants regulate various defense mechanisms to overcome the destructive effects of stress, which includes production of antioxidants like catalase, ascorbate peroxidase, super oxide dismutase and peroxidase in order to scavenge ROS, thereby preventing lipid peroxidation. Among these enzymes, catalase is the most active enzyme, since it play a major role in scavenging the oxidant H_2O_2 present in peroxisomes which will be converted to water and molecular oxygen with the activity of catalase. Among different treatment combinations, the treatment combination of M_2 – Moisture stress at reproductive stage with S_2 – Proline (50 mM) showed highest catalase activity of 9.65 μg H_2O_2 g^{-1} min^{-1} at 40 Days (Figure 3). Similarly, proline enhances catalase activity in basil plants (Agami *et al.*, 2016), cowpea (Merwad *et al.*, 2018) under water limited conditions and also in maize (De Freitas *et al.*, 2018), chilli (Butt *et al.*, 2016) under saline conditions.

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