Drought-induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench.

Beemarao Sankar, Cheruth A. Jaleel, Paramasivam Manivannan, Ashok Kishorekumar, Ramamurthy Somasundaram*, Rajaram Panneerselvam

Department of Botany, Annamalai University, Annamalainagar 608 002, Tamilnadu, India.

The research was performed to define the effect of water deficit on early growth, biomass allocation and biochemical constituents, proline metabolism and yield of five varieties of bhendi (*Abelmoschus esculentus* (L.) Moench.) plants. We found that there were significant differences in early growth, dry matter accumulation, biochemical constituents and proline metabolism among the five varieties. The root length, shoot length, total leaf area, fresh weight and dry weight were significantly reduced under drought-induced stress treatment. The proline content and γ-glutamyl kinase were significantly enhanced and proline oxidase activities were reduced. Drought stress caused an increase in the free amino acid and glycinebetaine content.

Key words: Drought, stress, growth, amino acid, glycinebetaine, proline, metabolism, *Abelmoschus esculentus*

Abbreviations: QAC – quaternary ammonium compound, GB – glycinebetaine, FC – field capacity, DAS – days after sowing, ROS – rain out shelter, DCPIP – 2,6-dichlorophenol indophenol, DMRT – Duncan's multiple range test.

Introduction

Plants may be subjected to environmental stresses that adversely affect growth, metabolism and yield (Lawlor 2002). Drought, temperature, salinity, air pollution, heavy metals, pesticides and soil pH are major limiting factors in crop production because they affect almost all plant functions (Hern-Ndez et al. 2001). Water deficit (commonly known as drought) can be defined as the absence of adequate moisture necessary for a plant to grow normally and complete its life cycle (Zhu 2002). The lack of adequate moisture leading to water stress is a common occurrence in rainfed areas, brought about by infrequent rains and poor irrigation (Wang et al. 2005).

A plant experiences drought stress either when the water supply to the roots becomes difficult or when the transpiration rate becomes very high, These two conditions often coincide under arid and semiarid climates. Water stress tolerance is seen in almost all plant species but its extent varies from species to species (Chaitanya et al. 2003). Although the

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* Corresponding author, e-mail: kalaisomu_20@rediffmail.com

general effects of drought on plant growth are fairly well known, the primary effects of water deficit at the biochemical and molecular levels are not well understood (CHAVES et al. 2003).

Drought occurs in many parts of the world every year, often with devastating effects on crop production (LUDLOW and MUCHOW 1990). Worldwide losses in crop yields from water deficits probably exceed the losses from all other causes combined (KRAMER 1983). Accumulation of proline has been advocated for use as a parameter of selection for stress tolerance (YANCY et al. 1982). Proline accumulation might respond to stresses such as temperature, drought and starvation (SAIRAM et al. 2002). High levels of proline enable a plant to maintain low water potentials. By lowering water potentials, the accumulation of compatible osmolytes involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortages within the organism (KUMAR et al. 2003). The occurrence of the quaternary ammonium compound (QAC) glycinebetaine (GB) in response to oxidative stress like salinity is a common phenomenon (DIONISIO-SESE and TOBITA 1998).

Bhendi (*Abelmoschus esculentus* (L.) Moench.) is a vegetable crop belonging to the family Malvaceae. An understanding of water stress tolerant varieties is necessary for breeders to identify plants suitable for drought areas. It is important to use a quick but reliable index for tolerance for water deficit that will enable screening of varieties.

The objectives of this study were to (i) determine the morphological traits that contribute to tolerance of water deficit, (ii) to estimate the changes that occur in proline metabolism under water stress, which is an important strategy in abiotic stress resistance and (iii) identify a variety of *A. esculentus* tolerant to water deficit.

Materials and methods

Five varieties of bhendi (okra) (*Abelmoschus esculentus* L. Moench.) ((SPHB 7, Saloni F_1, JK Haritha, Sakthi 101 and Mahyco$^®$) were used for the experiments. The seeds were surface sterilized with 0.2% $HgCl_2$ solution for five minutes with frequent shaking and thoroughly washed with tap water. The experiments were carried out by gravimetric method in containers. Each pot was weighed on an electronic weighing device (Model-Citizen XK3190-A7M).

Control pots were maintained to field capacity (FC) during the entire growth period. Till 30 days after sowing (DAS) all the pots were maintained at 100% FC and 60% FC during the treatment periods (30 to 70 DAS). Computation was done for pot weight and amount of water needed for the desired soil moisture regime. Treatments were carried out on alternative days.

Stress was imposed from 30 to 70 DAS using a rain out shelter (ROS) device. The pots were covered with ROS whenever rainfall was anticipated and immediately after rain, ROS was pulled back so that, pots received maximum sunlight. Further, the pots were regularly covered with ROS during nighttime. Using this system the pots were protected from rainfall and any external moisture entry.

Plants were uprooted and separated into leaves, stems, roots and pods and fresh weight (FW) was recorded. The samples were dried in an oven at 60°C until constant dry weight (DW) was obtained. The FW and DW were expressed in g per plant. Shoot and root length

were recorded on 50 and 70 DAS. Total leaf area was measured with LI-COR Photoelectric leaf area meter (Model LI-3100, Lincoln, U.S.A) and expressed in cm^2 per plant.

Total free amino acid content was extracted and estimated by following the method of MOORE and STEIN (1948) and expressed in mg g^{-1} DW. GB content was extracted and estimated by the method of GRIEVE and GRATTEN (1983) and expressed in $\mu g\ g^{-1}$ DW. Proline content was extracted and estimated by the method of BATES et al. (1973) and expressed in mg g^{-1} DW. Proline metabolizing enzyme like γ-glutamyl kinase [ATP: L. Glutamate--5-phosphotransferase (EC 2.7.2.11)] activity was assayed by the method of HAYZER and LEISINGER (1980) and expressed as μg of γ-glutamylhydroxamate formed min^{-1} (mg protein)$^{-1}$. Proline oxidase (L. Proline: O_2 oxidoreductase, EC 1.4.3.1) activity was determined according to the method outlined by HUANG and CAVALIERI (1979). Proline oxidase activity was expressed in mM 2,6-dichlorophenol indophenol (DCPIP) reduced min^{-1} (mg protein)$^{-1}$.

The pot culture was carried out in completely randomized design. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Values are given as mean ± SD of three experiments in each group.

Results

Morphological responses of varieties grown under water deficit

The root length was increased in drought-stressed (60% FC) bhendi varieties as compared to control (100% FC) on 50 DAS (Tab. 1). The root length decreased in the drought-stressed bhendi varieties on 70 DAS when compared to control. When compared to other varieties, JK Haritha showed better root growth under drought stress and the reduction was only 26 and 5 per cent over control on 50 and 70 DAS respectively.

Water deficit condition caused decreased stem length to a larger extent when compared to control on 50 and 70 DAS. Among the varieties, JK Haritha decreased stem length in 79 and 65 per cent with respect to the control on 50 and 70 DAS respectively. Mahyco® variety was severely affected when compared to other varieties and it was 55 and 53 per cent with respect to the control on 50 and 70 DAS under drought conditions.

Drought stress reduced the total leaf area when compared to control. Among the varieties, JK Haritha reduced leaf area up to 95 and 87 per cent with respect to the control on 50 and 70 DAS respectively. SPHB 7 variety was severely affected when compared to other varieties and it was 81 and 75 per cent with respect to the control on 50 and 70 DAS.

Drought stress decreased the whole plant FW to a larger extent. Among the five varieties, JK Haritha showed a minimum decrease and it was 88 and 84 per cent with respect to the control on 50 and 70 DAS respectively. The FW was low in the SPHB 7 variety when compared to other varieties and it showed a reduction of 71 and 69 per cent with respect to the control in 50 and 70 DAS under drought conditions. Water deficit condition decreased the whole plant FW to a larger extent in all the bhendi varieties.

Drought stress caused decreased DW accumulation in all the varieties of bhendi. The JK Haritha variety was the highest responder to water stress and it showed a reduction of 90 and 78 per cent with respect to the control on 50 and 70 DAS respectively. The SPHB 7 va-

Tab. 1. Drought stress-induced changes in root, stem length (cm per plant), total leaf area (cm^2 plant^{-1}), whole plant fresh and dry weights (g per plant) of five varieties of bhendi. Values are given as mean ± SD of three experiments in each group. * – significant difference from control at p≤0.05 (DMRT). DAS – days after sowing; FC – field capacity

Variety	50 DAS		70 DAS	
	100% FC (control)	60% FC (stressed)	100% FC (control)	60% FC (stressed)
Root length (cm plant^{-1})				
SPHB 7	15.2 ± 0.5	17.0 ± 0.6*	17.9 ± 0.6	17.4 ± 0.6
Saloni F$_1$	16.6 ± 0.5	18.8 ± 0.6*	18.1 ± 0.6	17.1 ± 0.6*
JK Haritha	14.2 ± 0.4	17.9 ± 0.6*	19.6 ± 0.6	20.6 ± 0.7*
Sakthi 101	14.8 ± 0.4	16.1 ± 0.5*	16.4 ± 0.5	15.7 ± 0.5*
Mahyco®	15.0 ± 0.5	17.4 ± 0.6*	17.6 ± 0.6	16.9 ± 0.6*
Stem length (cm plant^{-1})				
SPHB 7	59.2 ± 1.9	37.1 ± 1.4*	54.6 ± 1.8	31.9 ± 1.2*
Saloni F$_1$	57.3 ± 1.9	33.6 ± 1.2*	63.3 ± 2.1	35.3 ± 1.4*
JK Haritha	45.6 ± 1.5	36.4 ± 1.3*	67.3 ± 2.2	43.8 ± 1.5*
Sakthi 101	57.0 ± 1.9	34.9 ± 1.3*	66.7 ± 2.3	36.9 ± 1.4*
Mahyco®	55.1 ± 1.8	30.6 ± 1.2*	60.8 ± 2.1	32.5 ± 1.3*
Total leaf area (cm^2 plant^{-1})				
SPHB 7	378.6 ± 12.6	307.4 ± 10.6*	426.8 ± 14.2	319.8 ± 10.3*
Saloni F$_1$	359.5 ± 11.6	302.4 ± 10.1*	378.5 ± 12.6	301.4 ± 9.7*
JK Haritha	333.7 ± 11.1	318.4 ± 11.4*	386.4 ± 13.3	338.2 ± 11.7*
Sakthi 101	366.6 ± 12.2	300.3 ± 10.0*	392.7 ± 12.7	309.3 ± 10.7*
Mahyco®	356.2 ± 12.3	292.7 ± 9.8*	391.4 ± 13.1	308.1 ± 10.3*
Whole plant fresh weight (g plant^{-1})				
SPHB 7	58.3 ± 2.1	41.6 ± 1.5*	70.5 ± 2.4	48.9 ± 1.6*
Saloni F$_1$	66.5 ± 2.4	53.0 ± 1.8*	78.5 ± 2.8	57.1 ± 2.0*
JK Haritha	75.3 ± 2.7	66.6 ± 2.3*	84.1 ± 3.1	70.6 ± 2.4*
Sakthi 101	59.4 ± 2.1	47.8 ± 1.7*	70.2 ± 2.4	49.1 ± 1.7*
Mahyco®	65.3 ± 2.3	53.7 ± 1.9*	68.9 ± 2.5	48.7 ± 1.7*
Whole plant dry weight (g plant^{-1})				
SPHB 7	9.8 ± 0.4	7.4 ± 0.2*	12.3 ± 0.4	8.5 ± 0.3*
Saloni F$_1$	10.8 ± 0.4	8.2 ± 0.3*	14.7 ± 0.5	9.1 ± 0.3*
JK Haritha	13.2 ± 0.5	11.9 ± 0.4*	15.3 ± 0.5	11.9 ± 0.4*
Sakthi 101	9.3 ± 0.3	7.2 ± 0.3*	13.9 ± 0.5	9.4 ± 0.3*
Mahyco®	10.9 ± 0.4	8.8 ± 0.3*	14.7 ± 0.5	9.1 ± 0.3*

riety showed the lowest DW when compared to other varieties and it was reduced 75 and 61 per cent with respect to the control on 50 and 70 DAS under drought condition.

Water stress and biochemical constituents

Drought stress caused increased amino acid content in roots when compared with control on 50 and 70 DAS in all the varieties of bhendi (Fig. 1). Among these varieties, JK
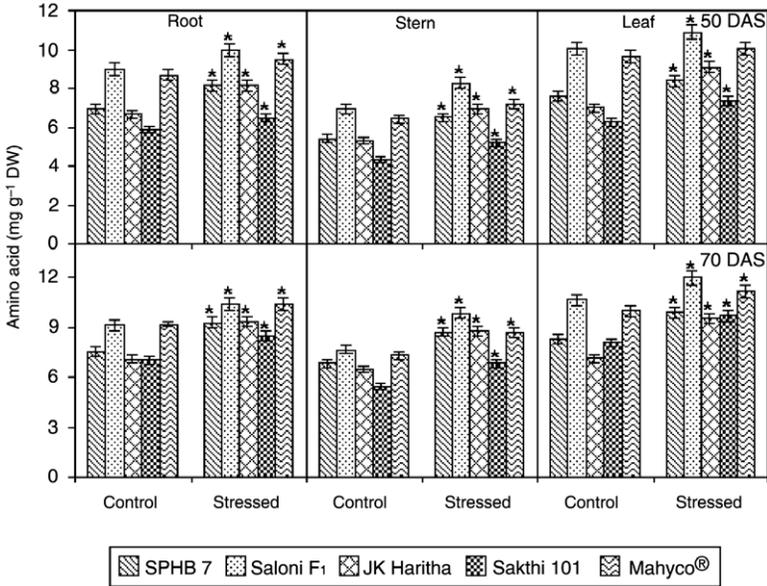
Fig. 1. Drought stress-induced changes in amino acid (mg g⁻¹ DW) contents of five varieties of bhendi. Values are given as mean ± SD of three experiments in each group. * the level of significance at $p \leq 0.05$ (DMRT).

Haritha showed higher accumulation and it was 22 and 32 per cent over control. Similarly the amino acid content was very low in Mahyco® variety, and it was 10 and 13 per cent over control on 50 and 70 DAS respectively. Under water deficit condition the amino acid content increased in stems of bhendi varieties. The JK Haritha variety showed increasing amino acid content of 30 and 35 per cent over control on 50 and 70 DAS respectively. The amino acid content showed lowest increase in the stem of Mahyco® variety and it was 12 and 19 per cent over control on 50 and 70 DAS under drought condition. Drought stress resulted in higher accumulation of amino acid content in leaves of all the varieties of bhendi when compared to control. Among the varieties studied, JK Haritha showed highest accumulation of amino acid in the leaves and the extent of increase was about 30 and 34 per cent over control on 50 and 70 DAS. The lowest rate of increase was noted in the leaves of Mahyco® variety and it was 4 and 11 per cent over control (100% FC) on 50 and 70 DAS in drought condition.

Glycinebetaine content showed an increase in all bhendi varieties under drought stress conditions (Fig. 2). Among the varieties, JK Haritha showed maximum increase and when compared to other varieties it was 51 and 60 per cent over control on 50 and 70 DAS respectively. The minimum increase was noted in Mahyco® and the increase was 43 and 44 per cent over control on 50 and 70 DAS. Drought stress has profound effects on the GB accumulation in stem of bhendi. Among these varieties, the JK Haritha the showed highest accumulation of GB in the stem and it was 40 and 49 per cent over control on 50 and 70 DAS samplings respectively. Similarly the lowest extent of increase was observed in Mahyco® variety and it was 21 and 26 per cent over control on 50 and 70 DAS. Drought stress caused increased GB accumulation in the leaves of all the varieties of bhendi. As in
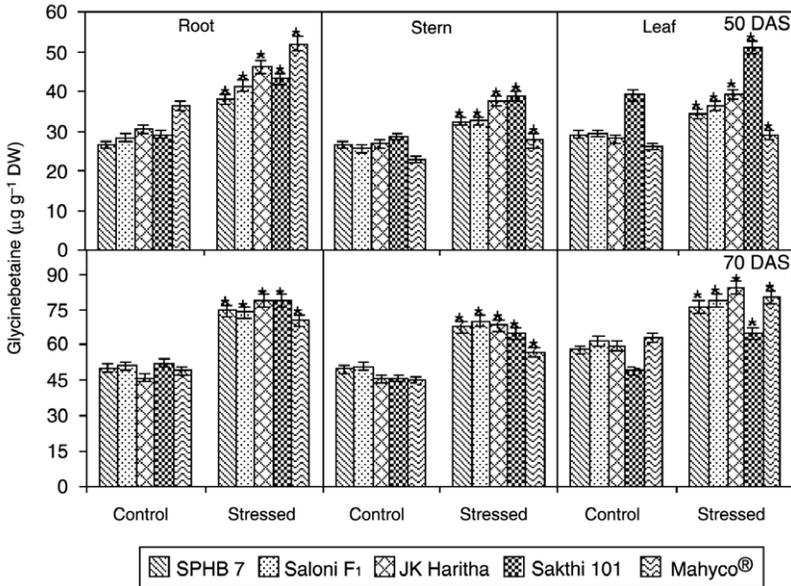
Fig. 2. Drought stress-induced changes in glycinebetaine (µg g^{-1} DW) contents of five varieties of bhendi. Values are given as mean ± SD of three experiments in each group. * = the level of significance at p≤0.05 (DMRT).

the earlier cases, the JK Haritha variety was the highest responder to drought stress when compared to other varieties and it showed 39 and 41 per cent over control on 50 and 70 DAS respectively, with Mahyco® having the lowest rate of GB content, showing 10 and 28 per cent over control on 50 and 70 DAS under drought condition. GB increased under drought stress in all varieties of bhendi. The extent of increase was higher in leaf followed by root and stem.

Proline and proline metabolizing enzymes

The proline content of the root increased at all stages of growth in all varieties of bhendi under drought conditions (Fig. 3). Among these varieties, JK Haritha showed the highest increase, of 44 and 49 per cent over control on 50 and 70 DAS respectively. The lowest rate of increase was recorded in Mahyco®, coming to 31 and 41 per cent over control on 50 and 70 DAS respectively. The proline content of stem increased in all bhendi varieties under drought stress. Drought stress altered the proline content of stem mainly in JK Haritha variety and it was 38 and 44 per cent over control at 50 and 70 DAS respectively. The lowest increase was observed in Mahyco® variety, of 20 and 34 per cent over control at 50 and 70 DAS respectively. The proline content of the leaves increased at all stages of growth in all the bhendi varieties. The JK Haritha variety was more active whence faced with drought stress and showed maximum accumulation of proline in leaves, 35 and 42 per cent over control on 50 and 70 DAS respectively. Mahyco® variety showed the lowest rate of increase in leaves and it was 19 and 29 per cent over control in 50 and 70 DAS under drought-stressed conditions. The effect was highly significant in JK Haritha and Mahyco®
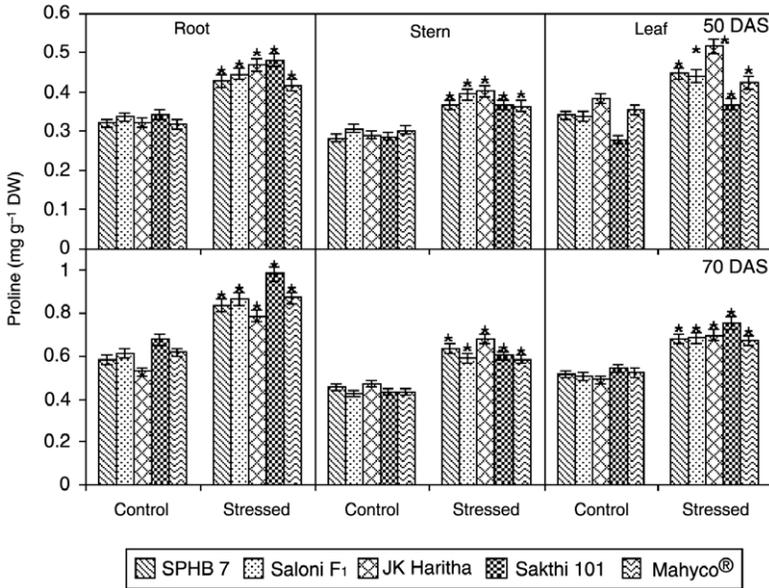
Fig. 3. Drought stress-induced changes in proline (mg g^{-1} DW) contents of five varieties of bhendi. Values are given as mean ± SD of three experiments in each group. * – the level of significance at p≤0.05 (DMRT).

varieties. Proline content increased under water deficit conditions in root, stem and leaf of all varieties of bhendi on 50 and 70 DAS. The increase was highly significant in the roots. Among the varieties the maximum proline content increase was noted in the roots of JK Haritha variety, when compared to other varieties on 50 and 70 DAS.

γ-Glutamyl kinase activity

The activity of γ-glutamyl kinase increased under drought stress in all bhendi varieties when compared to control (Fig. 4). The extent of increase was higher in JK Haritha, coming to 68 and 85 per cent over control on 50 and 70 DAS respectively. The lowest rate of increase was noted in Mahyco®, of 42 and 56 per cent over control under drought stress on 50 and 70 DAS. Under water deficit conditions, the γ-glutamyl kinase activity increased in stem of all bhendi varieties, JK Haritha variety showing the highest rate of activity, which was 64 and 59 per cent over control on 50 and 70 DAS under drought condition. The Mahyco® variety showed the lowest rate of activity, of 37 and 34 per cent over control on 50 and 70 DAS under drought conditions. The γ-glutamyl kinase activity increased in the leaves of all the bhendi varieties under drought conditions. The γ-glutamyl kinase increased greatly in JK Haritha leaf, by 81 and 33 per cent over control on 50 and 70 DAS. The lowest accumulation was observed in Mahyco®, of 64 and 7 per cent over control in 50 and 70 DAS, under drought-stressed conditions. Proline metabolizing enzymes like γ-glutamyl kinase were found to be increased under water stress in all the five bhendi varieties on 50 and 70 DAS. It was highly significant in the leaves, followed by root and stem.
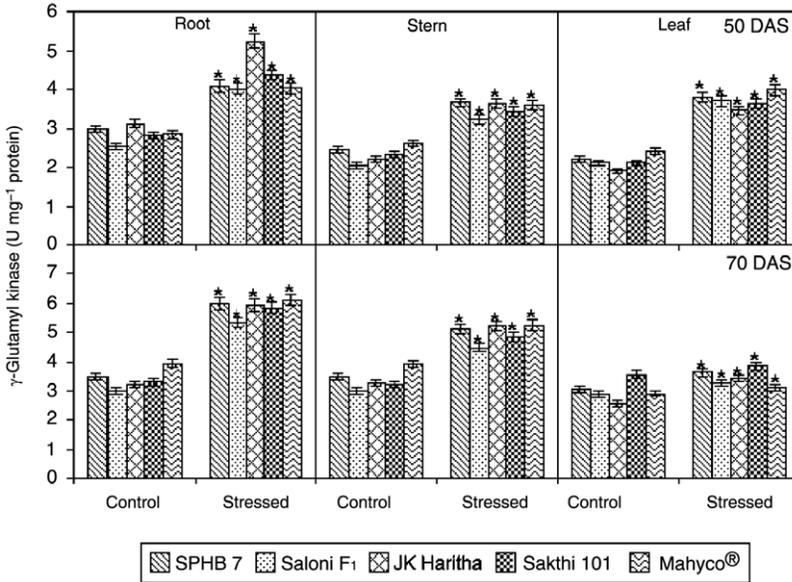
Fig. 4. Drought stress-induced changes in γ-glutamyl kinase (μg of γ-glutamylhydroxamate formed min^{-1} mg^{-1} protein) activities of five varieties of bhendi. Values are given as mean ± SD of three experiments in each group. * – significant difference from control at p≤0.05 (DMRT).

Proline oxidase activity

The level of the proline-degrading enzyme proline oxidase activity was inhibited in the roots by drought stress when compared to control in all the bhendi varieties (Fig. 5). Among the varieties JK Haritha showed the lowest rate of reduction in proline oxidase activity, when compared to other varieties under drought stress, coming to 90 and 84 per cent with respect to the control in 50 and 70 DAS respectively. In Mahyco® variety proline oxidase activity was inhibited greatly when compared to other varieties, coming to 67 and 72 per cent with respect to the control on 50 and 70 DAS. Proline oxidase activity was found to be greatly affected by drought stress in the stem of all the bhendi varieties. Among the varieties JK Haritha showed the lowest reduction in proline oxidase activity, coming to 84 and 79 per cent with respect to the control in 50 and 70 DAS respectively. The proline oxidase activity was highest decreased in Mahyco® when compared to other varieties and it was 70 and 69 per cent with respect to the control on 50 and 70 DAS under drought conditions. Proline oxidase activity decreased in the leaves of all the bhendi varieties under drought conditions. JK Haritha showed the lowest decrease in proline oxidase activity, of 86 and 84 per cent with respect to the control on 50 and 70 DAS under drought conditions. The proline oxidase activity showed the highest decrease in Mahyco® variety of 72 and 76 per cent with respect to the control on 50 and 70 DAS under drought conditions.

Discussion

In our results, we noted a reduction in growth parameters under drought stress. Growth is influenced by various internal and external factors besides its genetic makeup and is an
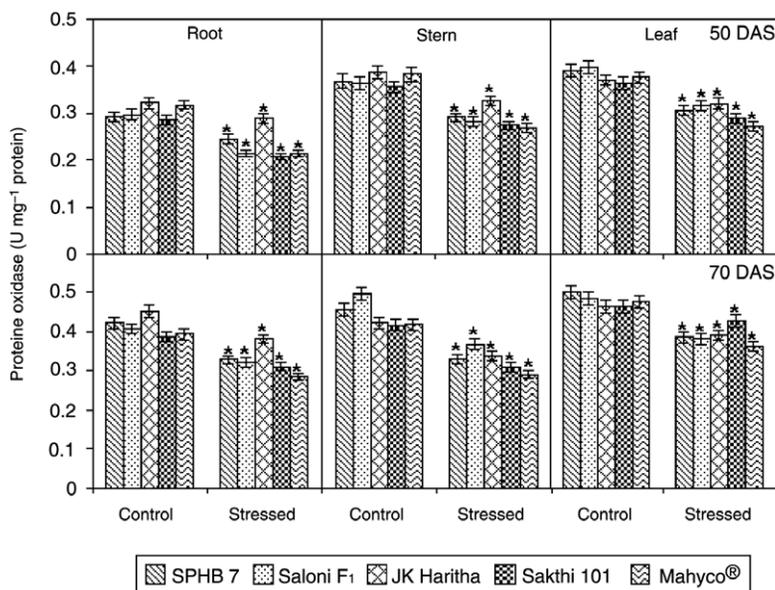
Fig. 5. Drought stress-induced changes in proline oxidase (μg of γ-glutamylhydroxamate formed min^{-1} mg^{-1} protein) activities of five varieties of bhendi. Values are given as mean $\pm$ SD of three experiments in each group. * – significant difference from control at p$\leq$0.05 (DMRT).

important tool for assessing crop productivity in various crops. Root characteristics especially root length, root length density and the number of thick roots, are important for a plant to have comparatively well-established above-ground parts by exploiting the available water (EKANAYAKE et al. 1985). A root system that enhances the ability of a plant to capture water is a fundamental adaptation mechanism to drought. Drought stress decreased the root and stem length in *Erythrina* (MUTHUCHELIAN et al. 1986), *Albizzia* seedlings (SUNDARAVALLI et al. 2005), *Eucalyptus microthea* seedlings (LI et al. 2000) and *Populus* species (YIN et al. 2005). The quantity and quality of plant growth depend on cell division, enlargement and differentiation and all of these events are affected by water stress (KUSAKA et al. 2005). This might be the reason for the reduced growth of plants under water deficit stress.

During water stress, total leaf area decreased significantly in *Eragrotis curvula* (COLOM and VAZZANA 2001) and in *Sorghum* (YADAV et al. 2005). Leaf area plasticity is important to maintain control of water use in crops. Leaf area reduced significantly under water stress. Reduction in leaf area by water stress is an important cause of reduced crop yield through reduction in photosynthesis (KRAMER 1983). The number of leaves per plant and individual leaf size and leaf longevity reduced by decreasing soil water potential, leaf area expansion depends on leaf turgor, temperature and assimilate supply for growth, which are all affected by drought (REDDY et al. 2003). Similar results were observed under drought stress in *Abelmoschus esculentum* (BHATT and SRINIVASA RAO 2005). The root growth was increased initially, but in later stages it was reduced because of severe drought stress. The reduction in plant height might be associated with declined cell enlargement and cell growth due to the low turgor pressure and also more leaf senescence under drought stress.

The FW was reduced under drought conditions in wheat (Rᴀɴᴇ et al. 2001). Similar results were observed in pearl millet (Kᴜsᴀᴋᴀ et al. 2005) and *Abelmoschus esculentum* (Bʜᴀᴛᴛ and Sʀɪɴɪᴠᴀsᴀ Rᴀᴏ 2005). The FW decrease under drought conditions might be the reason for the suppression of cell expansion and cell growth due to the low turgor pressure.

Drought stress decreased the plant biomass in wheat (Pᴀɴ et al. 2003), *Arachis hypogeae* (Nᴀᴜᴛɪʏᴀʟ et al. 2002), *Asteriscus maritimus* (Rᴏᴅʀɪɢᴜᴇᴢ et al. 2005) and in *Albizzia* seedlings (Sᴜɴᴅᴀʀᴀᴠᴀʟʟɪ et al. 2005). Decreased total DW may be due to the considerable decrease in plant growth, photosynthesis and canopy structure as indicated by leaf senescence during drought stress in *Abelmoschus esculentum* (Bʜᴀᴛᴛ and Sʀɪɴɪᴠᴀsᴀ Rᴀᴏ 2005).

The amino acid content has been shown to increase under drought conditions in sorghum (Yᴀᴅᴀᴠ et al. 2005). Similar results were obtained in pepper (Nᴀᴛʜ et al. 2005), coconut (Kᴀsᴛᴜʀɪ Bᴀɪ and Rᴀᴊᴀɢᴏᴘᴀʟ 2000), wheat (Hᴀᴍᴀᴅᴀ 2000) and *Arachis hypogaea* (Asʜᴀ and Rᴀᴏ 2002). The accumulation of amino acids may be due to the hydrolysis of protein and also may occur in response to the changes in osmotic adjustment of their cellular contents (Gʀᴇᴇɴᴡᴀʏ and Mᴜɴɴs 1980). Free amino acid accumulation is more important to account for most of the changes in osmotic potential. The accumulation of free amino acids under stress at all the growth stages indicates the possibility of their involvement in osmotic adjustment (Yᴀᴅᴀᴠ et al. 2005). Osmotic adjustment is one of the important mechanisms alleviating some of the detrimental effects of water stress (Mᴏʀɢᴀɴ 1984).

The glycinebetaine content increased under drought stress in barley (Nᴀᴋᴀᴍᴜʀᴀ 2001) and in higher plants (Jᴜɴ et al. 2000). Aliphatic QAC such as GB, stachydrine, homostachydrine, trigonelline have been found to accumulate in a large number of plants exposed to salt and water stress. Glycophytes like tomato, peas and beans showed an increase in GB with increasing salinity (Sᴜᴅʜᴀᴋᴀʀ et al. 1993; Gɪʀɪᴊᴀ et al. 2002).

Glycinebetaine is synthesized in chloroplasts, as two enzymes, choline monoxygenase and betaine aldehydehydrogenase, are responsible for GB synthesis chloroplastically. The accumulation of GB might serve as an intercellular osmoticum of GB and could be closely correlation with elevation of osmotic pressure (Sᴛᴏʀᴇʏ and Wʏɴ-Jᴏɴᴇs 1978). GB may maintain the osmoticum, provided that the basal metabolism of the plant can sustain a high rate of synthesis of these compounds to facilitate osmotic adjustment for tolerance to water stress (Kᴀᴠɪᴋɪsʜᴏʀᴇ et al. 1995).

Increased proline accumulation was reported in water-stressed sorghum (Yᴀᴅᴀᴠ et al. 2005), bell pepper (Nᴀᴛʜ et al. 2005), *Gossypium hirsutum* (Rᴏɴᴅᴇ et al. 1999), wheat (Hᴀᴍᴀᴅᴀ 2000) and in salt-stressed *Catharanthus roseus* (Jᴀʟᴇᴇʟ et al. 2007). Increased proline in the stressed plants may be an adaptation the purpose of which is to overcome the stress conditions. Proline accumulates under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress (Cʜᴀɴᴅʀᴀsʜᴇᴋᴀʀ and Sᴀɴᴅʜʏᴀʀᴀɴɪ 1996). Under abiotic stress like ultra violet light the proline content showed an increase in wheat (Dᴇᴍɪʀ 2000). NaCl stress showed increased proline content in rice (Lɪɴ et al. 2002) and peanut (Gɪʀɪᴊᴀ et al. 2002). Proline accumulation in plants might having a scavenger function and act as an osmolyte. The reduced proline oxidase may be the reason for increasing proline accumulation.

In abiotic stresses like NaCl, stress results in an increased accumulation of proline in to-bacco cells at the γ-glutamyl kinase level (LA ROSA et al. 1991). The induction of proline accumulation may be due to an activation of proline synthesis through the glutamate pathway involving γ-glutamyl kinase, glutamyl phosphate reductase and Δ′–pyrroline-5-carboxylate reductase activities (BRAY 1990) in peanut (GIRIJA et al. 2002) and in tomato (FUJITA et al. 2003). The proline accumulation in drought-stressed bhendi varieties can be attributed to the increased level of γ-glutamyl kinase activity.

Under water deficit conditions proline metabolizing enzymes like proline oxidase were found to decrease in all bhendi varieties. Among the organs the root showed lower decreased activity of proline oxidase than stem and leaf, under drought-stressed conditions. The reports coincide with earlier reports concerning water stress in tomato (FUJITA et al. 2003). The decrease in proline oxidase activity with increasing γ-glutamyl kinase activity might be the reason for higher proline accumulation in drought-stressed bhendi plants.

From the results of this investigation, it can be concluded that among the varieties tested, the JK Haritha variety of *A. esculentus* showed good resistance to drought stress. This information is of great importance in plant breeding experiments.

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