Journal of Advances in Science and Technology
Vol. 15, Issue No. 1, March-2018, ISSN 2230-9659

Characterization of Some Potent Drought Tolerant Tree Legumes for Agroforestry in Bankura, West Bengal

Arpita Banerjee[1*] Arijit Sinhababu[2]

[1,2] Department of Botany, Bankura Christian College, Bankura, West Bengal

Abstract – Availability of water in nature is a determining factor for terrestrial plant production on a global scale. In dry climates, low water availability limits productivity. Seedlings (30 days old) of Acacia auriculiformis A. Cunn., Delbergia sissoo Roxb., Leucaena leucocephala (Lam,) de Wit. and Saraca indica (Roxb.) Willd. of the family Fabaceae were subject to PEG- induced water stress (- 0.5 and -1.0 MPa) to assess their relative water content and the contents of chlorophyll, protein, soluble sugars and proline in leaves. Chlorophyll and protein contents in leaves of the seedlings showed a gross decline with increasing level of PEG-induced water stress in case of all species whereas amount of carbohydrate and proline content increased in all species with increasing levels of water stress. Considering the comparative biochemical analysis L. leucocephala and A. auriculiformis, these two plants, showed higher potential for drought tolerance.

Key Words: Agroforestry, Bankura, Biochemical Analysis, Fabaceae, Water Stress.

----------♦----------

1. INTRODUCTION

In India, agroforestry programme started in 1976. Under this programme, trees were planted in and around agricultural fields, roadsides, canal banks, village common lands and government waste land. The goal of social forestry or agroforestry is plantation by the people who can meet the growing demand for fuel, timber, fodder and some other uses. Drought resistance is generally defined as the maintenance of plant production during moderate water deficit. Water stress leads to substantial variation in morphology, anatomy, physiology and biochemistry of plants, which is ultimately reflected on the yield potential. Physical and biochemical responses of plants to environmental stress have been studied in a great detail for over a century, with particular reference to plant's adaptation to water deficits (Janardhan and Bhojaraja, 1999; Bhattacharjee and Mukherjee, 2002; Kar, 2002; Panda, 2002). Methods employed for imposing water stress under experimental conditions exhibit a range of variations, laboratory experiments are conducted to record changes in water relations of growing tissues through alterations in external water potential brought about by incubating them in osmotic solutions like mannitol and polyethylene glycols. Polyethylene glycol is suggested to have an advantage over other osmotic agents like mannitol as it does not enter the apoplastic space (Cress and Johnson, 1987; Money, 1989). Water stress severely modifies the metabolism to an extent depending upon species, duration and intensity of stress. Decline in leaf chlorophyll and protein as a consequence of water stress is a very common observation (Aspinall and Paleg, 1981; Nilsen and Orcutt, 1996). Water stress induced chlorophyll loss is ascribed mainly due to degradation, although a retardation of synthesis may also be equally important. One common observation associated with water stress is a gross decline in protein level, which may be ascribed to a decrease in protein synthesis and/ or an increase in protein hydrolysis (Nilsen and Orcutt, 1996; Chakraborty et al., 2001). Various metabolites and ions accumulate in plant tissues in response to water stress. Such an accumulation is usually associated with the osmotic adjustment of cells as a part of plants tolerance to tress (Aspinall and Paleg, 1981; Hanson and Hitz, 1982; Read, 1984). Osmotic adjustment not only helps cells of higher plants to withstand salt and water deficit by maintaining sufficient turgor, it also involves transport, accumulation, compartmentation of inorganic and organic solutes (Spickett et al., 1992) and stabilizing protein structure and functions (Bohnert et al., 1995). The concentration soluble carbohydrates found to be increased during water stress (Chowdhury and Choudhuri, 1986; Irigoyen et al., 1992).

2. MATERIALS AND METHODS

Plant materials

In the present investigation, four tree species (*Acacia auriculiformis* A. Cunn., *Delbergia sissoo* Roxb., *Leucaena leucocephala* (Lam,) de Wit. and *Saraca indica* (Roxb.) Willd. of the family Fabaceae were used as plant materials. These plants grow in and around Bankura (22°38' and 23°38' north latitude and 86°36' and 87°46' east latitude), where the soil type is dry lateritic, sandy and raddish in colour having average annual rainfall about 130 cm. These plants are commonly used by the local people as fuel, timber, fodder and some other uses. Since the legume seeds generally lose viability in prolonged storage, fresh seeds were collected from the adjacent forest of the Bankura town at the peak period of seed ripening from healthy tree.

Seedlings (30 days old) were taken as experimental plant materials to assess responses to water stress. Seedlings were raised by germinating healthy seeds (surface sterilized by sodium hypochlorite, as described earlier) initially on moist filter paper followed by transfer to sand beds where acid washed sand was used. Healthy seedlings were subjected to water stress induced by polyethylene glycol (PEG-6000), which is a non-permeating osmoticum used popularly for this purpose. Two levels of water stress (-0.5 and -1.0 MPa) were adjusted by the concentrations of PEG solutions, 19.6% and 29.6%, respectively according to Michel and Kaufman (1973) and was imposed to seedlings of the investigated species by dipping roots in solutions. A control set containing distilled water was maintained parallel. Incubation was done for 24 hours under 8 h light / 16 h dark cycles. At the end of experimental period leaves were collected from respective plants. Then the leaf samples were analysed for physiological and biochemical parameters.

***Relative water content (RWC)**:*

The relative water content of the leaves from seedlings of investigated plant species was estimated following the formula of Weatherly (1950). Leaves of the seedlings, stressed or unstressed control, were taken, washed with distilled water, blotted the surface solution and fresh weight was taken. Then isolated leaves were immersed in distilled water for 4 hours, blotted again the surface solution and the turgid weight were taken. For dry weight measurement respective plant materials were oven dried at 80 ºC for 3 days. Relative water content was calculated according to the following formula:

RWC = (Fresh weight-Dry weight) / (Turgid weight-Dry weight) X 100.

***Chlorophyll**:*

Leaf samples (50 mg) of the seedlings were initially preserved in 5ml methanol and kept overnight in a refrigerator. Subsequently, they were homogenized in dark with the same methanol and centrifuged at 5000 rpm for 10 minutes. The total chlorophyll content was estimated using the supernatants (10 ml) following the method of Arnon (1949).

***Protein**:*

For the determination of protein content, the residues of the samples, from which the chlorophyll was removed, were washed successively with 80% ethanol, 10% cold trichloro acetic acid, ethanol, ethanol: chloroform (3:1) and finally with ether to remove the phenolic compounds (Kar and Mishra, 1976). The washed pellets were then digested with 2 ml of 1N NaoH in water bath at 80 ºC for 1 hour. After centrifugation of the digest, the supernatant was taken and total protein content was estimated according to the method of Lowry et al., (1951). The protein content was expressed as mg g^{-1} dry weight.

***Carbohydrate**:*

For the determination of carbohydrates 50 mg leaf sample from each set was crushed in 5 ml of hot 80% ethanol and centrifuged at 5000 rpm for 10 minutes. The supernatant containing ethanol-soluble carbohydrates was then evaporated to dryness. Chlorophyll was removed by rinsing with solvent ether and the soluble carbohydrates were then eluted again with hot 80% ethanol. To 1 ml of this extract, 3 ml of 0.2% anthrone reagent was added in cold condition and the grass-green colour was stabilized by heating the tubes in a boiling water bath for 7 minutes (McCready et al., 1950). Content was expressed as mg glucose equivalents g^{-1} dry weight.

Proline:

Free proline content of leaf tissue was estimated according to the method of Bates et al., (1973). Plant material (200 mg) was first homogenized in 5 ml of 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 5000 rpm for 10 min. Two ml of this supernatant was added to 2 ml of acid ninhydrin reagent and incubated for 1 hour at 100 ºC. The reaction was terminated in an ice-bath. The reaction mixture was extracted with 4 ml of toluene in a separating funnel by vigorous shaking. The chromophore containing toluene layer was removed from the lower aqueous phase and its absorbance was read at 420 nm and expressed as µ mol g^{-1} dry weight.

3. RESULTS

Plant can adopt several mechanisms to cope with the adverse effect of water stress. Responses of plants

to drought seems to be regulated by the manner in which tissue moisture replacement and maintenance takes place. Table 1.1 showed the effect of short-term (24 h) water stress (0,-0.5 and -1.0 MPa) simulated by PEG-6000 on relative water content of the leaves of seedlings of selected plant species. In general, water stress significantly reduces RWC in all species as compared to control the effect being greater with increased level of water stress. The extent of decrease of RWC from respective control values was lower in *L. leucocephala* and *A. auriculiformis* at both the levels of water stress, while the other species showed maximum decline at -1.0 MPa level of water stress.

Effect of short term treatment (24 h) of different levels of water stress (0, -0.5 and -1.0 MPa) simulated by PEG-6000 on the contents of cellular macromolecules and metabolites (e.g. Chlorophyll, protein, sugar and proline) of the leaves of seedlings of selected plant species has been represented, for better comparison among species changes in these parameters in terms of percentage over control have been depicted in figures 1 (A-D).

Changes in the contents of chlorophyll in leaves of the seedlings showed a gross decline with increasing level of PEG-induced water stress in case of all species. Chlorophyll level in unstressed leaves (control) was highest in *L. leucocephala* (5.48 mg g^{-1} DW), while it was lowest in *S. indica* (4.41 mg g^{-1} DW). As can be revealed from the fig. 1A, percentage decline in chlorophyll level (from control level) due to water stress was comparatively less at -1.0 MPa. In the seedlings of *L. leucocephala* decline in such content over control was comparatively less at -1.0 MPa.

Total protein content of the leaves at seedling stage was also lower in stressed leaves than unstressed controls in all species. Protein content of unstressed control leaves was highest in *L. leucocephala* (283.21 mg g^{-1} DW), while least in *D. sissoo* (179.55 mg g^{-1} DW). When percentage decreased over control was considered, decline in protein content (over control) in *L. leucocaphala* was found to be comparatively less at both the levels of water stress (Fig. 1B).

In case of untreated leaves, content of sugars was variable in these species ranging from 52.77 mg g^{-1} DW in *S. indica* to 72.82 mg g^{-1} DW in *A. auriculiformis*. Sugar contents of the leaves subjected to water stress were found to be higher over control in all cases. Percentage increase over control (Fig. 1C) was found to be higher in *L. leucocephala* and *A. auriculiformis* at both levels of water stress. Such increase over control was minimum in *S. indica* at both level of water stress (134.12% at -0.5 MPa and 159.46% at -1.0 MPa level).

There was a wide variation in the proline content of the seedlings, content being highest in *S. indica* (397.75 mg g^{-1} DW) and least in *A. auriculiformis* (203.68 mg g^{-1} DW). Under water stress, content of proline increased significantly in all cases. The rise in proline content (% control) was remarkable in *L. leucocephala* (234.45% at -0.5 MPa and 399.47% at -1.0 MPa level) as revealed from Fig. 1D.

4. DISCUSSION

In the present study, four selected plant species were subjected to assessment for their relative drought tolerance. As those plants are tree by habit, it was not feasible to judge their performance at maturity against water stress under field condition. As an alternative, seedlings of those species were used for assessment of relative tolerance against water stress simulated by PEG-6000 solution under laboratory condition.

Relative water content of any plant tissue express the existing water status of the tissue in relation to its maximum water holding capacity. So, it is an important parameter in water stress experiment since it reflects the cellular capacity to maintain water status under stress. In the present study, water status of all the species was significantly affected by water stress imposed by PEG-6000 at both the levels of water stress. However, comparative less decline in RWC due to water stress in case of *L. leucocephala* and *A. auriculiformis* at both the levels of water stress revealed better maintenance of water status in these species. At particular water potencial higher RWC is an indicator of drought tolerance through osmoregulation (Roy Chowdhury and Choudhuri, 1985, 1989). On the other hand, higher decline in RWC in other species points to their poor capacity for osmotic adjustment.

Decline in leaf chlorophyll and protein as a consequence of water stress is a very common observation (Nilsen and Orcutt, 1996; Chakraborty et al., 2001; Kar, 2002). Drought tolerant plants are usually characterized by slower rate of loss of such macromolecules. Changes in leaf contents of chlorophyll and protein of the investigated seedlings showed a gross decline (over control) with increasing level of PEG induced water stress in all species. Water stress induced chlorophyll loss is ascribed mainly due to degradation, although a retardation of synthesis may also be equally important. Maintenance of relatively high level of chlorophyll in *L. leucocephala* suggests for its relative capacity for drought tolerance. Protein loss during water stress is also regarded as due to decline in synthesis and / or an increase in protein hydrolysis (Nilsen and Orcutt, 1996). Among the investigating plants, in case of *L. leucocephala* decline in protein content under water stress condition was less it may be due to presence/synthesis of some water stress tolerant proteins that would have mitigated ill effect of water stress.

Water stress causes accumulation of various metabolites and ions, which may be associated with tolerance of the plants through osmotic adjustment (Barthakur et al., 2001; Taiz and Zeiger, 2002). Both sugars and proline are compatible osmolites and play a role in osmotic adjustment (Irigoyen et al., 1992; Singh et al., 2000; Kar, 2002; Arora et al., 2002). In the present study, leaves of the seedlings subjected to two levels of PEG-induced water stress (-0.5 MPa and -1.0 MPa) showed significant amount of sugar accumulation as compared to control. Accumulation of soluble sugar as a consequence of water stress has been demonstrated by several workers (Gupta et al., 2000; Kusturi Bai and Rajagopal, 2000). Among the investigated plant species *L. leucocephala* and *A. auriculiformis* showed high accumulation of sugars compared to other species.

These investigated species also showed a considerable amount of proline accumulation in the leaves of stressed seedlings. Accumulation of proline has been reported by several authors as one of the marked responses to water deficit stress (Gupta et al., 2000; Singh et al., 2000; Barthakur et al., 2001). The rise in proline content in case of investigated plant species was once again remarkable in *L. leucocephala* and *A. auriculiformis.* An apparent correlation between accumulation of soluble sugars and proline may be a consequence of possible dependence of proline biosynthesis on carbohydrate metabolism (Irigoyen etal., 1996). Physiological significance of proline accumulation may be associated with drought tolerance probably through osmotic adjustment.

Table 1

Relative water content in the leaves of seedlings of four investigated plant species subjected to different levels (0, -0.5 and -1.0 MPa) of PEG induced water stress. CD values within species at 5 (%) level 3.22.

Water stress	AA	DS	LL	SI
0 MPa	90.11	90.23	90.57	90.33
-0.5 MPa	40.21	37.02	42.65	36.42
-1.0 MPa	31.34	27.90	35.42	29.90

(AA = *A. auriculiformis*; DS = *D. sissoo*; LL = *L. leucocephala*; SI = *S. indica*)

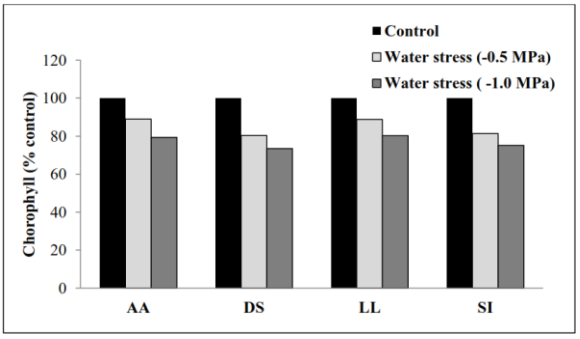

Fig. 1A. Effect of different levels (0, -0.5 and -1.0 MPa) water stress induced by PEG-6000 on chlorophyll contents in the leaves of seedlings of investigated plant species. (AA = *A. auriculiformis*; DS = *D. sissoo*; LL = *L. leucocephala*; SI = *S. indica*)

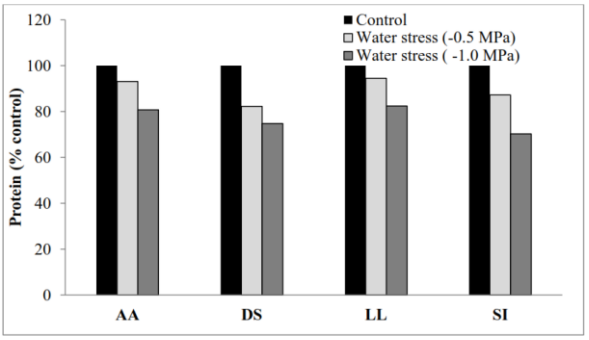

Fig. 1B. Effect of different levels (0, -0.5 and -1.0 MPa) water stress induced by PEG-6000 on protein contents in the leaves of seedlings of investigated plant species. (AA = *A. auriculiformis*; DS = *D. sissoo*; LL = *L. leucocephala*; SI = *S. indica*).

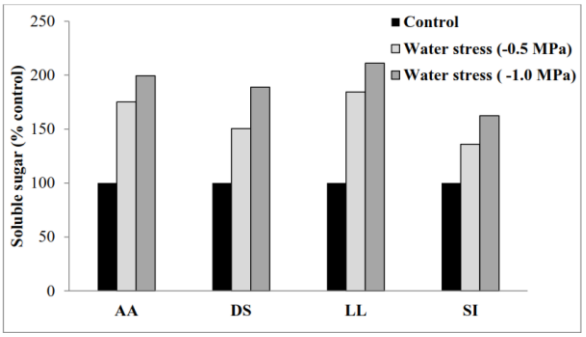

Fig. 1C. Effect of different levels (0, -0.5 and -1.0 MPa) water stress induced by PEG-6000 on soluble sugar contents in the leaves of seedlings of investigated plant species. (AA = *A. auriculiformis*; DS = *D. sissoo*; LL = *L. leucocephala*; SI = *S. indica*)

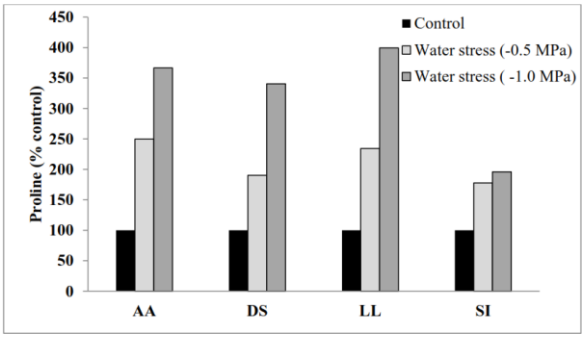

Fig. 1D. Effect of different levels (0, -0.5 and -1.0 MPa) water stress induced by PEG-6000 on proline contents in the leaves of seedlings of investigated plant species. (AA = *A. auriculiformis*; DS = *D. sissoo*; LL = *L. leuocephala*; SI = *S. indica*)

5. CONCLUSION

The report of the Task Force of Greening India for Livelihood Security and Sustainable Development (Planning Commission 2001) has suggested that 10 million ha of irrigated land and 18 million ha of rain-fed land should be managed under agroforestry system. Agroforestry through plantation of legume trees warrants a selection of species that cope well with the prevailing constrains of such wastelands. The major problem of the local wastelands that are characterized by dry lateritic soil is the occurrence of drought condition. Thus, before undertaking any agroforestry or afforestation programme in such areas, drought resistance capacity of the selected plants has to be considered. In the present case, selected plants were tested for their potential to tolerate water stress at seedling stage. For such assessment, certain stress sensitive biochemical parameters, viz. contents of chlorophyll, protein, soluble sugars and proline were considered for analysis. Finally, considering the comparative biochemical analysis (i.e., loss of chlorophyll and protein, accumulation of sugars and proline) in leaves of seedlings of four investigated plant species, *L. leucocephala* and *A. auriculiformis*, these two plants, showed higher potential for drought tolerance.

REFERENCES:

1. Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxides in *Beta vulgaris*. Plant Physiol., 24: pp. 1-15.

2. Arora, A., Sairam, R.K. and Srivastava, G.C. (2002). Oxidative stress and antioxidative system in plants. Curr. Sci., 82: pp. 1227-1238.

3. Aspinall, D. and Paleg, L.G. (1981). Praline accumulation, physiological aspects, In: Physiology and Biochemistry of Drough Resistance in Plants (ed. Paleg, L.G. and Aspinall, D.). Academic press, New York, pp. 205-240.

4. Barthakur, S., Babu, S. and Bansal, K.C. (2001). Over-expression of osmotin induces proline accumulation and confers tolerance to osmotic stress in transgenic tobacco. J. Plant Biochem. Biotechnol., 10: pp. 31-37.

5. Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free praline for water stress studies. Plant Soil, 39: pp. 205-208.

6. Bhattacharjee, S. and Mukherjee, A.K. (2002). Abiotic stress induced membrane damage in plants: a free radical phenomenon. In: Advances in Stress Physiology of Plants (ed. Panda, S.K.). Scientific Publishers, Jodhpur, India, pp. 15-35.

7. Bohnert, H.J., Nelson, D.E. and Jensen, R.G. (1995). Adaptation of environmental stresses. Plant Cell, 7: pp. 1099-1111.

8. Chakraborty, U., Dutta, S. and Chakraborty, B. (2001). Drought induced biochemical changes in young tea leaves. Indian J. of Plant Physiology, 6: pp. 103-106.

9. Chowdhury, S.R. and Choudhuri, M.A. (1986). Proline accumulation under water deficit stress and its utilization following stress release in two jute (*Corchorus*) species. Indian J. Exp. Biol., 24: pp. 605-607.

10. Cress, W.A. and Johnson, G.V. (1987). The effect of three osmotic agents on free praline and amino acid pools in *Atriplex canescens* and *Hilaria jamesii*. Can. J. Bot., 65: pp. 799-801.

11. Gupta, S.C., Rathore, A.K., Sharma, S.N. and Saini, R.S. (2000). Response of chickpea cultivers to water stress. Indian J. Plant Physiol., 5: pp. 274-276.

12. Hanson, A.D. and Hitz, W.D. (1982). Metabolic responses of mesophytes to plant water deficits. Annu. Rev. Plant Physiol., 33: pp. 163-203.

13. Irigoyen, J.J., Emerich, D.W. and Sanchez Diaz, M. (1992). Water stress induced changes in concentrations of praline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiol. Plant., 84: pp. 55-60.

14. Janardhan, K.V. and Bhojaraja, R. (1999). Plant responces and adaptation to water

deficit. In: Advances in Plant Physiology (ed. Hemantarajan, A.). vol. 2. Scientific Publishers, Jodhpur, India, pp. 113-135.

15. Kar, R.K. (2002). Studying plant responses to water stress: an overview. In: Advances in Stress Physiology of Plants (ed. Panda, S.K.). Scientific Publishers, Jodhpur, India, pp. 61-79.

16. Kar, R.K. and Mishra, D. (1976). Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. Plant Physiol., 57: pp. 315-319.

17. Kusturi Bai, K.V. and Rajagopal, V. (2000). Osmotic adjustment as a mechanism for drought tolerance in coconut (*Cocos nucifera* L.). Indian J. plant Physiology, 5: pp. 320-323.

18. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin-phenol reagent. J. Biol. Chem., 193: pp. 265-275.

19. McCready, R.M., Guggloz, J., Silviera, V. and Owens, H.S. (1950). Determination of starch and amylase in vegetables. Analyt. Chem., 22: pp. 1156-1158.

20. Michel, B.E. and Kaufmann, M.R. (1973). The osmotic potential of polyethylene glycol-6000. Plant physiol., 51: pp. 914-916.

21. Money, N.P. (1989). Osmotic pressure of aqueous polyethylene glycols. Relationship between molecular weight and vapour pressure deficit. Plant Physiol., 91: 766-769.

22. Nilsen, E.T. and Orcutt, D.M. (1996). The Physiology of Plants Under Stress. John Wiley & Sons Inc., New York, pp. 336.

23. Panda, S.K. (2002). The biology of oxidative stress in green cells: a review. In: Advances in Stress physiology of Plants (ed. Panda, S.K.). Scientific Publishers, Jodhpur, India, pp. 1-13.

24. Read, R.H. (1984). Use and abuse of osmo-terminology. Plant Cell Environ., 7: pp. 165-170.

25. Reddy, P.V., Asalatha, M. and Babitha, M. (2000). Relationship of mineral ash and chlorophyll content with transpiration efficiency in groundnut under different moisture regimes. Indian J. Plant Physiol., 5: pp. 59-63.

26. Roy Chowdhury, S. and Choudhuri, M.A. (1985). Hydrogen peroxide metabolism as an index of water stress tolerance in jute. Physiol. Plant., 65: pp. 503-507.

27. Roy Chowdhury, S. and Choudhuri, M.A. (1989). Effect of $CaCl_2$ and ABA on changes in H_2O_2 metabolism in two jute species under water deficit stress. J. Plant Physiol., 135: pp. 179-183.

28. Singh, D.V., Srivastava, G.C. and Abdin, M.Z. (2000). Effect of benzyladenine and ascorbic acid and ascorbic acid content and other metabolites in sena (*Cassia angustifolia* VAHL.) under water stress conditions. Indian J. Plant Physiol., 5: pp. 127-131.

29. Spickett, C.M., Smirnoff, N. and Radcliffe, R.G. (1992). Metabolic responses of maize root to hyper osmotic shock. Plant Physiol., 99: pp. 856-863.

30. Taiz, L. and Zeiger, E. (2002). Stress physiology. In: Plant Physiology. Third Edition. Sinauer Associates Inc. Publishers, Sunderland, Massachusetts, pp. 591-632.

31. Weatherley, P.E. (1950). Studies in the water relations on the cotton plant I. The field measurement of the water deficit in the leaves. New Phytol., 49: pp. 81-97.

Corresponding Author

Arpita Banerjee*

Department of Botany, Bankura Christian College, Bankura, West Bengal

arpitabanerjeebcc@rediffmail.com