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Selection of fuel wood yielding trees for agro-forestry in dry lateritic area.

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ABSTRACT

Twenty five day old seedlings of five fuel wood yielding tree species (*Acacia nilotica* Willd., *Butea monosperma* Roxb., *Delonix regia* (Hook.) Raf., *Peltophorum pterocarpum* (DC.) K. Heyne) and *Tamarindus indica* Linn.) of the family Leguminosae (Fabaceae) were subject to polyethylene glycol (PEG)-induced water stress (-0.5 and – 1.0 MPa) for 24 h in order to assess their relative tolerance to water stress. Leaves were analyzed for relative water content, chlorophyll, protein, carbohydrate and proline content. Considering the comparative biochemical analysis (i.e., loss of chlorophyll and protein, accumulation of sugars and proline) in leaves of seedlings of five investigated plant species, *A. nilotica* and *B. monosperma*, these two plants, showed higher potential for drought tolerance.

Keywords: Biochemical changes, Fuel wood, Legume tree, Water stress.

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INTRODUCTION

Recently, agro-forestry is gaining much importance, as it has a vast scope to mitigate several problems including problem of fuel, fodder, timber and shade production. It can also be adopted on agricultural land, marginal and sub marginal wastelands, lands not presently available for cultivation of arable crops etc. In many parts of the world, especially in the tropics, vast area of forest lands are now denuded of trees destroying the fragile environment leaving extensive areas unproductive. These areas, which are otherwise unsuitable for cultivation of crop species, can be utilized well by growing fuel wood yielding plants, an alternative to fossil fuels. Energy availability and consumption pattern in rural areas reveal that irrespective of socio-economic factor, most of them are dependent upon traditional fuels, which were obtained by them free of cost. However, such areas often suffer from water deficit. In dry climates, low water availability limits productivity. Water stress leads to substantial variation in morphology, anatomy, physiology and biochemistry of plants, which is ultimately reflected on the yield potential. Agro-forestry requires precise research work in a particular area based on climatic and edaphic conditions of the locality. 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 [1-4]. 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 [5,6]. Water stress severely modifies the metabolism to an extent depending upon species, duration and intensity of stress. One common observation associated with water stress is a gross decline in chlorophyll and protein level, which may be ascribed to a decrease in protein synthesis and/ or an increase in protein hydrolysis [7,8,9]. 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 [7,10]. Therefore attempts are being made in different concerns of our country and abroad to formulate suitable package of practice for growing such plants.

MATERIAL AND METHODS

In the present investigation, five tree species (*Acacia nilotica* Willd., *Butea monosperma* Roxb., *Delonix regia* (Hook.) Raf., *Peltophorum pterocarpum* (DC.) K. Heyne and *Tamarindus indica* Linn.) of the family Leguminosae (Fabaceae) were used and seedlings (25 days old) were taken as experimental plant materials to assess responses to water stress. Seeds of plant species were obtained from the local forest of Bankura (lies between 22° 38ˈ and 23° 38ˈ North latitude and between 86° 36ˈ and 87° 46ˈ East longitudes). The soil is mainly dry lateritic type, reddish in colour and coarse sandy in texture with pH ranges from 5.5 to 6.5 at different regions of the district. Seedlings were raised by germinating healthy seeds (surface sterilized by sodium hypochlorite) 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 [11] 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 [12]. 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 °C 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 [13].

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 trichloroacetic acid, ethanol, ethanol: chloroform (3:1) and finally with ether to remove the phenolic compounds [14]. The washed pellets were then digested with 2 ml of 1N NaoH in water bath at 80 °C 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.* [15]. Protein content was calculated by comparing O.D. values with a standard curve prepared for bovine serum albumin. 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 [16]. The absorbance was measured at 610 nm in a UV-VIS spectrophotometer (Systronics) and compared with a standard curve prepared from glucose. 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* [17]. 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 °C. 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. The proline content was calculated by comparing absorbance with a standard curve prepared from L-proline and expressed as μ mol g^{-1} dry weight.

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 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 *A. nilotica* and *B. monosperma* at both the levels of water stress, while the other species showed maximum decline at -1.0 MPa level of water stress.

Table 1: Relative water content in the leaves of seedlings of five legume trees subjected to different levels of PEG induced water stress.

Water stress	Name of the plants				
	AN	BM	DR	PP	TI
0 MPa	90.34	91.75	90.53	89.92	90.18
- 0.5 MPa	45.12	49.43	39.84	37.06	40.02
- 1.0 MPa	34.67	37.15	31.90	29.75	27.98

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 except *B. monosperma and A. nolotica* where instead of decline chlorophyll level increased over control. Chlorophyll level in unstressed leaves (control) was highest in *B. monosperma* (4.98 mg g $^{-1}$ DW), while it was lowest in *Peltophorum pterocarpum* (3.87 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 and *B. monosperma* decline in such content over control was comparatively less at -1.0 MPa.

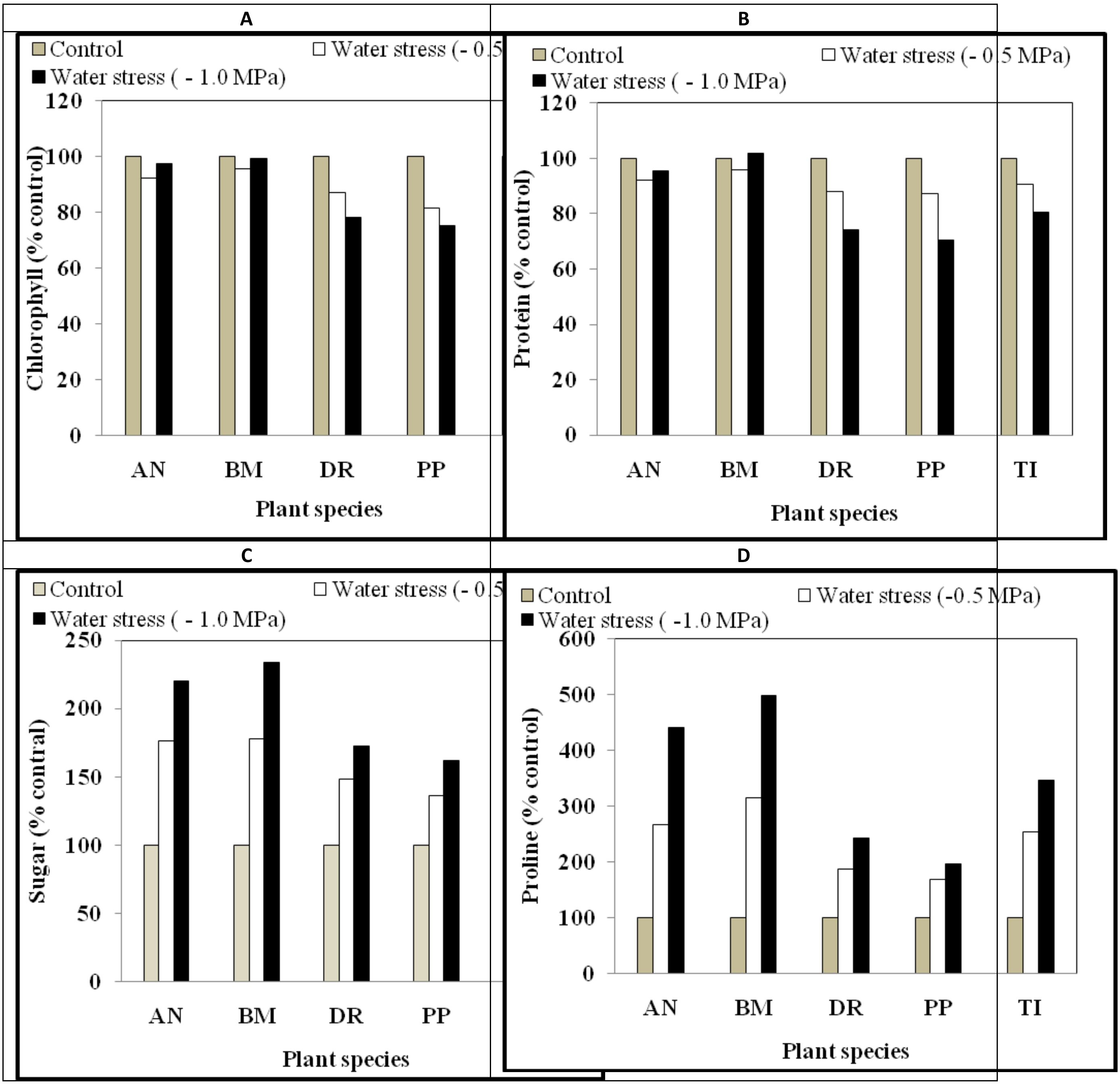

Fig 1: Changes in the contents of chlorophyll (A), protein (B), Soluble sugar (C) and proline (D) in the leaves of seedlings of five fuel wood tree species (*Acacia nilotica* = AN, *Butea monosperma* = BM, *Delonix regia* = DR, *Peltophorum pterocarpum* = PP and *Tamarindus indica* = TI)) in response to different levels of water stress (0. – 0.5 and – 1.0 MPa) induced by PEG-6000.

[Content in control:
Chlorophyll (mg g $^{-1}$ DW) – AA = 4.36, BM = 4.98, DR = 4.43, PP = 3.87, TI = 4.67
Protein (mg g $^{-1}$ DW) – AA = 160.56, BM = 315.19, DR = 188.78, PP = 190.57, TI = 215.10
Soluble sugar (mg g $^{-1}$ DW) – AA = 50.70, BM = 46.13, DR = 43.95, PP = 48.44, TI = 52.00
Proline (µmol g $^{-1}$ DW) – AA = 610.38, BM = 356.28, DR = 380.11, PP = 391.90, TI = 410.75
Respective CD value (at P = 0.05) for interaction: chlorophyll = 0.82, protein = 06.85, soluble sugar = 4.15, proline = 26.20].

Total protein content of the leaves at seedling stage was also lower in stressed leaves than unstressed controls in all species except *B. monosperma* seedlings where the protein content of the leaves becomes significantly higher under water stress than in control. Protein content of unstressed control leaves was highest in *B. monosperma* (315.19 mg g $^{-1}$ DW), while least in *A. nilotica* (160.56 mg g $^{-1}$ DW). When percentage decreased over control was considered, decline in protein content (over control) in *B. monosperma* 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 52.00 mg g $^{-1}$ DW in *T. indica* to 43.95 mg g $^{-1}$ DW in *D. regia*. 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 A. nilotica and *B. monosperma* at both levels of water stress. Such increase over control was minimum in *P. pterocarpum* at both level of water stress. Changes in the proline content of leaves of the seedlings of the selected plants upon water stress were shown a wide variation in the proline content of the seedlings, content being highest in *A. nilotica* (610.38 mg g $^{-1}$ DW) and least in *B. monosperma* (356.28 mg g $^{-1}$ DW). Under water stress, content of proline increased significantly in all cases. The rise in proline content (% control) was remarkable in *B. monosperma* as revealed from Fig. 1D.

DISCUSSION

Drought resistance is generally defined as the maintenance of plant production during moderate water deficit. In the present study, five 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 *A. nilotica* and *B. monosperma* 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 [18,19]. 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 [3,8,9]. 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, except *B. monosperma* where both chlorophyll and protein content were found to be higher than unstressed seedlings. Decline in leaf chlorophyll and protein as a consequence of water stress is a very common observation [7,8]. 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 *B. monosperma* suggests for its relative capacity for drought tolerance. However, a higher level of chlorophyll in leaves of *B. monosperma* seedlings under water stress was very unusual. It can only be explained by possible increase in synthesis due to water stress [20]. Protein loss during water stress is also regarded as due to decline in synthesis and / or an increase in protein hydrolysis [8]. Among the investigating plants, *B. monosperma* synthesized protein under water stress condition it may be 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 [21,22]. Both sugars and proline are compatible osmolites and play a role in osmotic adjustment [3,23-25]. 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 [26,27,28]. Among the investigated plant species *A. nolotica* and *B. monosperma* 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 [21,24,26,28]. The rise in proline content in case of investigated plant species was once again remarkable in *A. nolotica* and *B. monosperma.* An apparent correlation between accumulation of soluble sugars and proline may be a consequence of possible dependence of proline biosynthesis on carbohydrate metabolism [23]. Physiological significance of proline accumulation may be associated with drought tolerance probably through osmotic adjustment.

CONCLUSION

Leguminous plants are found throughout the world, but the greatest variety is found in tropics and sub-tropics. Legumes are crucial to the balance of nature, as most of them are able to convert nitrogen from air in to ammonia, which is readily utilized by plants. Plants usually encounter a number of stresses under field conditions. Agro-forestry 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 afforestation programme in such areas, drought resistance capacity of the selected plants has to be considered. In the present study, 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 five investigated plant species, *A. nolotica* and *B. monosperma*, these two plants, showed higher potential for drought tolerance.

REFERENCES

[1] Janardhan KV, Bhojaraja R. Advances in Plant Physiology. Scientific Publishers, Jodhpur, India, 1999, pp. 113-135.
[2] Bhattacharjee S, Mukherjee AK, Advances in Stress Physiology of Plants. Scientific Publishers, Jodhpur, India, 2002, pp. 15-35
[3] Kar RK, Advances in Stress Physiology of Plants, Scientific Publishers, Jodhpur, India, 2002, pp. 61-79.
[4] Panda SK. Advances in Stress physiology of Plants .Scientific Publishers, Jodhpur, India, 2002, pp.1-13.
[5] Cress WA, Johnson CV. Can J Bot 1987; 65: 799-801.
[6] Money NP. Plant Physiol 1989; 91: 766-769.
[7] Aspinall D, Paleg LG. Physiology and Biochemistry of Drough Resistance in Plants. Academic press, New York, 1981, pp. 205-240.
[8] Nilsen ET, Orcutt DM. The Physiology of Plants under Stress. John Wiley & Sons Inc., New York, 1996, pp. 336.
[9] Chakraborty U, Dutta S, Chakraborty B. Indian J. of Plant Physiology 2001; 6: 103-106.
[10] Hanson AD, Hitz WD. Annu Rev Plant Physiol 1982; 33: 163-203.
[11] Michel BE, Kaufmann MR. Plant Physiol 1973; 51: 914-916.
[12] Weatherley PE. New Phytol 1950; 49: 81-97.
[13] Arnon DI. Plant Physiol 1949; 24: 1-15.
[14] Kar M, Mishra D. Plant Physiol 1976; 57: 315-319.
[15] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. J Biol Chem 1951; 193: 265-275.
[16] McCready RM, Guggloz J, Silviera V, Owens HS. Analyt Chem 1950; 22: 1156-1158.
[17] Bates LS, Waldren RP, Teare ID. Plant Soil 1973; 39: 205-208.
[18] Roy Chowdhury S, Choudhuri MA. Physiol Plant 1985; 65: 503-507.
[19] Roy Chowdhury S, Choudhuri MA. J Plant Physiol 1989; 135: 179-183.
[20] Reddy PV, Asalatha M, Babitha M. Indian J Plant Physiol 2000; 5: 59-63.

[21] Barthakur S, Babu S, Bansal KC. J Plant Biochem Biotechnol 2001; 10: 31-37.
[22] Taiz L, Zeiger E. Plant Physiology Sinauer Associates Inc. Publishers, Sunderland, Massachusetts, 2002, pp. 591-632.
[23] Irigoyen JJ, Emerich DW. Physiol Plant 1992; 84: 55-60.
[24] Singh DV, Srivastava GC, Abdin MZ. Indian J Plant Physiol 2000; 5: 127-131.
[25] Arora A, Sairam RK, Srivastava GC. Curr Sci 2002; 82: 1227-1238.
[26] Gupta SC, Rathore AK, Sharma SN and Saini RS. Indian J Plant Physiol 2000; 5: 274-276.
[27] Kusturi Bai KV, Rajagopal V. Indian J. plant Physiology 2000; 5: 320-323.
[28] Sinhababu A, Kar RK. Acta Physiologia Plantarum 2003; 25: 403-409.