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Determining Legume Plants that Tolerate Dryness and Grow in Dry Lateritic Soil

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In arid regions, productivity is restricted by an abundance of water. The objective of the research is to screen of suitability of some legume plants in the dry laterite soil of Purulia and adjoining districts in West Bengal, India. Seedlings (25 days old) of *Acacia mangium* Willd., *Albizia procera* (Roxb.) Benth, *Bauhinia acuminata* Linn. of the family Leguminosae (Fabaceae) is 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 as well as activities of enzymes catalase, peroxidase and superoxide dismutase (SOD). In leaves of the seedlings, chlorophyll and protein contents decline with increasing levels of PEG-induced water stress in the case of all species whereas the amount of soluble sugar and proline content increases in all species with increasing levels of water stress. The enzyme activity of catalase, peroxidase and SOD decreased with increased level of water stress. Such decline in the activity of these enzymes was least in *B. acuminata*. Apparently, *B. acuminata* is potentially most tolerant to water stress among the plants studied.

Key words: Legume tree, drought stress, Biochemical changes

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 is a limiting factor of productivity. Agro-forestry requires proper 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 (Janardhan *et al.,* 1999; Bhattacharjee *et al.,* 2002). Polyethylene glycol has an advantage over other osmotic agents as it does not enter the apoplastic space (Cress and Johnson 1987; Money 1989). Water stress severely modifies the metabolism which depends on 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 (Aspinall and Paleg 1981; Nilsen *et al.,* 1996). Under water stress various metabolites and ions accumulate in plant tissues. Such an accumulation is usually associated with the osmotic adjustment of cells (Hanson and Hitz 1982). In maize plants, severe drought during the seedling stage results in noticeable leaf curling and stunted growth (Effendi *et al.,* 2019). Previous research has demonstrated that drought stress during this early growth phase of a plant's life cycle can significantly increase tolerance to water scarcity in later growth stages, even though the seedling establishment stage is one of the most vulnerable growth stages (Auler *et al.,* 2021). Therefore, during the seedling establishment stage, choosing and identifying drought-tolerant cultivars can help increase crop yield (Ru *et al.,* 2022). In wastelands particularly deficient in moisture and nutrition requires some adaptive mechanism in the species for survival under such constraints. Thus, a screening of legume species for drought tolerance is essential for undertaking any afforestation programme in dry wastelands. Considering the above mentioned facts, the present study on the leguminous trees is thus an attempt to assess the potential of the seedling tolerance to drought by studying metabolic responses**.** Besides, stress condition induces generation of active oxygen species (AOS) as a result of biochemical and physiological reaction within the cell. Tolerant plants efficiently scavenge free radicals and peroxides involving enzymes like catalase, peroxidase and SOD. Hence activities of these enzymes were also studied in the seedlings of plants studied.

MATERIALS AND METHODS

In the present investigation, three species *Acacia mangium* Willd., *Albizia procera* (Roxb.) Benth, *Bauhinia acuminata* Linn. of the family Leguminosae (Fabaceae) were used. Seedlings (25 days old) were taken as experimental plant materials to assess responses to water stress. Initially on moist filter paper seedlings were raised followed by transfer to sand beds where acid washed sand was used. Selected plant's seedlings were subjected to water stress induced by polyethylene glycol (PEG-6000). Two levels of water stress (-0.5 and -1.0 MPa) were adjusted according to Michel and Kaufman by the concentrations of 19.6% and 29.6% of PEG Solutions, respectively. By dipping roots in solutions, stress was imposed to seedlings of the investigated species. A control set was maintained using distilled water (0 Mpa). Incubation was done for 24 hours under 8h 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. Leaves of the seedlings under stressed or control were taken, washed with distilled water, then surface solution was blotted and fresh weight was taken. After that 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 with the same methanol and centrifuged at 5000 rpm for 10 minutes. The total chlorophyll Content was estimated using the supernatants (10 m) following the method of Arnon.

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: chloroform (3:1) and finally with ether to remove the phenolic compounds. 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, taking the supernatant total protein content was estimated according to the method of Lowry *et al.,* 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: To determine carbohydrate content 50 mg leaf sample from each set was crushed in 5 ml of hot 80% ethanol and then centrifuged at 5000 rpm for 10 minutes. The supernatant containing ethanol-soluble carbohydrate was then evaporated to dryness. Chlorophyll was removed by rinsing with solvent ether. 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.

The absorbance was measured at 610 nm in a UV-VIS spectrophotometer and compared with a standard curve prepared from glucose. Content was expressed as mg glucose equivalents g^{-1} dry weight.

Proline: Proline content of leaf tissue was determined by the method of Bates *et al.,* Plant material (200 mg) was first homogenized in 5 ml of 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 5000 pm 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 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 mol / g dry weight.

Enzyme activities: For the determination of activities of catalase and peroxidase, the enzymes were extracted homogenizing leaf tissue in cold 0.1 M phosphate buffer(pH 7.0) containing 1% PVPP followed by centrifugation at 10,000 rpm at 4^0 C. The supernatant was used as enzyme source and the assay for catalase activity was carried out according to the method described by Biswas and Choudhury. The activity of peroxidase was determined following the rate of oxidation of pyrogallol. The activity of enzyme SOD was determined by measuring its ability to inhibit photochemical reduction of NBT according to the method of Giannopolitis and Ries (1977) with some modification by Roy Chowdhury and Choudhuri (1985). The 3 ml reaction mixture contained 0.05 M Na2CO3, 0.1mM EDTA, 63 µM NBT, 13 µM methionone, 20 µl enzyme extract and 1.3 µM riboflavin. The riboflavin was added last. Five ml distilled water was added to reaction mixture and shaken. The assay mixture was then incubated under light at 20 µmol m-2 s-1 for 30 minutes. After 30 minutes of incubation, absorbance was taken at 560 nm immediately in a UV-VIS spectrophotometer. The non-irradiated sample served as control and was deduced from A560.

RESULTS

Relative water content: Plant can adopt several mechanisms to cope with the adverse effect of water stress. 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 *B. acuminata* at both the levels of water stress, while the other species showed maximum decline at -1.0 MPa level of water stress. Table.1. shows the relative water content of the plants under experimental conditions.

Analysis of variance showed that the difference between plants was statistically significant (P= <0.05) under both the stress level. Post hoc comparison using the Tukey test indicated that the mean relative water content of the plant *B. acuminata* (M= 56.2) was significantly higher than those of other plants.

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 in the tables 2 to 5. For better comparison among species changes in these parameters in terms of percentage over control have been depicted in figures 1-4.

Chlorophyll: 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 (Table.2). Chlorophyll level in unstressed leaves (control) was highest in *A. mangium* (5.40 mg g^{-1}DW), while it was lowest in *B.acuminata* (4.90 mg g^{-1} DW). As can be revealed from the fig. 1, due to water stress percentage decline in chlorophyll level from control level was less at -1.0 MPa. In the seedlings of *B.acuminata* decline in such content over control was comparatively less at-1.0 MPa.

Total protein: Total protein content of the leaves at seedling stage (Table.3) was also lower in stressed leaves than unstressed controls in all species. Protein content of unstressed control leaves was highest in *B.acuminata* (309.68 mg g^{-1} DW), while least in *Acacia mangium* (184.22 mg g^{-1} DW). When percentage decreased over control was considered, decline in protein content (over control) in *B.acuminata* was found to be comparatively less at both the levels of water stress (Fig. 2).

Analysis of variance showed that the difference between plants was statistically significant (P= <0.05) under both the stress level. Post hoc comparison using the Tukey test indicated that the mean protein content of the plant *B.acuminata* (M=286 , SD=.0327) was significantly higher than those of other plants. Plants *A. mangium* and *B.acuminata* differ significantly with greater value of mean difference.

Carbohydrate: Changes in soluble sugar content during water stress of seedlings of selected plant species have been shown in Table 4. In case of untreated leaves, content of sugars was variable in these species ranging from 36.12 mg g^{-1} DW in *B.acuminata* to 72.62 mg g^{-1} DW in *Acacia mangium*. Leaves subjected to water stress have higher sugar contents over control in all cases. Percentage increase over control (Fig. 3) was found to be higher in *B.acuminata and A. procera* at both levels of water stress.

Analysis of variance showed that the difference between plants was statistically significant (P= <0.05) under stress levels. Post hoc comparison using the Tukey test indicated that the mean carbohydrate content of the plant *Acacia mangium* (M=109.6) was significantly higher than those of other plants. *B.acuminata* outperforms the other two plants under increased stress condition.

Proline: There was wide variation in the proline content of the seedlings, content being highest in the *Albizia procera* (570.24 mg g^{-1}DW) and least in *Acacia mangium* (204.60 mg g^{-1}DW). Changes in the proline content of leaves of the seedlings of the selected plants upon water stress were shown in Table.5. Under water stress, content of proline increased significantly in all cases. The rise in proline content (% control) was remarkable in *B.acuminata* (250.81 % at -0.5Mpa and 434.22 % at -1.0 Mpa level) as revealed from fig.4.

Enzyme activities: Catalase activity of seedling leaves (Fig.5A) decreased with increasing level of water

stress in all the plants investigated (Table.6). In terms of percentage over control, Catalase enzyme activity was least affected by water stress in *B. acuminata* at both the level of water stress. Peroxidase activity (Fig.5B) becomes lower under stress conditions (Table.7). All the plants show more or less similar pattern of reduction in enzyme activity under -0.5 Mpa level. When percent decrease over control was considered, it was found that stress induced lowering of activity was less in *B. acuminata*. In case of untreated seedling SOD activity (Table.8) did not vary much among species (ranging from 102 to110 unit h^{-1} g^{-1} DW).The rate of decline in the activity due to stress was not same in all species as can be revealed from percentage decrease over control.

Table 1: Relative water contents (mg g^{-1} DW) in the leaves of seedlings of investigated plant species to different levels (0, -0.5 and -1.0 MPa) of PEG induced water stress.

Water stress	AM	AP	BA
0 Mpa	92.31	90.18	91.20
-0.5Mpa	37.02	37.12	43.21
-1.0Mpa	27.23	30.65	34.23

Table 2: Chlorophyll contents (mg g^{-1} DW) in the leaves of seedlings of investigated plant species to different levels (0, -0.5 and -1.0 MPa) of PEG induced water stress.

Water stress	AM	AP	BA
0 Mpa	5.40	5.20	4.90
-0.5Mpa	4.36	4.72	4.30
-1.0Mpa	4.10	4.15	4.00

Table 3: Protein contents (mg g-1 DW) in the leaves of seedlings of investigated plant species to different levels (0, -0.5 and -1.0 MPa) of PEG induced water stress.

Water stress	AM	AP	BA
0 Mpa	184.22	203.72	309.68
-0.5Mpa	164.10	189.00	288.14
-1.0Mpa	152.60	143.20	260.56

Table 4: Carbohydrate contents (mg g^{-1} DW) in the leaves of seedlings of investigated plant species to different levels (0, -0.5 and -1.0 MPa) of PEG induced water stress.

Water stress	AM	AP	BA
0 Mpa	72.62	38.20	36.12
-0.5Mpa	118.2	65.82	63.92
-1.0Mpa	138.12	81.24	90.00

Table 5: Proline contents (mg g^{-1} DW) in the leaves of seedlings of investigated plant species to different levels (0, -0.5 and -1.0 MPa) of PEG induced water stress.

Water stress	AM	AP	BA
0 Mpa	204.60	570.24	330.22
-0.5Mpa	500.64	1227.12	828.24
-1.0Mpa	716.34	1480.28	1433.90

Table 6: Catalase enzyme activity (unit min^{-1} g^{-1} DW) in the leaves of seedlings of selected plants subjected to different level (0,-0.5 and-1.0 MPa) of PEG induced water stress.

Water stress	AM	AP	BA
0 Mpa	40.40	106.56	36.85
-0.5Mpa	17.20	56.25	22.30
-1.0Mpa	14.65	36.00	16.20

Table 7: Peroxidase enzyme activity (unit min^{-1} g^{-1} DW) in the leaves of seedlings of selected plants subjected to different level (0,-0.5 and-1.0 MPa) of PEG induced water stress.

Water stress	AM	AP	BA
0 Mpa	28.45	33.20	19.75
-0.5Mpa	17.74	20.55	12.45
-1.0Mpa	10.00	12.85	10.20

Table 8: SOD enzyme activity (unit h^{-1} g^{-1} DW) in the leaves of seedlings of selected plants subjected to different level (0,-0.5 and-1.0 MPa) of PEG induced water stress.

Water stress	AM	AP	BA
0 Mpa	106.45	102.20	109.84
-0.5Mpa	45.80	41.35	64.20
-1.0Mpa	40.20	24.44	52.67

Abbreviation :(AM= *Acacia mangium, AP= Albizia procera, BA= Bauhinia acuminata,)*

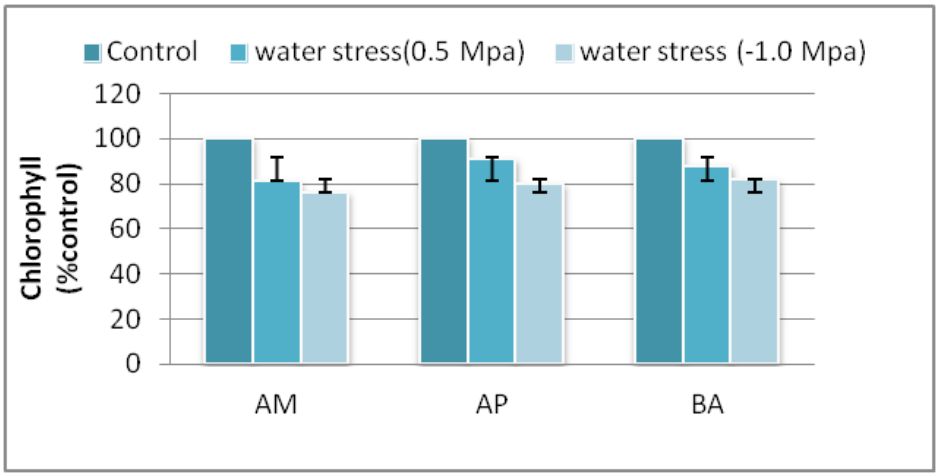

Figure 1: Effect of different levels (0, -0.5 and -1.0 MPa) of PEG induced water stress on chlorophyll contents in the leaves of plant species investigated.

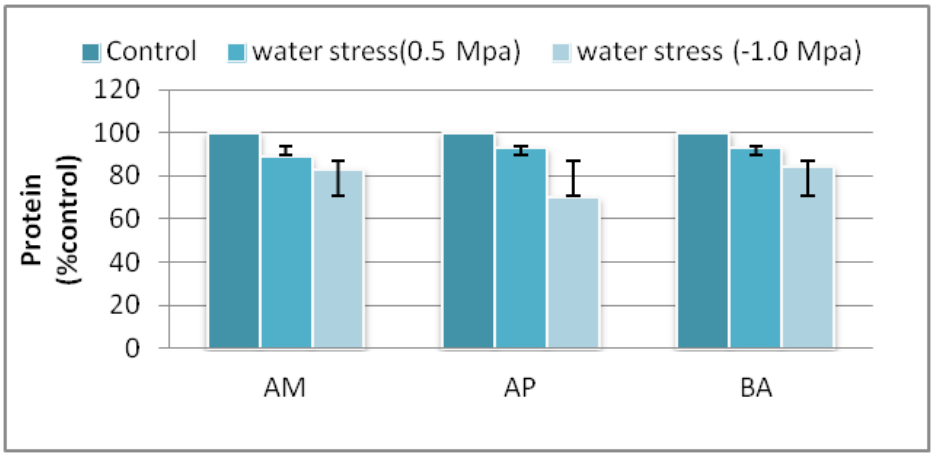

Figure 2. Effect of different levels (0, -0.5 and -1.0 MPa) of PEG induced water stress on protein contents in the leaves of plant species investigated.

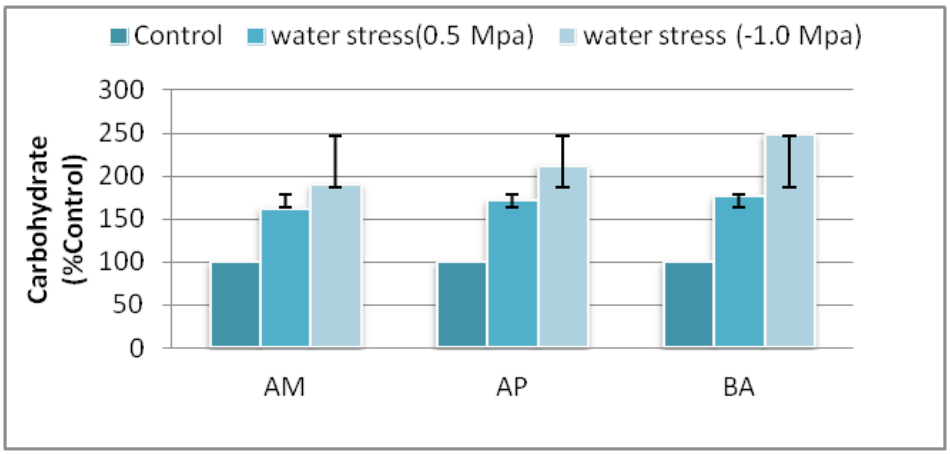

Figure 3: Effect of different levels (0, -0.5 and -1.0 MPa) of PEG induced water stress on Carbohydrate contents in the leaves of plant species investigated.

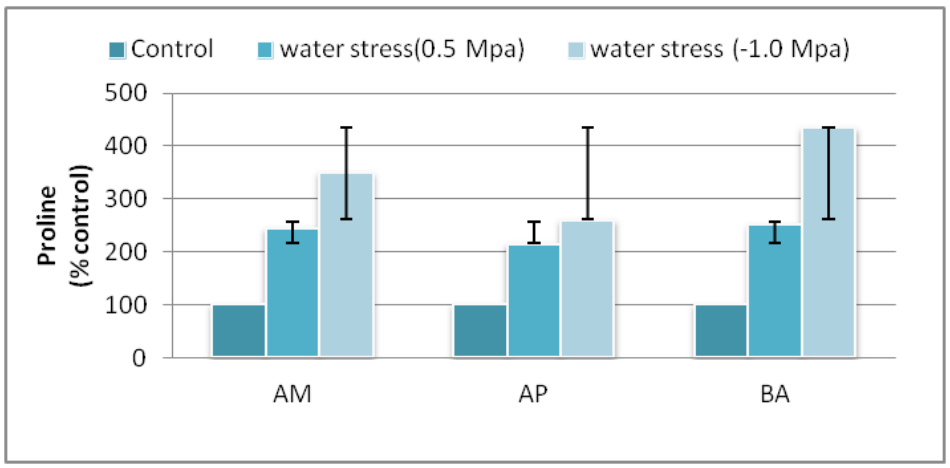

Figure 4: Effect of different levels (0, -0.5 and -1.0 MPa) of PEG induced water stress on Proline contents in the leaves of plant species investigated.

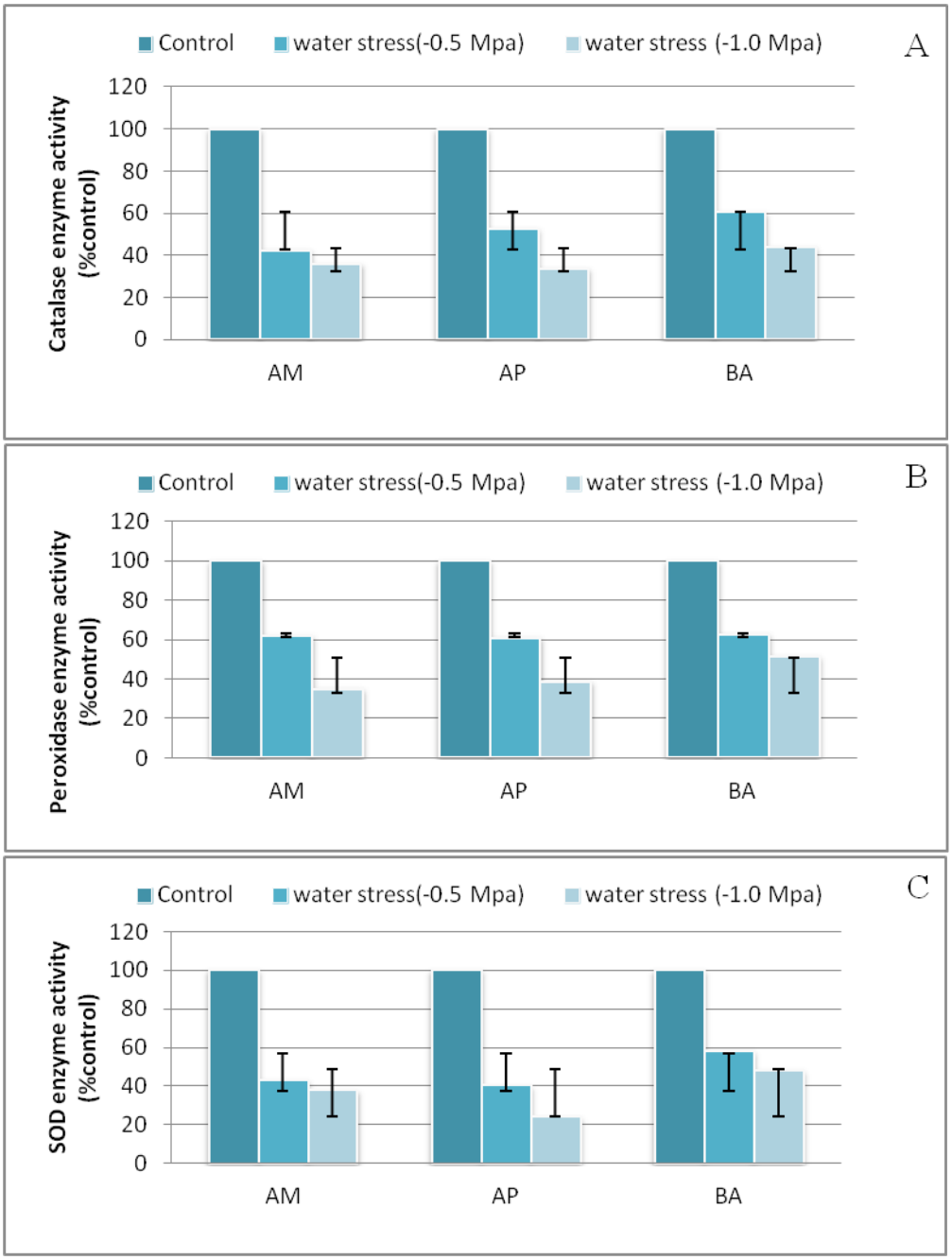

Figure 5: Changes in the activities of Catalse(A), Peroxidase(B) and Superoxide dismutase (C) In the leaves of seedling of three plants (AM= *Acacia mangium,AP= Albizia procera, BA= Bauhinia acuminata)* in response to different levels of water stress (0,-0.5 and -1.0 MPa) induced by PEG-6000.

DISCUSSION

In the present study, relative drought tolerance of four plant species was analyzed. As those plants are tree by habit, it was not feasible to judge their performance at mature condition against water stress under field condition. Assessment of relative tolerance against water stress induced by PEG-6000 solution was done using seedlings of those species under laboratory conditions.

Relative water content is an important parameter in water stress experiment since it reflects the cellular capacity to maintain water status under stress. In the present study, all the species was significantly affected by PEG-6000 induced water stress at two levels of water stress (-0.5 and -1.0 MPa). However, the extent of decrease of RWC from respective control values was

lower in *B. acuminata* that revealed better maintenance of water status in this species. On the other hand, higher decline in RWC in other species reflects their poor capacity for osmotic adjustment. At particular water potential higher RWC is an indicator of drought tolerance through osmoregulation.

Decline in chlorophyll and protein content is a very common observation under water stress (Nilsen and Orcutt 1996; Chakraborty *et al.,* 2001).Water stress induced chlorophyll loss is mainly due to degradation, although a retardation of synthesis may also be equally important. Plant seedlings showed a gross decline (over control) in leaf contents viz. chlorophyll and protein with increasing level of PEG induced water stress in all species.

However, maintenance of relatively high level of chlorophyll in *B.acuminata* suggests for its relative capacity for drought tolerance.

Both sugars and proline are compatible osmolites and play a role in osmotic adjustment (Irigoyen *et al.,* 1992; Arora *et al.,* 2002). In the present investigation, leaves of the seedlings subjected to water stress 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 (Sinhababu and Kar 2003; Kusturi *et al.,* 2000; Gupta *et al.,* 2000). Among the investigated plant species *B.acuminata and A. procera* showed high accumulation of sugars compared to other species. Stressed seedlings also showed a considerable amount of proline accumulation in the leaves. Several authors reported accumulation of proline as one of the marked responses to water deficit stress (Barthakur *et al.,* 2001; Gupta *et al.,* 2000; Sinhababu and Kar 2003). The rise in proline content in case of investigated plant species was once again remarkable in *B.acuminata and A. procera.* An apparent correlation between accumulation of soluble sugars and proline may be a consequence of possible dependence of proline biosynthesis on carbohydrate metabolism (Irigoyen *et al.,* 1992). Physiological significance of proline accumulation may be associated with drought tolerance probably through osmotic adjustment.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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