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Research Article

MORPHOLOGICAL AND BIOCHEMICAL STUDIES ON DROUGHT INDUCED TIRUNELVELI SENNA (*Senna alexandrina* Mill.)

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ABSTRACT

The present investigation was aimed at evaluating the relative performance of different morphological, biochemical and protein for drought tolerance in *Senna alexandrina* Mill. To achieve the objectives, pot experiment was conducted. There is no significant decrease in leaves and the leaves were very rigid and healthy. We couldn't find any wilting even in the severe drought stress condition. The chlorophyll content got increased when the stress level increases which is good sign for drought tolerance on contrary to other plants. The glycine betaine content was present highly in control plant (350.8 µg g^{-1} FW) itself which is a new report. The antioxidant level and its activity were very stable among the drought stressed *Senna alexandrina* Mill. The proteolytic activity was increased when drought stress increases which is a protective effect to the plants to cope up the drought and the protein content also increased which correlates with the proteiolytic activity. The protein banding pattern also showed that the plant has more drought tolerance capacity. 70, 65, 40, 30, 13 and 3 kDa protein bands has significant decrease in expression and 19, 15, 8 kDa protein bands expression are increased over increased stress levels of *Senna alexandrina* Mill.

Key Words: Tirunelveli Senna, Drought, Biochemical, Protein, Glycine- betaine

INDTRODUCTION

The drought tolerance mechanism involved in the plant systems differs in plant types and even in species level. So understanding the mechanism and the network participating in the drought resistance is inevitable. For that, some necessary parameters have to be evaluated to narrow down its regulatory system and its mechanism. Different parameters like relative water content (RWC), proline and protein pattern are considered as markers for stress analysis. *Senna alexandrina* Mill. is drought tolerant plant. However, studies on biochemical factors and protein pattern during drought stress have not been carried out as per our knowledge. Therefore, the present study was framed to compare the biochemical and protein responses in leaf tissue of a control and a drought stressed plant of *Senna alexandrina* Mill.

MATERIALS AND METHODS

Experimental design of drought induction under Green House Condition

The *Senna alexxandrina* Mill. plant variety KKM (Se) 1, a drought tolerant cultivar from Tirunelveli, was procured from Agriculture College and Research Institute, Killikulam and local seed shops in Tirunelveli, Tamilnadu (Fig 1a). The same has been authenticated by the Botanical survey of India, Southern Region, Coimbatore.

Experimental studies were carried out on 30 days old healthy plants of *Senna alexandrina* Mill. The drought was induced in experimental plants for 21days duration by lowering the water level (Fig 1d & 1e).

Completely randomised design (CRD) was followed for the experiment with three replicates. The experiment consists 25 experimental units comprised of five treatments and five blocks (5 treatments x 5 blocks) (Fig 1g). Average of 3 blocks for each treatment is taken into account (Fig 1h). Before the experiment, field capacity of soil in pots was determined to apply different levels of drought stress. Field capacity has been defined as that amount of water which is held by soil against gravity [1]. Therefore, Field Capacity (%) = (Amount of Water (g)/Dry Soil Weight (g)) × 100. The amount of water held by the soil was calculated from the difference between dry and wet soil weight. Drought treatments were determined by using the amount of water held by the soil as follows:

Experiment outline [Lowering the water level [2]]

Treatment 1 (Control) : 100% amount of water held at field capacity.

Treatment 2: 75% amount of water held at field capacity.

Treatment 3: 50% amount of water held at field capacity.

Treatment 4: 25% amount of water held at field capacity

Treatment 5: 0% amount of water held at field capacity

During drought treatment, each pot was weighed daily at 8 a.m. and watered until the pot weight reached to pre-determined weight, which was calculated as follows;

$$P = Pf - (Wf\,(100-D)/100)$$

Where:

P = pot weight (g);

Pf = pot weight at field capacity (g),

Wf = amount of water held by a pot at field capacity (g);

D = drought level (%)

Statistical Analyses

Data were analysed statistically analysed using one-way analysis of variance (ANOVA). Data are presented as a Mean ± Standard error. The Mean separations were carried out using Duncan's multiple range tests and significance was determined at 5% level with Sigma Stat 3.5 (Systat Software Inc, California, USA).

Morphological Analysis

Number of Leaves

The leaves of the plant were counted by the average leaf number of the control plant. The data were obtained in 7, 14 and 21-day intervals during the drought stress treatment. The yellow colour leaves were not considered for the subsequent analysis.

Shoot length

Plant height was measured from the soil surface to the base of the first leaf of the main shoot the average height of a single plant was recorded in cm. The data were obtained in 7, 14 and 21-day intervals during the drought stress treatment.

Root Length

The root length was measured by sacrificing the plants on 7, 14 and 21 days of intervals during drought stress treatment. Plants were washed in water and the root length was measured in cm using a scale and thread. Five observations were made from randomly selected samples from each treatment and the average was found out.

Biomass

Fresh Weight

The uprooted entire plant was washed with water and by without damaging any part of the plant. Then using muslin cloth the excess water is removed completely [3]. The roots were weighed separately for each treatment and control in an electronic balance. The data were analysed and mean taken from five observations from each treatment of 21 days after the stress treatment.

Dry Weight

Dry weight was determined from the washed plants after drying at 70° C for 48 hours in a dry oven until a constant weight was obtained [4]. Relative water content was estimated as the difference between dry weight and fresh weight.

Biochemical Analysis

Estimation of Chlorophyll

The chlorophyll content was analysed by the method of Witham *et al.*, 1971 [5]. 100-150 mg of plant tissue was taken and homogenized in 1.5 ml of a cold solvent mixture of acetonitrile/methanol/water (72/8/1, v/v/v) by using cold mortar. The extract was centrifuged for 1 minute at 10,000x g. And diluted part of the supernatant ten times with acetone in the spectrophotometric cuvette and measured the absorbance of the solution at 661.6 nm and 644.8 nm. The values correspond to the absorption maxima of chlorophyll a and chlorophyll b in acetone respectively. Calculate the total chlorophyll (a+b) concentration using the formula:

Total chlorophyll (µg/ml) = 7.05A661.6 + 18.05A644.8

Calculate total chlorophyll (a+ b) content based on the leaf:

Total chlorophyll (mg/g FW) = d x V extr x (Chl a + b) / (1000 x LW)

Where,

LW = leaf weight (g)

V extr =Extract volume (ml)

d =dilution

Proline content

Freeze stored leaf tissue (0.5 g) was homogenized in 10 ml of 3% sulphosalicylic acid (Himedia, Mumbai, India) using pre-chilled mortar and pestle. The homogenate was filtered through Whatman No. 1 filter paper and the filtrate was used for the estimation of proline content. 2 ml of extract was reacted with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin reagent. The reaction mixture was kept in a water bath at 100°C for 1 hour to develop brick red colour. By placing the tube in an ice bath, the reaction was stopped. After cooling the reaction mixtures, 4 ml of toluene was added and mixed vigorously with a test tube stirrer for 15-20 sec then transferred to a separating funnel. After thorough mixing, the chromophore-containing upper toluene phase was aspirated from the lower aqueous phase, warmed to 30°C and the absorbance read at 520nm using UV-visible spectrophotometer against toluene (Qualigens, Mumbai, India) blank. The assay was performed with three replicates. The standard curve of proline was prepared. Free proline content in the sample was estimated by referring to a standard curve made from known concentrations of proline using the following formula [6]. The free proline content was expressed as µmoles g−1 FW of leaves.

Proline content µmol g−1 FW

$$\frac{\frac{\mu g of\ proline/ml \times ml of toluene}{115.5 \mu\ g/\mu\ mol}/g of sample}{5}$$

Estimation of Glycine –betaine

GB was determined with some modifications of Grieve and Grattan, 1983 [7]. 1 g of leaf sample was grounded in 10 ml of distilled water and filtered. The extracts were diluted 1:1 with 2 N H_2SO_4. Aliquots of 0.5 ml were put in test tubes, cooled in ice water for one h before a cold KI-I2 reagent (200 µl) was added. The tubes were stored at 0-4°C for 16 h and then centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was aspirated. The peridotite crystals were dissolved in 5 ml of 1,2-dichloroethane. After 2-2.5 h, the absorbance was

measured at 365 nm. Standard betaine corresponding to a concentration ranging between 0.2 and 1 mg m-1 was prepared.

DPPH radical-scavenging Assay

The DPPH radical scavenging by the test solution is analysed spectrophotometrically [8]. 50µl of sample extract in methanol was mixed with 450 µL Tris-HCL buffer (at 50mmol L-1, pH 7.4) and 1.0 ml DPPH (0.1mmol L-1 in methanol), the resultant absorbance was recorded at 517nm after 30 mins incubation at 37ºC in the dark. The percentage of incubation was calculated from the following equation:

$$\% \text{ of inhibition} = [(A0 - A1) / A0] \times 100$$

Where,

A0 - absorbance of the control (blank, without test solution)

A1 - absorbance in the presence of the test solution.

Proteolytic Activity

Leaf material (0.5 g FW), grounded in liquid nitrogen and was homogenized in 2.5 ml or 3 ml ice-cold 50 mM Tris-HCl buffer pH 7.5 containing 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM β-mercaptoethanol, 0.005% Triton X 100, 50 mg Polyclar AT and centrifuged at 14000 g for 4 min at 4ºC. Total soluble leaf protein content was measured [9]. Proteolytic activity was assayed spectrophotometrically using casein as a substrate. To the test samples, 5 ml of 0.65% casein pH 7.5, 1 ml of protease enzyme was added, mixed and incubated at 37ºC for 10 min. Then 5 ml of 110mM TCA was added.To the blank 5 ml of 0.65% casein pH 7.5, and incubated at 37ºC for 10 min. Then 5 ml of 110 mM TCA 1 ml of protease enzyme was added and incubated for 30 min at 37ºC. After incubation, the test samples and the blank were filtered through a 0.45µm filter. For the colour development of standard curve different concentrations of 1.1mM L-Tyrosine was used. Distilled water was added to make the final concentration of 2 ml. to this 5 ml of 500mM Sodium Carbonate and 1ml of folin & ciocalteu's phenol reagent was added. 5 ml of 500mM Sodium Carbonate and 1ml of folin & ciocalteu's phenol reagent was added 2ml test and 2ml blank filtrate. Mixed and incubated at 37ºCfor 30 mins. Absorbance was measured at 660nm.

$$\frac{units}{ml}\ enzyme = \frac{\mu moleoftyrosineequivalentsreleased \times 11}{1 \times 10 \times 3}$$

Where,

11= Total volume (in millilitres) of assay

10= Time of assay

1=Volume of the enzyme (in millilitres) used

3 = Volume (in millilitres) used in the colorimetric determination.

$$units/mg\,protein = \frac{Units/ml\,enzyme}{mg\,protein/ml\,enzyme}$$

Determination of Protein Content

Soluble proteins were measured by the method proposed by Bradford (1976). This method is based on the principle that protein Coomassie brilliant blue G-250 complex in the acid solution whose extinction coefficient (λ_{max}=595nm) is much greater than the free dye (λ_{max}=465nm). This dye binds strongly to a positively charged group of proteins and also to hydrophobic regions in the protein. The proteins were extracted using the method of [10]. To 20µl of protein samples, 180µl of distilled water was added to make up the volume to 200µl. To this 3ml of Bradford's reagent was added and incubated for 5 mins at room temperature. Absorbance was read at 595 nm. Different concentration of BSA was used as a standard. The concentration of total protein content was determined from a standard curve.

Extraction of Protein and SDS-PAGE

The protein for pattern analysis was extracted by Wu *et al.*, 2014 method [10]. 0.25g of leaf sample was ground in liquid nitrogen using a pestle and mortar. 10 ml of TCA / Acetone was added to homogenize the mixture. The mixture was centrifuged at 15000g x for 5minutes at 4ºC. The pellets obtained was resuspended in 1-1.5ml of cold TCA/Acetone and centrifuged at 15000g x for 5 minutes at 4ºC. The TCA/Acetone step was repeated until the pellet becomes white. The pellet was again resuspended with cold acetone and centrifuged at 15000g x for 5 minutes at 4ºC.the pellets are resuspended in SDS extraction buffer containing 1%SDS,0.15M Tris-HCl Ph8.8, 0.1M DTT,1mM EDTA,2mM PMSF, Protease inhibitor and 0.001% bromophenol blue and incubated at 65°C for 1hour. Then the tubes were centrifuged at 15000g x for 10 minutes at RT. To the supernatant equal volume of Tris buffered phenol was added and centrifuged at 15000g x for 5 minutes at RT. The phenol phase obtained after centrifugation was washed with wash buffer I and wash buffer II. After this, the phenol phase was mixed with 0.1M ammonium acetate in methanol to a total volume of 2 ml. This mixture

was kept at -20°c for overnight. Centrifuge the mixture at 15000g x for 5 minutes at 4°c.the protein pellets obtained was washed with 80% acetone and are stored at -20°C.

An aliquot of the extract was used to determine its protein content by using known concentration of BSA as standard [9]. Denaturing polyacrylamide gel was made with the different percentage of separating gel [11]. The separating gel containing 30% acrylamide mix [Acrylamide 29.2 g and Bisacrylamide 0.8 g], 375 mM Tris-HCl (pH 8.8), 0.05% (w/v) ammonium persulfate (APS) and 0.4 µl ml−1 TEMED was used for resolving the polypeptides, whereas a 5% stacking gel containing 30% acrylamide mix [Acrylamide 29.2 g and Bisacrylamide 0.8 g], 125 mM Tris-HCl (pH 6.8), 0.05% (w/v) APS and 0.5 µl ml−1 TEMED (Sigma, St. Louis, USA), was used to concentrate (stack) the polypeptides. The electrophoresis running buffer consisted of 25 mM Tris, 192 mM glycine and 0.1% SDS (pH 8.3) [Sigma, St. Louis, USA]. Electrophoresis was accomplished at 35 mA for 16 h at 4°C [12]; using a cooled vertical electrophoresis unit, model SE600, Hoefer®, Inc. (Holliston, Massachusetts(USA). The gels were stained with staining solution containing Coomassie blue R-250, Methanol and Glacial acetic acid for 20 minutes followed by destaining the gel in acetic acid /methanol (1:3).After complete destaining, the gel is stored in a solution containing 10% glacial acetic acid and 10% methanol. The photographic images of the gels were taken with the use of a Nikon colour digital camera P80. The net intensity quantification of protein patterns for all experiments was obtained using gel analyser 2010 Software.

RESULTS AND DISCUSSION

Morphological Parameters

Number of Leaves

The *Senna alexandrina* Mill. plant tolerated to the drought stress treatment. There is no much difference between control and stressed plant in 21 days stress treatment except the severe stress (100% drought stress). When the stress percentage increases the leaf number not reduced significantly (Fig.1e & Table.1). Only 30% of the leaf was able to emerge in the severe drought stress (100% drought stress) when compared to the control. There is no significant difference between the day interval (7, 14 & 21) except the severe stress (100% drought stress). The leaves were very rigid and healthy. Notably, the chlorophyll content got increased in the severe stresses.

Shoot length

Shoot length of the plant not reduced significantly when the drought stress increases. The length of the internodes has been reduced when compared to the control plant. Axillary branches were not able to see in the severe drought-stressed plants (75% and 100% drought stress) 35% of shoot length got reduced in the severe drought (Table 1) There is no significant difference between the day interval (7, 14 & 21) except the severe stress (75% and 100% drought stress) but we couldn't find any wilting even in the severe drought stress condition.

Root length

Root length of the treated plants showed significant difference with the control. Normally, during drought stress, there will be an increase in root length but on contrary *S.alexandrina* Mill. maintained its root length to 20-24 cm (Table 1) invariably to less, moderate and severe drought stress. But the numbers of auxiliary roots got increased during the stress which may help in getting more water from the surrounding. There is significant difference between the day interval (7, 14 & 21). The root has grown longer in 75% drought stress.

Fresh weight

S.alexandrina Mill plants showed 20% decrease in fresh biomass to control during the moderate stress (50% drought stress) and severe stress (75% drought stress), but 80% of fresh biomass was lost when compared to the control in the severe stress (100% drought stress) (Table 1). The plants were able to withstand up to 75% drought stress.

Dry weight

S.alexandrina Mill plants showed 50% water loss when compared to the fresh weight and dry weight in control plant this indicates that the plant has less water content. Whereas other plant may have more than 70 % of water content. When we see within the group there is no significant different between the fresh and dry weight with only the loss of 50% water weight. 20% decrease in dry biomass to control during the moderate stress (50% drought stress) and severe stress (75% drought stress), but 80% of dry biomass was lost when compared to the control in the severe stress (100% drought stress) (Table 1). The plants were able to withstand up to 75% drought stress.

Relative water content

S.alexandrina Mill plants showed 50 % of water loss as overall that is when we compare between the fresh and dry weight of same treatment almost all reads shows 50% decrease in weight. That indicates the Relative water content of *Senna alexandrina* Mill. is 50% of the fresh weight.

Biochemical Assays

Chlorophyll content

The total chlorophyll content was found to be increased significantly during the increase of drought stress. During the moderate stress (50% drought stress) and severe stress (75% drought stress) about 25% of chlorophyll content increased over control plant (Table.2). This is a new scenario and there is no such report of hike in the chlorophyll content due to abiotic stress that may be the effect of nitrogen metabolism and increased level of glutamic acid, a precursor of 5-aminolevulinic acid (ALA) involved in the biosynthesis pathway of chlorophyll.

Proline

The free proline content has significant difference among the drought stressed *S.alexandrina* Mill. There is a steady increase when the drought stress increases, in the severe drought stress (100% drought stress) 10 fold sudden increase was observed. Whereas the less, moderate and severe drought (75% drought stress) stress has free proline of 2.6 to 4.7 µmol g^{-1} FW and severe drought (100% drought stress) showed 47.011 µmol g^{-1} FW (Table 2). The less accumulation of proline up to 75% drought implies that the *S.alexandrina* Mill has the more drought tolerance capacity.

Glycine Betaine

Glycine betaine accumulation was high in *S.alexandrina* Mill. Control plant has a high content of 350 µg g^{-1} FW (Table 2) which indicates that the plant has more osmoprotectants which facilitate the drought tolerance. Whereas maximum of 80µmol g^{-1} DW in salt tolerant indica rice variety was observed[13]. Another report says that wild variety of cotton showed only 120 µmol g^{-1} DW in the control plant.[14] In our study, there is a significant change in the glycine betaine during the increased stress, but statistical analysis revealed that there is a small amount of increase only observed and stability was maintained among the less, moderate and severe drought stress.

DPPH Total antioxidant assay

Total antioxidant level and its inhibition activity were stable among the less, moderate and severe drought stress which indicates the stability of plant against the drought stress. In severe drought stress of 100%, the inhibition activity reduced to 49 from 87 of control plant (Table 2).

Proteolytic activity.

The proteolytic activity was increased significantly when the drought stress increases which indicates the increased activity of protease enzyme in clearing the unwanted proteins formed during the stress which is a protective effect to facilitate the drought tolerance capacity. There is 10units/ml increase in the less, moderate and severe stress (Table 2). *S.alexandrina* Mill did not show sudden hike or fluctuation suggesting the efficiency of drought tolerance.

Protein content

The total protein content got increased during the increase of drought stress. This correlates with the proteolytic activity. There is a 1µg of protein increase over the control plant (Table 2). The protein extraction method used in this study gave a good quantity and quality of protein. 50µg of protein were obtained from 0.1gm of leaf sample.

Protein pattern analysis by PAGE

The drought treated *S.alexandrina* Mill plant proteins were subjected to PAGE analysis and we were able to get 15 prominent bands that ranges between 3 to 250 kDa. (Fig 2 a)

During the increased levels of stress (25, 50, 75 &100%) the following proteins 70, 65, 40, 30, 13 and 3 kDa has significant decrease in expression on the contrary the 19, 15, 8 kDa proteins expression were increased. To separate different sized proteins, we used three different percentage gels, but the banding patterns were similar in all the three different percentage gels. The 12% gel gives better resolving and visibility (Fig 2 b). Among the highly expressed protein during high stress the 40 kDa protein quantity was high and it significantly reduced in the severe drought stress level of 75% and 100%. The 19 kDa and 8 kDa protein were increased highly when the stress is high (75% and 100%). The 19 kDa and 8 kDa may have a protective effect on the drought stress. It may involve in the drought tolerance mechanism. The protein has to be targeted and its functional studies may reveal more information on the drought tolerance mechanism in the plant *S.alexandrina* Mill.

CONCLUSION

The above experiments and assay revealed the drought tolerance capacity of *S.alexandrina* Mill. This plant showed prominent results, the morphological parameters, biochemical analysis, and protein analysis through PAGE confirmed that the plant has high drought tolerance capacity over the other plant systems. Still, many in-depth works have to be carried out to better understand and to find special mechanism involved in this plant. Some of the results were very different in this study as the chlorophyll content got increased during the stress level increases; the glycine betaine content was highly present in control plant itself which were new scenarios. As concluding remarks, this plant showed high drought tolerance to heavy drought stress. This plant *S.alexandrina* Mill has to be explored in transcriptomic and proteomic approaches to better understand the mechanism involved in drought tolerance to cater the other crops and plants susceptible to water.

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Table: 1. Effect of different levels of water induced drought stress on the growth of *Senna alexandrina* Mill. were evaluated by leaf number, shoot length, root length, fresh weight and dry weight. The data was recorded after 21 days of stress treatment.

TREATMENT (Water at Field Capacity)	Number of Leaves	Shoot Length (cm)	Root Length (cm)	Fresh Weight of plant (g)	Dry Weight of plant (g)
100%	39.333 ± 0.667^{a}	18.008 ± 0.295^{a}	24.906 ± 0.077^{a}	0.820 ± 0.012^{a}	0.443 ± 0.006^{a}
75%	$38.000 \pm 1.155^{a*}$	17.434 ± 0.302^{a}	$24.130 \pm 0.086^{b*}$	0.791 ± 0.013^{a}	0.423 ± 0.013^{a}
50%	$36.000 \pm 1.155^{b*}$	17.333 ± 0.290^{a}	$24.026 \pm 0.127^{c*}$	$0.601 \pm 0.007^{b*}$	$0.354 \pm 0.007^{b*}$
25%	$35.333 \pm 0.667^{c*}$	9.948 ± 0.149^{b}	20.471 ± 0.274^{d}	$0.571 \pm 0.008^{c*}$	$0.357 \pm 0.007^{c*}$
0%	12.667 ± 0.667^{d}	3.804 ± 0.140^{c}	13.110 ± 0.402^{e}	0.341 ± 0.007^{d}	0.135 ± 0.006^{d}

Data are Mean ± standard error of 3 independent replicates with 5 plants for each replicate of each treatment. Values followed by the same letters and symbols within a column are not significantly different at 5 % level according to Duncan's multiple range tests.

Table: 2. Biochemical analysis on different levels of water induced drought stress in *Senna alexandrina* Mill. were evaluated after 21 days of stress treatment.

TREATMENT (Water at Field Capacity)	Chlorophyll ($mg^{-1}g$ FW)	Glycine Betaine (µg of Glycine Betaine g^{-1} FW)	Proline ($\mu mol\ g^{-1}$ FW)	DPPH (% of Inhibition)	Proteolytic Activity units/ml enzyme	Protein content (in µg)
100%	0.653 ± 0.002[a]	350.8 ± 1.514[a]	2.570 ± 0.115[a*]	87.747 ± 0.247[a]	105.980 ± 0.748[a]	4.883±0.042[a]
75%	0.702 ± 0.004[b]	338.0 ± 4.214[b*]	2.827 ± 0.093[b*]	86.993± 0.498[a]	116.257 ± 1.959[b]	5.250±0.049[b]
50%	0.850 ± 0.007[c]	333.7 ± 3.697[c*]	3.017 ± 0.187[c*]	87.913 ± 0.290[a]	123.493 ± 1.250[c]	4.716±0.043[a]
25%	0.879 ± 0.009[d]	326.9 ± 4.516[d*]	4.730 ± 0.083[d]	84.327 ± 0.433[c]	133.817 ± 1.433[d]	5.348±0.031[c]
0%	0.633 ± 0.007[a]	305.0 ± 4.649[e]	41.343 ± 0.462[e]	49.227 ± 0.327[b]	141.150 ± 1.055[e]	6.312±0.025[d]

Data are Mean ± standard error of 3 independent replicates with 5 plants for each replicate of each treatment. Values followed by the same letters within a column are not significantly different at 5 % level according to Duncan's multiple range tests.

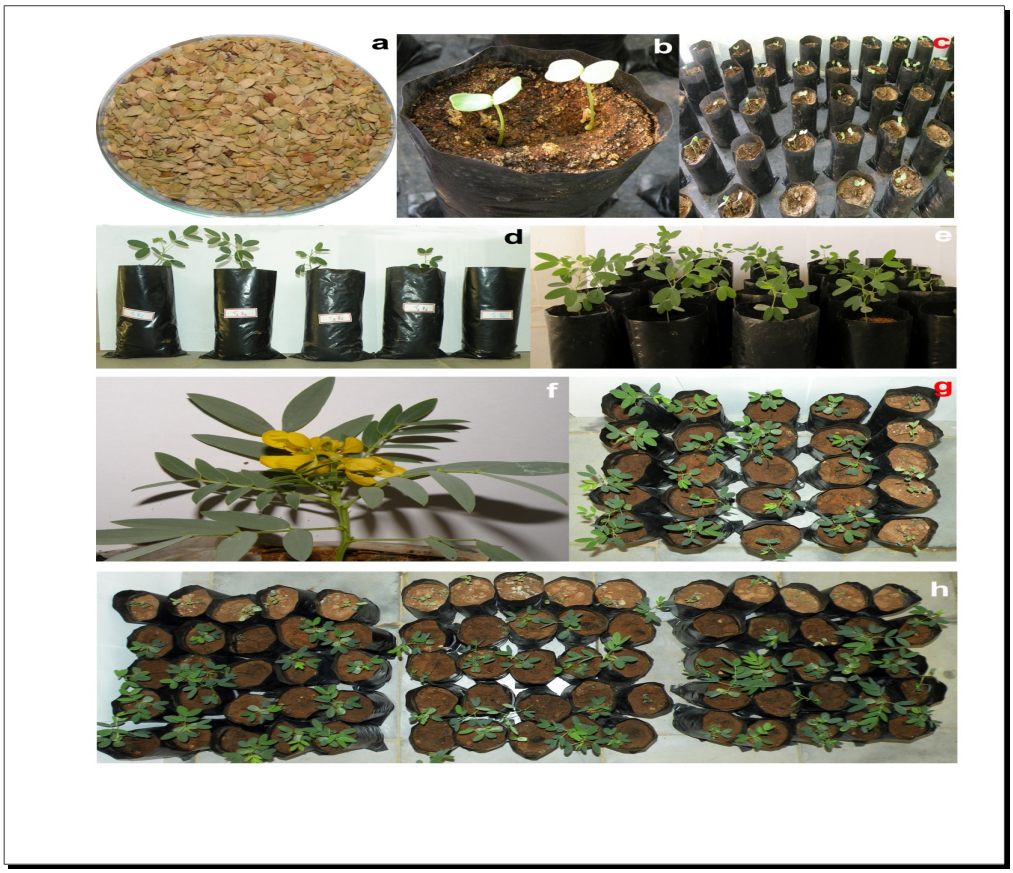

Fig.1. Experimental setup of *S.alexandrina* Mill. for drought stress treatment **a.** Seed; **b&c**. Seedlings; **d.** Different treatment levels viz., 0%, 25%, 50%, 75% and 100% drought stress; **e&g**. Experimental unit consist of 5 plants for each treatment and 5 blocks totally 25 plants; **f.** Plant with flower; **h.** 3 experimental units were used for the whole study with 75 plants in RCD (Randomised Complete Design).

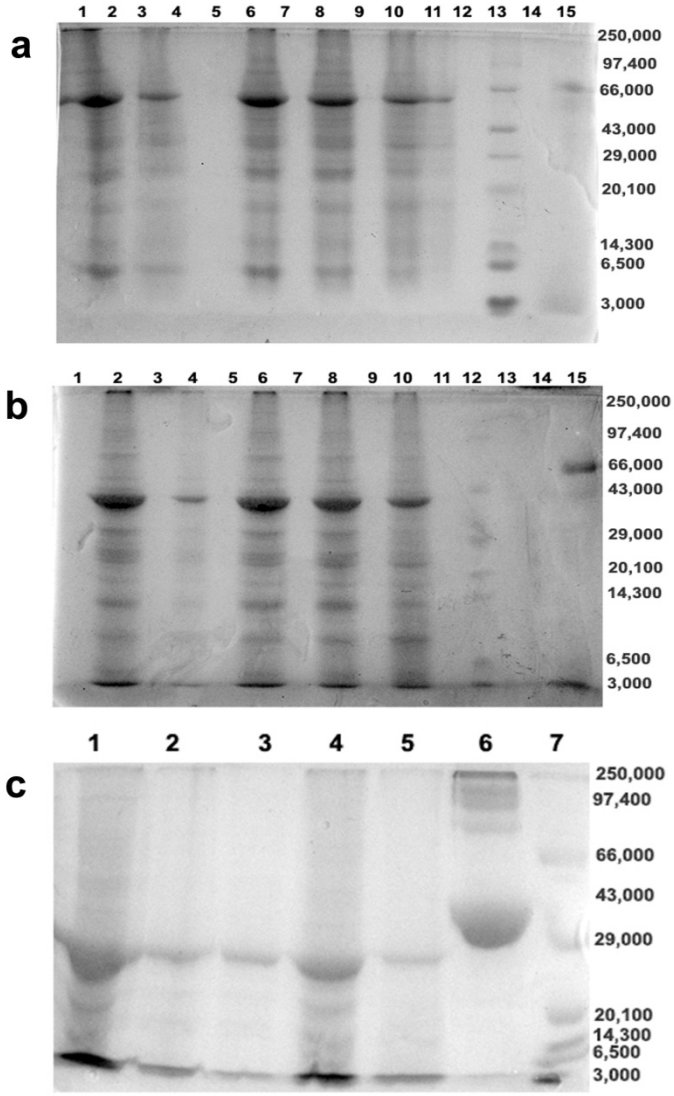

Fig 2 Protein pattern analysis of *S. alexandrina* Mill under drought stress treatment. **a.** 15 % PAGE gel with samples T1,T2,T3,T4,T5, Protein Marker and BSA standard in the lanes 1,3,6,8,10, 13,15 respectively. Lane 2, 4, 5, 7, 9, 11, 12, 14 are blank. **b.** 12% PAGE gel with samples T1,T2,T3,T4,T5, Protein Marker and BSA standard in the lanes 2,4,6,8,10,12 & 15 respectively. Lane 1,3,5,7,9,11,13 are blank. **c.** 10% PAGE gel with samples T1,T2,T3,T4,T5, Protein Marker and BSA standard in the lanes 1,2,3,4,5,6,&7 respectively. Protein Marker: 3KDa to 250Kda.