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SHORT COMMUNICATION

Effect of salinity stress on seed germination of *Leucaena leucocephala: a multipurpose tree*

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Leucaena leucocephala is a highly drought and salinity tolerant plant species tolerating as high temperature as 48°C (Hocking, 1993). It is a species of small Mimosoid tree that is native to southern Mexico and northern Central America but now naturalized throughout the tropics. In India, it is also known as *Subabul* and grown for multiple uses, such as green manure, charcoal making, hedge plants besides being widely used in agro-forestry system and wind break plantation for its tremendous ability to survive amidst a wide range of environment and soil factors .Except being high in sodium and iodine content, it is a balanced livestock fodder that provides supplemental protein along with minerals in tropical areas. Its leaves have high nutritive fodder for live stock thriving in arid regions. Plant is used for soil conservation and crop intensification (Shelton and Brewbaker,1994). It is fast growing leguminous, nitrogen fixing multipurpose plant species (Halliday and Somasegaran, 1983).

The impact of various abiotic stresses in plants is important for understanding their physiology, ecology and to improve crop production under stress. Soil salinity is one of the most serious limiting factors for growth and production of plants in the arid regions. Salts are natural component of soil and salinity in soil comes from weathering of minerals, addition of inorganic fertilizers, manures and irrigation water. At present,11.22 lakh ha land is affected by salinity and sodicity in the state comprising districts of Pali, Jodhpur, Barmer, Bikaner, Ajmer, Nagaur, Sri Ganganagar and Hanumangarh (Verma *et al.*,2005). An excess of salt inhibits metabolic processes including protein synthesis. The toxic ions (Na^+ and Cl^-) interfere in balanced absorption of essential nutritional ions (Tester and Davenport, 2003) and with the acquisition of nitrates. Salinity stress adversely affects seed germination(Berrichi *et al.*, 2010). It causes growth inhibition, senescence and death of plant during prolonged exposure or extreme salinity shock. Effect of salinity is more realized by a plant in a particular stage of growth. A vast area of the western border of the India is protected by the Rajasthan's arid region, which is primarily barren. To provide a counterside of camouflage *Leucaena leucocephala* is considered a good alternate for the problem, for its ability to invade the clear wasteland areas forming dense thick covers, fast growth and weedy properties. Soil as well as water salinity is common in arid region, which inhibits growth and development in many plants. The EC of ground water of border districts of Rajasthan varies from 3.0 to 9.0dSm^{-1} and saline water is more problem in western Rajasthan (Verma *et al*, 2005).

Keeping in view, the fast growth of *subabul* over the slow growing indigenous plant species such as ker, babul and khejri, a study was undertaken to investigate the effect of different concentration of NaCl on seed germination, growth, physiological and biochemical responses of *Subabul*.

The experiment was conducted at DRDO Laboratory, Jodhpur during the year 2009 to investigate the effect of diffent concentration of NaCl on seed germination, growth and development of plant. Uniform size seeds of *Leucaena leucocephala* were randomly taken and washed with tap water, air dried at room temperature and then stored at 4°C before being used. Seeds were scarified by rinsing them in hot water at 75°C for 10 minutes, followed by concentrated sulphuric acid washing for 10 minutes. Seeds are then rinsed with distilled water. The experiment was laid out in completely randomized design (CRD) with 3 treatments having 3 replication each having 15 seeds. Seeds were salinized in petri dishes with moistened cotton of varying concentrations of 50 mM, 100 mM and 200 mM sodium chloride (NaCl) along with a suitable control (only distilled water). Petri dishes were kept in a temperature controlled incubator for germination of seeds. Number of seeds germinated were observed and noted everyday till seventh day. The final germination percentage was calculated according to the equation described by Elis and Roberts (1981).

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds tested}} \times 100$$

For biochemical study, plantlets were uprooted carefully after seven days and were washed with distilled water. After recording length of shoot and root of each plantlet, were separated into root, shoot and cotyledon. Data for the following parameters were also recorded.

Growth analysis of germinated seeds

Plants germinated out of ten were observed for

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length of the root, shoot and cotyledon for different salt concentration for 7 days after which they were oven dried at 70°C for 4 days for recording dry weight.

Sample preparation for enzyme extraction

The required quantity (1 gm) each of *subabul* (*Leucaena leucocephala*) roots, shoots, cotyledons were separately crushed in prechilled mortar- pestle with acid washed sand in 1 ml Tris-HCl and centrifuged at 10,000 rpm for 10 mins. The supernatant was used for biochemical estimation of protein, peroxidase, acid phosphatase and proline activity.

Estimation of protein

Quantitative Estimation of Total Soluble Protein Using Bradford's Dye Binding Method (Bradford, 1976). The series for sample was made by taking sample from 0.1 to 1 ml. Volume was made to 1ml by Tris-Cl and 3ml dye was added in each tube. A blank was also prepared containing only Tris- Cl and CBBG dye. OD at 595 nm was recorded. Readings in the standard curve were marked and the amount of protein present was calculated.

Estimation of Peroxidase Activity

Enzyme was extracted as described in sample preparation and peroxidase was estimated using H_2O_2 oxidoreductase method (Putter, 1974). Series was made by taking 0.1ml sample extract, 3 ml Tris-HCL, 0.05 ml Guaiacol (20 mM), 0.5 ml H_2O_2 (1%). The assay mixture was shaken and then optical density at 436nm was taken.

Estimation of acid phosphatase

Enzyme was extracted as described in sample preparation. Series was made by taking 0.1ml sample extract and the volume was made upto 2 ml by adding reaction mixture, 2 ml sodium bicarbonate is also added. Tubes were shaken and incubated for 10 minutes. The optical density at 495nm was taken.

Estimation of proline

Free proline was estimated as per the method of Bates *al.* (1973). Enzyme was extracted as described in sample preparation. 0.1to 1ml of standard proline was taken and made up to 1ml by acetic acid. 1ml of Ninhydrin was added into it. The mixture was heated at 100°C. OD at 520nm was taken to draw a standard curve. 100µl of sample (shoot and roots extract) was taken in a vial. Then 1ml of Ninhydrin was added and volume was made to 2ml by glacial acetic acid. Heated for 1hour at 100°C and then the reaction was terminated in an ice bath. The reaction mixture was extracted with 1ml toluene (to stop the reaction). The chromophore containing toluene was warmed at room temperature (RT) and its OD was measured at 410 nm.

SDS-PAGE- Protein sample were resolved on SDS-PAGE as described by Laemmli (1970) and bands were stained with CBBG R - 250 dye in 50% methanol and 10% acetic acid solution and destained by frequently changing the same solution but without dye. The protein bands acquired blue color after sufficient staining and destaining procedure.

Native PAGE For detection of activity of isoenzyme-peroxidase native PAGE was carried out (without SDS) on 7% separating and 4% stacking gel. After the suitable migration of enzyme bands, they were incubated in benzidine solution for 2 minutes for the bands to turn brown. The gel was immediately transferred to distilled water to stop the reaction. The gel was then photographed (Fig. 7).

Seed germination

The data obtained on seed germination under different concentration of NaCl is presented in Table 1. Perusal of data on seed germination reveals that in control the seed germination was to the tune of 93.33% which dropped to 80.00% in 50 mM NaCl solution. Further increase in NaCl concentration affected the seed germination drastically and dropped to 53.33% at 100 mM and 0.06% at 200 mM. The results revealed that germination of seeds can be achieved upto 50 mM NaCl solution. The results are in line with these reported earlier in by Midaoui *et. al.* (2002).

Morphological parameters

After 7 days treatment of various concentrations of NaCl, the length of roots, shoots and cotyledons gradually decreased in *Leucaena leucocephala. Perusal of data in Fig. 1 reveals that the magnitude of shoot, root and cotyledon was maximum under control conditions. Subjecting the seeds to NaCl treatment demonstrated drop in magnitude of these parameters. This is illustrated by the fact that when seeds were subjected to the treatment of 50 mM NaCl solution, the magnitude of root, shoot and cotyledon was* from 8.4 cm to 2.8 cm, from 6.2 cm to 1.5 cm and from 5.2 cm to 1.9 cm respectively, 0.4 cm, 0.3 and 0.3 respectively when seeds were subjected to treatment 200 mM NaCl solution suggesting the toxic effects due to prolonged salt stress (Table 1).

The data on peroxidase activity in root, shoot and cotyledon at different concentration of NaCl is presented in Fig. 2. Perusal of data reveals that at 50 mM NaCl, the peroxidase activity was at par in all the three parts of the seedling. However, at 100 mM NaCl treatment the peroxidase activity in root and shoot are at par with those at 50 mM NaCl, but peroxidase activity is very high in cotyledon. Further, increase in NaCl concentration to 200 mM demonstrated increase in peroxidase activity in all the three parts of seedlings. The increase in peroxidase activity in cotyledon at 7 days after sowing may be on account of fact that this is the main center of biochemical activities and hence to protect the vital enzymes, there is an increase in antioxidant enzyme so that the metabolic activities may be performed at optimal rate. Similar findings have been reported by Bybordi *et.al.*(2010).

The data on proline content under different treatment in NaCl presented in Fig. 3. of data reveals that

proline content at 50mM treatment was lowest in roots, shoots and cotyledon. This increase slight at 100 mM but increased drastically at 200 mM. Accumulation of proline in response to stress has been reported from time to time. In the present study also accumulation of proline has been demonstrated which may be an adaptive mechanism to keep the pace of metabolic activity in the germinating seeds. Similar results have also been reported by Tyagi *et al.* (1999) in *Lathyrus*.

The data on acid phosphatase activity (Fig. 4) also revealed that at 50mM treatment, the acid phosphatase activity also high in root and lowest in cotyledon. However, at 100 mM NaCl treatment, it become at par in all the three parts, but at 200 mM concentration the maximum acid phosphatase activity was recorded in root followed by shoot and least in cotyledon. The increase in acid phosphatase activity in seedlings may be in order to metabolize the Pi in the plant system or may be due to starvation of plants in salinity stress which leads to *de novo* system of acid phosphatase in intra and extra cellular portion (Stephen *et. al.*, 1994).

The data on protein content in root, shoot and cotyledon at different concentration of NaCl demonstrated that total soluble protein drastically decreased from 70 µg/mg sample at 50mM to 36 µg/mg sample at 200mM NaCl, 66 µg/mg sample to 36 µg/mg sample and 64 µg/mg sample to 49 µg/mg sample in root, shoot and cotyledon of seedling respectively. However, at 50 mM NaCl, the root shows highest concentration of protein followed by shoot and least in root and shoot at 200 mM. Results illustrated that plant is able to maintain the protein biosynthesis at lower concentration of NaCl but at higher concentrations of NaCl the protein synthesis is hampered.

Thus, from the foregoing account, it can be concluded that during salinity stress, the physiological processes in the plant are affected as the proteins and acid phosphatase level decreases while there is a substantial increase in the level and activity of proline and isoenzyme peroxidase. The role of these enzymes can be attributed in a plant's defence response against abiotic (salinity) stress and can act as a stress marker in them. Higher salt concentration is also detrimental to the growth and germination of seedling.

Table 1: Germination percentage on different concentration of NaCl after 7 days.

S.No.	Conc. of NaCl (mM)	No. of seed germinated	Germination (%)
1.	Control	14	93.33
2.	50	12	80.00
3.	100	8	53.33
4.	200	1	0.066

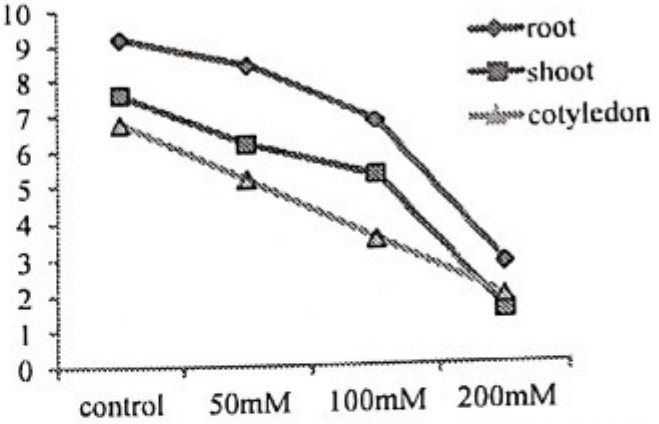

Fig. 1. Effect of various concentration of NaCl on roots, shoots and cotyledons (Y axis- length in cms, X axis- various NaCl concentrations)

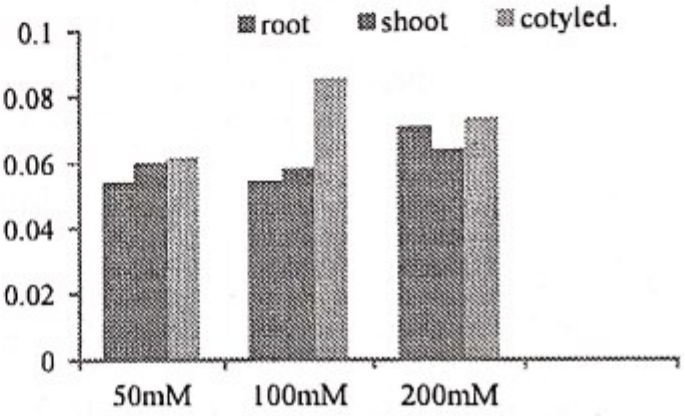

Fig. 2. Peroxidase activity [Y axis- absorbance at 436 nm, X axis- molarity of NaCl concentration]

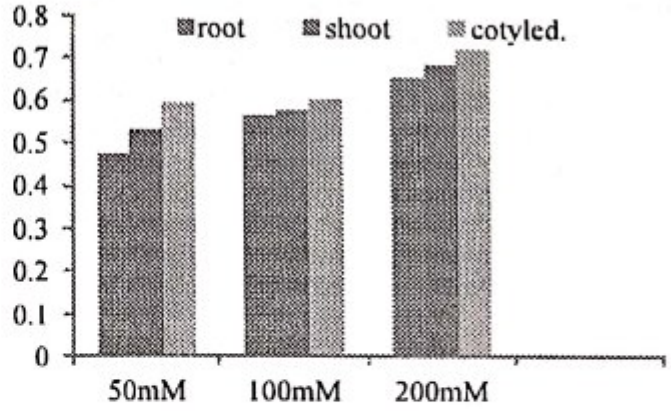

Fig.3. Proline activity (Y axis- absorbance at 520 nm, X axis- molarity of NaCl concentration)

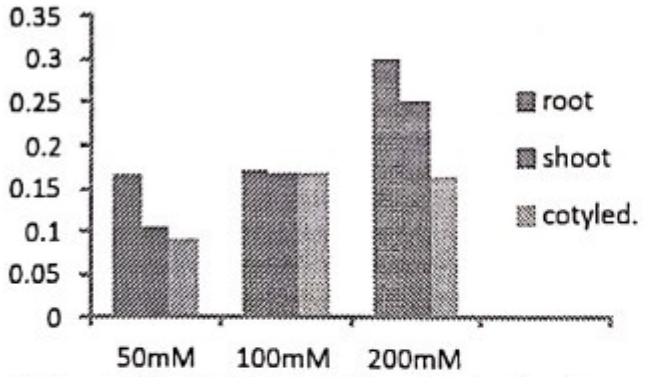

Fig. 4. Acid phosphatase activity (Y axis- absorbance at 495 nm, X axis- molarity of NaCl concentration)

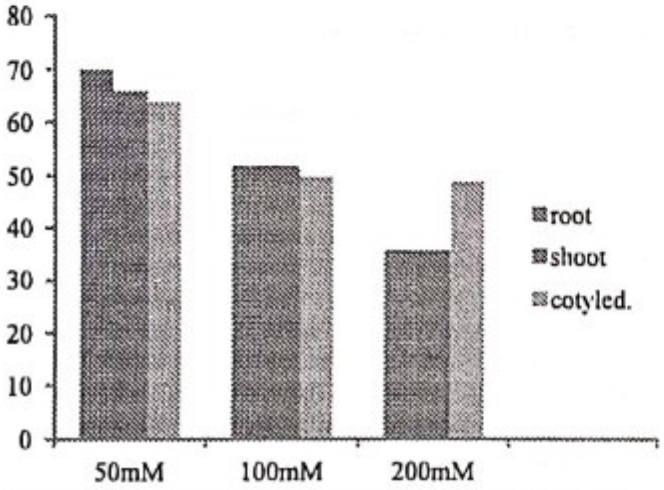

Fig. 5. Total soluble protein (Y axis- Protein µg/mg, X axis-molarity of NaCl concentration)

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