Salt tolerance screening of Bamboo genotypes (bamboo sps.) using growth and organic osmolytes accumulation as effective indicators

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Abstract

Bamboo is known as wonder grass due to its rapid growth, high economic value and varied applications. In this study, three bamboo species namely *Dendrocalamus strictus (S1)*, *Dendrocalamus longispathus* (S2) and *Bambusa bambos*(S3)were investigatedfor salinity tolerance in hydroponic culture system. The vegetative growth parameters; shoot height (SH), root height (RH), number of leaves (NL) & fresh weight (FW) were monitored after 14 days of salt treatment; and found not significantly affected or slightly reduced at higher salt concentrations in case of S3 and S1 species whereas in case of S2 species all the growth parameters were found retarded with increase in NaCl showing salt sensitiveness in this species. Proline accumulation was conspicuous than glycine betaine (GB) in the salt tolerant species. Salt susceptible S2 species treated at 100mM NaCl had, 7.57 and 22.85 folds higher proline than that of S1 and S3 species treated under similar concentration. Whereas, GB content showed a completely different trend with increasing NaCl concentration, its concentration did not vary significantly in S2 species but was found to decrease in S3 species and increased in S1 species. Co-relating the different parameters; S3 and S1 species were identified as salt tolerant while S2 species was classified as salt sensitive.

Keywords: salinity, bamboo, growth parameters, photosynthetic pigments, osmolytes

Theme: Propagation, Plantations & Management

Abbreviations: SL - Shoot length, RL - Root length, NL - Number of leaves, FW- Fresh weight, GB- Glycine betaine, S1- *Dendrocalamus strictus,* S2-*Dendrocalamus longispathus,* S3- *Bambusa bambos*

Introduction

Detrimental effect of salinity stress on plants' growth and development is one of the major concerns of plant scientists and environmentalists. It is the most prevalent form of abiotic stress among the various other forms such as drought, temperature, heavy metals, heat and chemical toxicity all of which bring about oxidative stress in plant. Soil salinization is increasing day by day due to various natural and anthropogenic activities. These activities lead to accumulation of harmful salts in soil such as sodium chloride (NaCl), calcium chloride ($CaCl_2$), sodium sulfate (Na_2SO_4), and magnesium sulfate ($MgSO_4$). These salts easily dissolve in water and release free cations such as Na^+, Ca^{2+}, and Mg^{2+} and anions like Cl^- and SO_4^{2-}. These ions accumulate within the plant cells and cause deleterious effects by creating water potential gradient that makes plants incapable of absorbing soil water (water stress). Salt stress also causes nutritional imbalance in plant (Ashraf 1994; Zhu et al. 2003; Turan et al. 2010). Sodium is the major absorbed cation in most of the saline soils (Marschner 1995) which are not essential for plant and becomes stressor for plant growth and development, ratherNa^+affects nutrient utilization by the plants (Rego et al. 2011). To overcome this problem, screening of salt tolerant plant varieties that could grow in saline areas is essential for ecorestoration and meaningful utilization of such waste lands and waste water. Bhutta et al. (2004) described salt tolerance as the ability of the plant species to grow under such stressful conditions without much penalty in the net productivity of the plant species. Growing of halophytes, which generally have less biomass is not an ideal solution to deal with soil salinity (Ravindran et al. 2007; Qadir and Khan 2008). Plantation of tree species having inherent ability to cope up with the stress is the best choice albeit; availability of such species is an R&D challenge.

Many plants accumulate organic and inorganic osmolytes such as proline, glycine betaine, mannitol, trehalose (Heidari et al. 2011; Su et al. 2011) that help in osmoregulation, maintaining the membrane integrity, enzyme activity and scavenging of reactive oxygen species. Thus, understanding the mechanism adopted by various plant systems will help in screening of the salt tolerant species and also give an overall concept to further enhance the tolerance capacity. Screening for salt tolerance has been carried out in woody genera like Pinus, Eucalyptus, Larix, Fraxinus, Acer, Olea, Quercus, Sequoia, Casuarina in which synthesis of appreciable amounts of polyols, amino acids, sugars and quarternary amines have been reported (Nguyen &Lamant 1988;Gucci et al. 1997; Popp et al. 1997; Cha-um et al. 2013).

Various studies have also been carried out to assess the detrimental effects of NaCl on the growth of other plant species such as populus (Gebre et al., 1998), wheat (Bashiti et al., 2005), cotton (Sarwar et al., 2006), sunflower (Heidari et al., 2011) , eucalyptus (cha-um et al., 2013). However similar studies conducted on bamboo species are meagre. Bamboo is one of fast growing highly desirable tree species with greater economic benefits having thousands of species with wide applications. It is one of the highly efficient tree having around 1500 known species under 87 genera (Ohrnberger 1999); with various applications for furniture making, pole construction, paper making, handicrafts, edible shoots, jewelry making, potential source in essential oils and medicines(Ganapalhy et al. 1995) . Bamboo is the strongest and fastest growing grass on earth due to its unique rhizome dependent growth pattern (Assaye et al. 2014). It has a great potential as a social forestry plant. India is in second position (after China) in having natural bamboo resources and many species growing as natural habitats among which *Dendrocalamus strictus, Dendrocalamus longispathus &Bambusa bambos* are found to be growing in larger areas.

This study was carried out to understand the salinity tolerance performance of three bamboo species; *Dendrocalamus strictus, Dendrocalamus longispathus &Bambusa bambos*by treating them at two different NaCl concentrations i.e. 50mM & 100mM for a period of 14 days. The aim of the study was to identify the salt tolerant variety among these three species based on two different parameters viz. morphological (SL, RL, NL, FW) and accumulation organic(Proline,GB) osmolytes.As there is a dearth of research on understanding the salt tolerance of bamboo species, the result of this study would not only help in identifying the salt tolerant variety, but also aid in understanding the inherent salt tolerance mechanisms evolved in bamboo species to combat the stressful conditions.

2. Materials & Methods

2.1.Plant Materials & Growth Conditions

Seeds of 9 bamboo species were collected from various Forestry Departments in India. Among the various species; seeds of *D. strictus, B. bambos, D. longispathus*used for the present study were collected from Maharashtra Forestry Department, Nagpur. Seedlings were developed in glasshouse at 32± 2^0C, 70±10 % relative humidity (RH) and natural day night cycle. One month old seedlings of 3~ 4cm height were chosen to conduct the stress treatment studies in hydroponicscondition at Environmental Biotechnology Division, CSIR-NEERI, India.

2.2. Experimental setup & Salt treatment in Hydroponics

The seedlings were removed carefully from soil; roots were washed with tap water before treatment in Hoagland solution (Figure 1). Prior to salt treatments, seedlings were acclimatized in Hoagland nutrient solutions for seven days. Thereafter, they were treated with three NaCl concentrations i.e. 0, 50 &100mM in 20% strength Hoagland nutrient solution. The pH of the solutions was maintained in the range of 5.7~5.8. For each concentration one bamboo seedling was placed in one tube with 50ml solution and was allowed to grow upto 14 days. The tubes were arranged in a tray using completely randomized block design with replicates for each treatment and were regularly made up with normal distilled water. The stress treatment was carried out for a period of 14 days. Growth and yield parameters (SL, RL, NL& FW) and accumulation of organic osmolytes (proline and GB) were determined in the treated plants in comparison to control (no salt). Comparative analysis of the parameters and their interactions were carried out among the three species with respect to control to assess their salt tolerance and determine the best suitable variety.

2.3. Measurement of growth parameters

After 2 weeks of salt treatment plants were harvested for growth measurements. SL, RL, NL and FW were measured manually. The shoot and root length was determined using meter rule and number of leaf by visual counting while the FWwas determined by using weighing balance (Schimadzu). .

2.4. Organic Osmolytes Determination

2.4.1. Proline Estimation

Free proline content was measured according to the method of Bates et al. (1973). Approximately, 0.25g of fresh leaf samples were homogenized with 3% sulphosalicylic acid and centrifuged to remove the residue, 2ml of leaf extract (supernatant) was boiled in water bath at 100ºC for 1hour after adding 2ml of ninhydrin and 2ml of glacial acetic acid, after which 4ml of cold toluene was added. Absorbance of the toluene layer was measured by UV- Visible spectrophotometer (Shimadzu UV-1800) at 520nm. Proline content was calculated using the formula: (µg proline in extract/115.5)/g sample = µmoles proline g-1 FW.

2.4.2. Glycine betaine Measurement

Glycine betaine content was estimated in the leaf samples by method described by Carillo et al. (2011). About 40- 50mg samples were frozen in liquid nitrogen, grinded using prechilled mortar and pestle. The finely ground powderwas transferred to precooled 1.5ml eppendorf tubes and was suspended in 1ml Milli Q water. This sample-water mixture was subjected to freeze thaw cycle by freezing in liquid nitrogen and thawing at 40^{o}C for 20 minutes, and left overnight at 4^{o}C. The extract was centrifuged at 14000g for 5mins. at 4^{o}C, clear supernatants was separated from pellets and was used for GB estimation.

Glycine betaine content was determined by HPLC (WATERS HPLC), approximately 20-25µl extract was injected into ODS2 C18 column (250 mm X 4.6 mm internal diameter) preceded by a guard column (10 X 1mm) packed with same phase. The mobile phase was composed of 13mM sodium heptane sulphonate and 5mM sodium sulphate in milliQ grade water with pH adjusted to 3.7 using H_2SO_4 at a flow rate of 0.8ml/min in isocratic mode for a run time of 10min. The eluted glycine betaine was detected by measuring the absorbance at 200nm using PDA detector. Glycine betaine was quantified by comparing sample peak areas with standard solutions ranging from 0.05-4mM prepared in MilliQ water.

2.5. Statistical Analysis

The experimental data were analyzed using one way Analysis of Variance (ANOVA) of Agres statistical software package version 3.01(Agres 1994). The Least significant difference test (LSD) was performed to compare the mean values and separate the groups when ANOVAs were significant at $p \leq 0.05$.

3. Results

The treatment set up of bamboo seedlings were maintained in glass house and monitored periodically as presented in Figure 1a, b and c.

Figure 1

(a) Dendrocalamus strictus(S1)

(b) Dendrocalamus longispathus(S2)

(c) Bambusa bambos(S3)

Table 1.Statistical significance of all morphological parameters and osmolytes content among the three species (S), treatments (T) and their interaction (S*T).

Parameters studied	Statistical significance of variations in means		
	S (Variations	T (Variations in	S X T (Variations in

		in mean data among species)	mean data among the treatments)	Mean data among the interaction of species to treatment)
Vegetative growth parameters	SL (cm)	**	**	**
	RL (cm)	**	**	NS
	NL	**	**	*
	FW (mg/plant)	**	**	**
Osmolytes	Proline content (µmol.g^{-1})	**	**	**
	GB Content (µmol.g^{-1})	*	NS	**
	**	*P*≤0.01	Highly significant	
	*	*P*≤0.05	Significant	
	NS		Not significant	

3.1. Growth Parameters

Analysis of variance has showed significant difference in all the treatments (Table 1). SL in all the three species was found to decrease with increasing NaCl concentration. In species S1 species,SL was significantly affected by increase in salt concentrations. Like there was 100% reduction in SL in case of species S2 in comparison to control. There was no increase in SL for the period of stress treatment in species S2. Similarly, the SL of S3 species also decreased as NaCl concentration increased and the effect was pronounced in T3 concentration (100mM), while T2 (50mM) and control SLs were not significantly different from each other. The RL in response to different salt treatments showed that salinity stress has considerable effect on RL of S1 and S2 species. The RL was found to decrease with increase in salt concentrations. The treatment concentrations also showed significant mean variations irrespective of species. However, in the case of species S3, it showed highest mean RL irrespective of treatments (Table 2), which infers that it has capability of producing lengthy roots which is one of the desirable trait in salinity tolerance. Number of leaves which is another important parameter for assessing salinity stress tolerance showed a significant trend. There was general reduction in the NL in response to salt concentration, but this response was varied among the different species. The S3 species performed better under T2 concentration with only 5% mean reduction in comparison to control plants whereas at T3 concentration, there was 73% reduction in NL which was 1.46 folds greater than that of S1 species grown in the same concentration(50% mean reduction). In case of S2species in both T1 & T2 treatment

concentrations, growth performances with respect to all parameters were retarded which was also similar in case of control. This indicate the growth inferiority in this species in control as well as treated plants, which needs further investigation to justify and draw a logical conclusion.

Table 2. The vegetative growth parameters under different NaCl concentrations in different bamboo species

Species	NaCl(mM)	SL(cm)	RL(cm)	NL	FW(mg)
D.strictus	0	12.17±0.29 a	4.3±2.1	6±1a	567±167a
	50	5±1b(59)	1±0(77)	4±1b (33)	507±82b(11)
	100	4.17±0.29c (66)	1±0(77)	3±1b (50)	307±94b(46)
D.longispathus	0	0.5±0d	1.06±0.1	0±0c	152±4d
	50	0±0e(100)	0.5±0(53)	0±0c(0)	127.2±34d(16.31)
	100	0±0e(100)	0±0(100)	0±0c(0)	92±9d(39.4)
B.bambos	0	5.7±1.04 b	7.5±0	6.3±0.6a	587±17b
	50	5.8±0.8b (1.7)	6.3±0.6 (16)	6±2a(5)	347±12b(41)
	100	1.3±0.3d (77)	3.2±1.3 (57)	1.7±0.6b (73)	193±11c (67)

Legends: SL (SL), RL(Root length), NL (Number of leaves), FW (Fresh weight/plant), RWC (Relative water content) respectively. Values in parenthesis are the mean reduction (% control) of growth parameters. Values represented by similar letters are not significantly different from each other ($p \leq 0.05$).

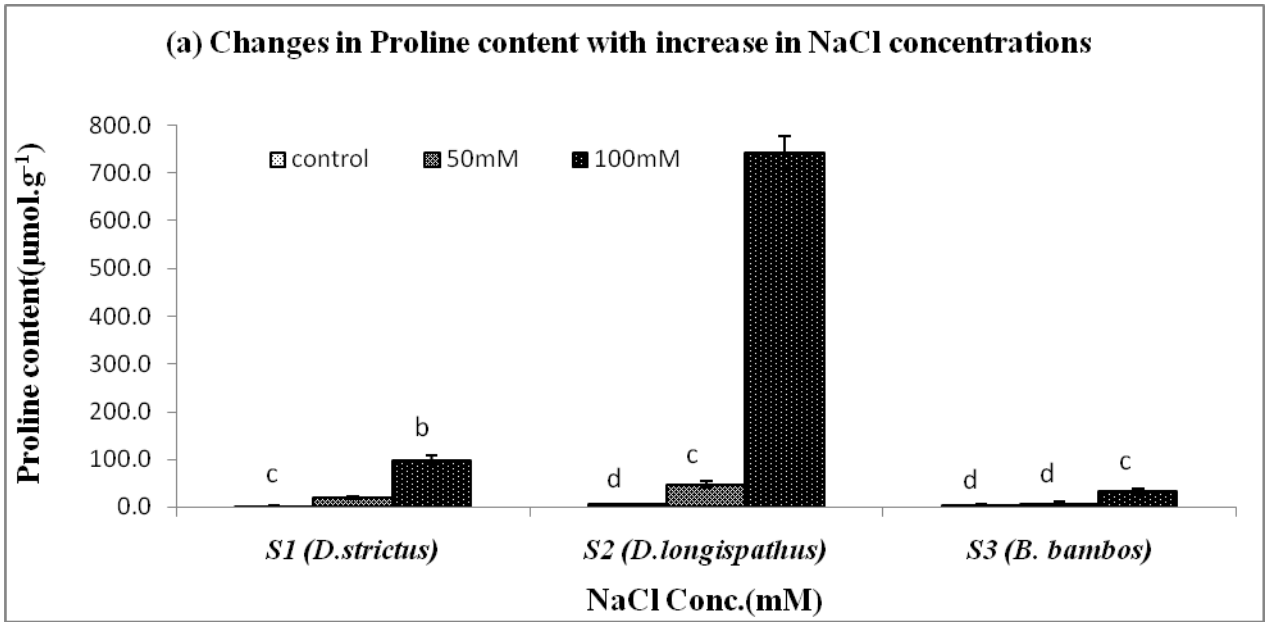
As observed for the growth parameters, significant decrease in the FWwas observed in all the bamboo species with increasing NaCl concentration (Table 1). Meanwhile, among all the species considered, reduction was highest in S3 species (67%) compared to S1 (46%) and S2 (39.4%) at T3 concentration. In the individual mean values, irrespective of the treatments, S2 species had the lowest FWrecorded in all the three treatments including control. Thus, the other two species (S1 & S3) performed better in comparison to S2 species.

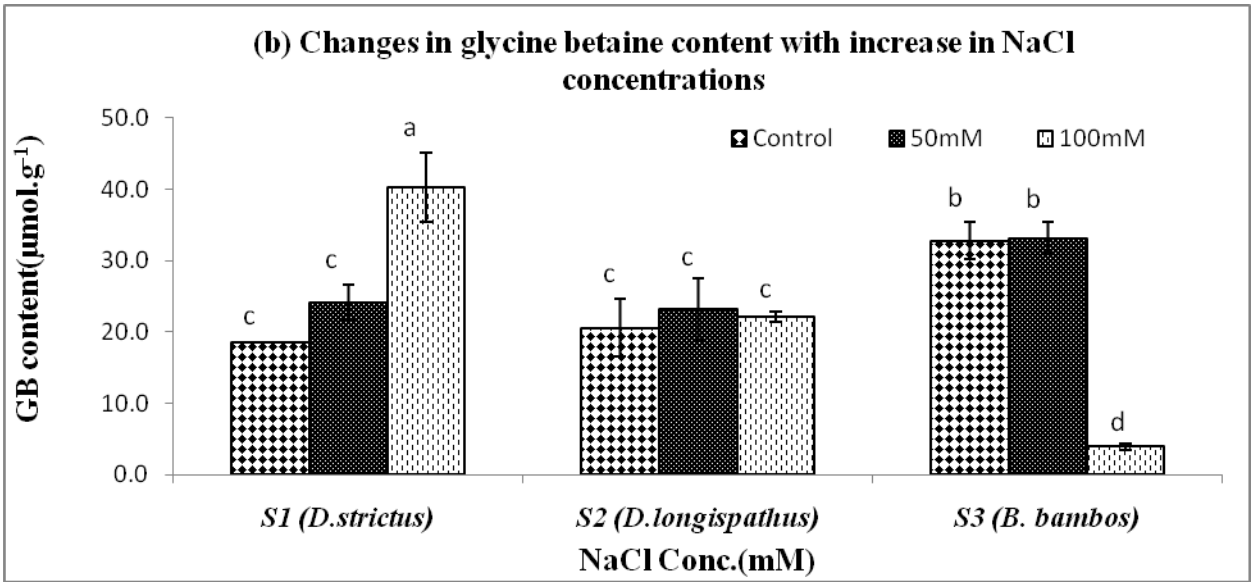
3.2. Organic Osmolytes Accumulation

Significant differences were observed in proline and glycine betaine contents of all treatment concentrations irrespective of different bamboo species in comparison to control. (Figure 2). Proline content increased with increasing NaCl concentration in all the three species. It was found that S2

seedlings accumulated highest proline (744.05±32.5) which was 7.57 folds higher than that of the S1 seedlings (98.17±9.8) and 22.85 folds higher than the S3 (32.56±5.3) seedlings treated with 100mM NaCl. Glycine betaine content had a wide variation in all the three species, unlike what was observed in case ofproline. The S1 species had the highest GB accumulation (40.21±4.8) at 100mM NaCl in comparison to other two species. In case of S2 species there was no significant variation in GB content, that was similar to that of control. Whereas in case of S3 species the pattern of GB accumulation was entirely different from other two species, at 50mMNaCl GB content was similar to that of control, but at 100mM NaCl, GB content declined 8.29 folds in comparison to control.

Figure 2





4. Discussion

Effect of NaCl ondifferent plant species such as populus (Gebre et al., 1998), wheat (Bashiti et al., 2005), cotton (Sarwar et al., 2006), sunflower (Heidari et al., 2011), eucalyptus (cha-um et al., 2013) has been reported with response to growth performance and osmolytes accumulation. To the best of our knowledge no such study is reported on bamboo species. Bamboo is one of fast growing desirable tree species with high economic benefits. Utilization of this species for pollution management, environmental benefit & ecological gain will be a win-win situation due to its diverse utility in domestic and industrial sector. In this context relevance of bamboo species for utilization of saline lands and waste water for eco-capital build up and resource recovery is a prospectiveproposition. In this particular study we have evaluatedgrowth performance of three bamboo species; S1, S2 & S3 under two different NaCl concentrations i.e. 50mM & 100mM for a period of 14 days.It was observed that salt stress upto 50mM did not have significant effect on vegetative growth of*Bambusa bambos* (S3) as determined through species all morphological parameters ; viz. SL, RL, NL and FW compared with that of control. Further increase in NaCl concentration also had no significant effect on all parameters. Thus,the extent of growth reduction was therefore minimal in case of S3 species.Whereas in case of S2 species growth performance was retarded in all the vegetative growth parameters and had the least growth rate under salt stress.

Proline is one of the most common stress indicators, which is produced inside plant body under salt stress, and known to function as a compatible solute that helps in osmotic adjustment, for nitrogen storage, scavenging of free radicles and protect macromolecules from salt damage (Chookhampaeng, 2011). Glycine betaine (GB) is the second low molecular weight quaternary ammonium compound which also acts as an osmolyte to combat stressful conditions in various plant systems. In this study, proline content increased significantly in all the three species with increasing NaCl concentration but highest proline content was recorded in S2 species treated under 100mM NaCl. Some earlier studies have reported increase in proline content with increasing salt concentration as a salt tolerant trait but in case of bamboo species an inverse relationship was observed. The species (S2) having highest proline (149 folds higher than control) content had poor vegetative growth performance. Whereas, in S1 and S3 genotypes, increase in proline content at 100mM NaCl with respect to control was only 49 to 11 folds respectively but had healthy vegetative growth performance compared to S2 species. Bolarin et al., (1995) and De Lacerda et al, (2005) have argued that the excessive levels of proline content may be due to leaf damage or a symptom of stress. Comparing the three bamboo species with respect to GB; increasing trend was observed in case of S1 species in treated plants compared to control seedlings, whereas in S2 species its content remained unchanged. But in S3 species the pattern was entirely different than the other two, GB content was similar in control and 50mM NaCl, butdecreased 8.25 folds in 100mM NaCl. These findings showed that proline played an important role in salt tolerance in comparison to GB in case of bamboo species. Taking into account all morphological parameters, proline and GB accumulation under 50mM and 100mM NaCl, it is inferred that healthy growth of salt treated S3 seedlings indicate its salt tolerance capacity and can sustain the salt stress and maintain vegetative growth under stressful conditions.

5. Conclusion

Our investigation shows indicative parameters which could be used to screen the salt tolerant variety, and added to understand the mechanisms adapted by bamboo to combat the stressful conditions. Based on the results of these parameters as scientific evidences it can be concluded that S3 (*Bambusa bambos*) and S1 (*Dendrocalamus strictus*) are salt tolerant bamboo varieties depicting better growth performance presumably due to optimum proline concentrations. Whereas, S2 (*Dendrocalamus longispathus*) species is the salt sensitive genotype with declined growth performance inspite of highest proline content. Further work are in progress to illustrate the role of proline versus GB and performance evaluation for longer duration and field evaluation.

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The authors declare no competing interests.

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Figure captions

Figure 1. Treatment of three bamboo species in different NaCl concentrations

Figure 2.The effect of increasing concentrations of NaCl (0,50 & 100 mM) on osmolyte accumulation in Bamboo genotypes.(a) Proline and (b) Glycine betaine of seedlings after 14 days of treatment. Vertical bars are standard errors. Values represented by similar letters are not significantly different from each other ($p \leq 0.05$).