



Full Length Article

Effects of Salt Stress on Seed Germination of Four Ornamental Non-halophyte Species

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Abstract

The seeds of *Pinus taeda*, *Pinus elliottii*, *Lagerstroemia indica* and *Fraxinus chinensis* were germinated at various salinity regimes (0, 50, 100, 150 and 200 mM NaCl). The results indicate that germinated seeds of four investigated tree species were inhibited at different saline levels. Germination speed and seed vigour, which were indicated by germination index and vigour index, decreased with increase in salt stress, whereas different descending patterns were observed among all the seeds. The salt-resistant capacity of seeds in four tree species were arranged in numerical order by their relative germination percentage and vigour index; however, they could not be clearly identified from each other when NaCl levels were 50 and 200 mM. At 100 mM NaCl, salt-tolerance was ranked from highest to lowest as follows: *Pinus elliottii*, *Fraxinus chinensis*, *Pinus taeda* and *Lagerstroemia indica*. However, *Pinus elliottii* ranked lower than *Fraxinus chinensis* at 150 mM NaCl, indicating that *Fraxinus chinensis* exhibited better adaptation to salt stress than *Pinus elliottii*. © 2018 Friends Science Publishers

Keywords: Salt stress; Germination characteristics; Ornamental trees; Seed vigour; Salt tolerance

Introduction

The salinization of agricultural and forestry lands occurs worldwide due to natural processes and human impact. The global primary salt-affected-soil is about 955 M ha, while secondary salinization due to human activities affects some 77 M ha land area (Metternicht and Zinck, 2003). Salinity limits the vegetative and reproductive growth of plants by inducing severe physiological dysfunctions and causing direct and/or indirect harmful by-effects, even at low salt concentrations (Shannon *et al.*, 1994). The salinity-induced vegetation loss and biodiversity reduction along coastal areas, where the irrigation water contains a higher salt concentration than inland places, makes it urgent for breeders to select salt-resistant plants. Furthermore, global warming may cause a rise in sea levels that would flood extensive coastal areas with salt water (Daniels, 1992; Wigley and Raper, 1993). In all, the land productivity for both agriculture and forestry is at risk owing to soil salinity.

Seed germination, seedling emergence, and early survival are particularly sensitive to substrate salinity (Baldwin *et al.*, 1996). Usually, seeds of the non-halophytic species show more reduction in percentage germination under higher levels of salinity as well as in the germinating seed (Tobe *et al.*, 2001, 2003; El-Keblawy, 2004; Afzal *et al.*, 2008; Archana *et al.*, 2010; Van Tran *et al.*, 2018), which can inhibit germination related metabolic progress (Pujol *et al.*, 2000), whereas species differ in their salinity

tolerance. Moreover, the evaporation and the capillary rise of water will make the salt concentration on the saline soil surface even higher (Almansouri *et al.*, 2001). Therefore, seeds and seedlings are exposed to higher NaCl when compared with mature plants (Munns and Tester, 2008). Consequently, the germination stage is crucial in the life circle of non-halophytes along coastal areas.

The polygenic nature of salt tolerance has clearly been established (Flowers, 2004), which makes it a huge task to engineer specific genes of salt tolerance into sensitive plants (Flowers, 2004; Ma *et al.*, 2009). In less developed areas, the determination of the germination of seeds in saline conditions could be a simple and useful parameter for breeders as germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants (Bybordi and Tabatabaei, 2009).

Four non-halophyte woody species of *Pinus taeda* Linnaeus, *Pinus elliottii* Engelm., *Lagerstroemia indica* Linnaeus, and *Fraxinus chinensis* Roxburgh are used for reforestation and urban greening in the coastal provinces of China because of their tolerance to abiotic stress. However, the salinity threshold was not clear among these four non-halophyte species. This research describes the germination characteristics of four woody species in various saline regimes and differentiates salt tolerance among these species. This study is expected to provide a reference for the selection of tree species in reforestation and urban greening and the theoretical basis of re-vegetation in salinized lands.

Materials and Methods

The experiments were conducted at the Southern Tree Seed Inspection Center of the National Forestry Administration, Nanjing Forestry University, Nanjing, PR China, from March to April, 2012.

Seed Materials and Pretreatment

Seeds of *F. chinensis* were collected in the autumn of 2011 from Ji'an, Jiangxi Province, PR China. Seeds were sealed in polyethylene bags and then subjected to dry storage at temperatures fluctuating from 3–5°C before the experiments were carried out. Prior to the germination tests, the seed wings of *F. chinensis* were removed and soaked in water for seven days.

Experimental Design

The two treatment factors were species (*P. taeda*, *P. elliotii*, *L. indica*, and *F. chinensis*) and concentrations of sodium chloride solutions (0, 50, 100, 150, and 200 mM), which were applied in a randomized complete design. The experiment consisted of twenty treatments.

Germination Assays

To homogenize the quality of the seed samples, seeds of each species were thoroughly mixed and then selected representatively using the method of quartering. Replicates of one hundred seeds from each treatment were sown uniformly onto one layer of degreasing cotton (2 mm thick) in rectangular, transparent polystyrene germination boxes with covers (24.5 cm × 14 cm × 5 cm) and moistened with 90 mL de-ionized water or corresponding NaCl solution. The weight of each box was recorded prior to incubation, so that water loss could be added to each box every other day to maintain a constant concentration of the corresponding NaCl solution. The seeds were incubated in a growth chamber (S6620LC, Seedburo Equipment Co., USA) at 25°C and provided with full daylight. Germination was recorded daily over 31 days. The seeds of all four species were not considered germinated until germinants—seeds which developed into normal seedlings as described by the International Seed Testing Association (ISTA, 2014). Germination recovery was unable to be tested owing to fungal infections. Each treatment was replicated four times. Germination percentage (GP) was calculated as described by El-Keblawy (2004).

Seed Vigour Evaluation

This was performed simultaneously with the germination tests. Germinants were evaluated daily as described previously by ISTA (2014). The germination index (GI) measurement was proposed by Tao and Zheng (1991).

At the final count, the mean seedling length (MSL) of the germinants from each treatment was determined by a scale ruler (minimum unit is mm). MSL was expressed in cm. The seed vigour index (VI) in each treatment involved measuring the seedling length and the germination index from the same treatment, which was calculated as described by Tao and Zheng (1991):

$$VI = GI \times MSL \quad (1)$$

Analysis of the Salt-tolerance Capacity

After germination and vigour evaluation, relative germination percentage (RGP) and relative vigour index (RVI) were calculated. The three indicators, which reflect the salt-tolerance of seeds in four woody species when germinated, were calculated as follows:

$$RGP = \frac{GP_n}{GP_0} \times 100\% \quad (2)$$

Where GP_n is the germination percentage under one certain treatment of salinity, and $n = 1, 2, 3,$ and 4 , representing the NaCl treatments which were 50, 100, 150, and 200 mM, respectively; and GP_0 is the germination percentage under 0 mM NaCl treatment.

$$RVI = \frac{VI_n}{VI_0} \times 100\% \quad (3)$$

Where VI_n is the vigour index under one certain treatment of salinity, and $n = 1, 2, 3,$ and 4 , representing 50, 100, 150, and 200 mM NaCl treatments, respectively; and VI_0 is the vigour index under 0 mM NaCl treatment.

The salt-tolerance in all four species was considered to be the same under the treatment of 0 mM NaCl.

Statistical Analysis

The statistical analysis was done with Excel 2003 and SPSS 18.0. All values were presented as the mean ± standard deviation (SD) of four replicates and then were subject to two-way analysis of variance (ANOVA). When the main and interaction effects were found to be of statistical significance, further analysis was made using one-way ANOVA and Duncan's multiple range tests at the 5% level (if necessary) within either species or salinities. Before the ANOVAs, values of germination indices and vigour indices were square root transformed.

Results

Effects of Salinity on Final Germination (GP) of Seeds

Final germination (%) was significantly affected by species ($F=3.825, 0.01 < P < 0.05$), salinity ($F=813.060, P < 0.01$), and species × salinity interaction ($F=84.030, P < 0.01$).

Further analyses within species showed that the effect of salinity was significant in all four species (Fig. 1). Final germination of seeds of four species was inhibited with increased salinity, and their changing patterns varied from each other. Despite the appearance of hypocotyl, *L. indica* seeds were not considered germinated under the salinity regimes of 150 and 200 mM, because the radicle emergence was not observed.

For *P. taeda* and *P. elliottii* seeds, germination at 50 mM NaCl was not inhibited significantly when compared with control (non-saline). From 50 to 150 mM NaCl, GP of seeds in two species varied significantly. With each 50 mM increment in salinity, germination rate dropped by 58.5% and 23.4% for *P. taeda* and *P. elliottii*, respectively, from 50 to 100 mM, and by 44.2% and 64.2% for *P. taeda* and *P. elliottii*, respectively, from 100 to 150 mM. As the degree of salt stress continued to rise, significant variation in GP was only observed for *P. elliottii* seeds, decreasing by 76.5% from 150 to 200 mM NaCl. For *L. indica* and *F. chinensis* seeds, the GP varied significantly with each 50 mM increment in salt stress (from 0 to 150 mM).

Effects of Salinity on Germination Index (GI) of Seeds

The effect of salinity within species is significant (Fig. 2). The germination index within seeds in four species was inhibited with increasing salt stress, but species behaved differently to salt medium. As seeds were considered ungerminated, the germination index of *L. indica* seeds was zero under salinity levels (150 and 200 mM NaCl).

For all seeds in four species, the GI varied significantly ($P < 0.05$) with each 50 mM increment in salinity. Within regimes from 0 to 50 mM, germination index dropped by 16.2%, 26.2%, 33.4% and 22.5% for *P. taeda*, *P. elliottii*, *L. indica*, and *F. chinensis*, respectively. From 50 to 100 mM, the GI of *P. taeda* seeds dropped by 75.0%, while GI of all other seeds reduced less than 50%. From 100 to 150 mM, all but *F. chinensis* seeds subjected to GI loss of more than 70%. As the degree of salt stress continued to rise, significant variation in the GI was observed for *P. taeda*, *P. elliottii*, and *F. chinensis* seeds, decreasing by 52.0%, 70.9 and 80.5% from 150 to 200 mM, respectively.

Effects of Salinity on Vigour Index (VI) of Seeds

Vigour index was significantly influenced by species ($F=37.8.663$, $P < 0.01$), salinity ($F=1239.211$, $P < 0.01$), and their interaction ($F=27.691$, $P < 0.01$) was also found significant (Fig. 3). Vigour index within seeds from four species was adversely affected with increased salinity. The vigour index of the *L. indica* seeds was zero under the salinity regimes of 150 and 200 mM as no seeds could germinate under these conditions. In general, expect for *L. indica*, VI variations for all seeds in the four

species resembled germination index. Within the salinity from 0 to 50 mM, the VI of *L. indica* seeds decreased dramatically upto 90%.

Comparison of Relative Germination Percentage (RGP) within Species

The effects of species were significant within different saline treatments (Fig. 4). At 50 mM NaCl, only the RGP of *L. indica* seeds was found less than 70%. At 100 mM NaCl, *P. elliottii* seeds maintained the highest RGP (68.8%). In contrast, the RGP of *F. chinensis* seeds dropped by 20.1% when compared with *P. elliottii* seeds. As the salinity level continued to rise to 150 mM, no significant variation was found ($P > 0.05$) between the RGP of *P. taeda* and *P. elliottii* seeds. When incubated in 200 mM NaCl, the RGP of *P. taeda* seeds was the highest, reaching 19%, and next, ranking from high to low, was that of *F. chinensis*, *P. elliottii*, and *L. indica* seeds, dropping by 56.1%, 30.4% and 100% in order from highest to lowest, respectively.

Comparison of Relative Vigour Index (RVI) within Species

According to the two-way ANOVA, species ($F=356.644$, $P < 0.01$), salinity ($F=1423.952$, $P < 0.01$) and their interaction ($F=61.951$, $P < 0.01$) have significant influence on RVI of four species. Further analyses within salinities disclosed that the effect of species was significant (Fig. 5).

Under 50 mM NaCl, *L. indica* had the lowest RVI (10%). An estimated 80% decrease of RVI of *L. indica* compared with *F. chinensis* seeds was found. Under 100 mM NaCl, *P. elliottii* seeds maintained the highest RVI, reaching 30.4%, and next, ranked from high to low, were that of *F. chinensis*, *P. taeda*, and *L. indica* seeds, dropping by 31.2%, 46.7% and 80.7% in order of highest to lowest. As the salinity level continued to rise to 150 and 200 mM, the seed RVI in four species resembled RGP, expect for that *F. chinensis* and *P. elliottii* seeds maintained the highest relative vigour index (though less than 3%) when incubated in 200 mM NaCl.

Discussion

Germination is a key stage in the life cycle of plants in saline environments as it determines whether the plants can establish successfully in certain areas (Song *et al.*, 2005). During germination stage of plants, a series of morphological and physiological changes take place, which may reflect parental genotypes and, to some extent, affect the post-germination processes. The better germinability of seeds under abiotic stresses indicates a much higher chance for its survival and vigorous growth of seedlings. In this study, exposure to saline conditions during germination resulted in a reduced germination percentage of four tree species (Fig. 1).

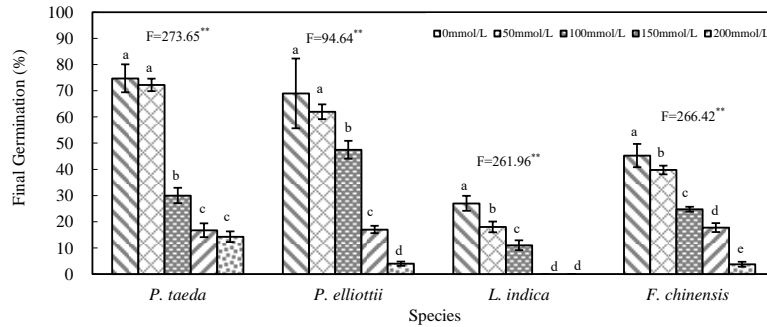


Fig. 1: Final germination (mean± SD) of *P. taeda*, *P. eliottii*, *L. indica* and *F. chinensis*, seeds in various salinities. Bars marked with the different lowercase letters within the same species indicate the significant difference among the relevant means at 5% level. ** indicates $P < 0.01$ within species

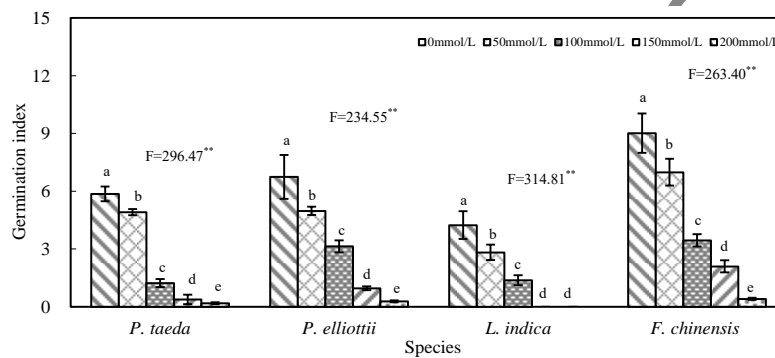


Fig. 2: Germination index (mean± SD) of *P. taeda*, *P. eliottii*, *L. indica* and *F. chinensis*, seeds in various salinities. Bars marked with the different lowercase letters within the same species indicate the significant difference among the relevant means at 5% level. ** indicates $P < 0.01$ within species

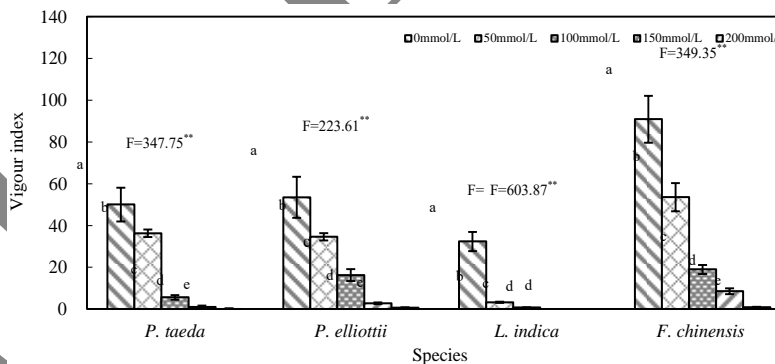


Fig. 3: Vigour index (mean± SD) of *P. taeda*, *P. eliottii*, *L. indica* and *F. chinensis*, seeds in various salinities. Bars marked with the different lowercase letters within the same species indicate the significant difference among the relevant means at 5% level. ** indicates $P < 0.01$ within species

In both non-halophytes and halophytes, salinity reduces total number of germinated seeds and postpones the initiation of germination processes (Kozłowski, 1997); however, within each group, the responses were variable and species-specific (Ungar, 1982, 1991). Salt and water stresses could reduce germination by either

limiting water absorption by the seeds (Dodd and Donovan, 1999), affecting the mobilization of stored reserves (Prakash and Prathapasanen, 1988; Bouaziz and Hicks, 1990; Lin and Kao, 1995) or directly affecting the structural organization or the synthesis of proteins in germinating embryos (Ramagopal, 1990).

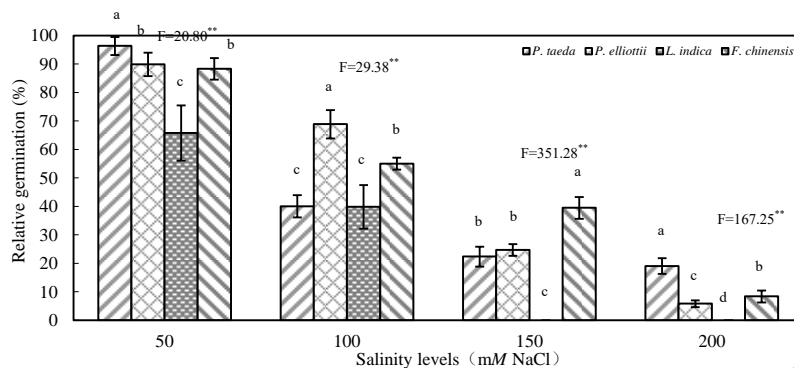


Fig. 4: Relative germination percentage (mean \pm SD) of *P. taeda*, *P. elliottii*, *L. indica* and *F. chinensis* germinants in various salinities. Bars marked with the different lowercase letters within the same species indicate the significant difference among the relevant means at 5% level. ** indicates $P < 0.01$ within species

For *L. indica* and *F. chinensis* seeds, the germinability was gradually reduced within the given saline regime and seeds of *L. indica* had long lost its ability to germinate above 150 mM NaCl, indicating no possibility for *L. indica* to establish a habitat under such high salinized conditions. However, it is noteworthy that since the *L. indica* seed has initial germination only upto 30% in non-saline conditions, it is quite difficult to judge its potential under stress. Further studies must be carried out to make sure whether the seed viability in *L. indica* has long lost.

However, for *P. taeda* and *P. elliottii* seeds, germinability was not affected when salt stress was under 50 mM NaCl, suggesting the better ability than *L. indica* and *F. chinensis* seeds to adapt to salinity at low level. Salinity stress influences seed germination primarily by lowering the osmotic potential of soil solution sufficiently to retard water absorption by seeds (Khan and Ungar, 1984), and also through toxicity to the embryo (Zekri, 1993). It could be interpreted by that the seeds take up Na^+ and/or Cl^- from NaCl solution, therefore lowering the osmotic potential of cells and maintaining a water potential gradient that enables continued water uptake at declined external water potentials (Bradford, 1995; Ungar, 1995; Khajeh-Hosseini *et al.*, 2003). While this uptake of Na^+ and/or Cl^- could lead to a toxic effect at higher salinities and poor germination of seeds (Khajeh-Hosseini *et al.*, 2003), which might help to explain the reduced germination percentage of *P. taeda* and *P. elliottii* seeds when subjected to salinity above 50 mM NaCl up to 200 mM NaCl. However, it is worthwhile noting that germination tolerance of plant species to salinity under laboratory conditions does not necessarily correlate with their response to salts under field conditions, and may be many times lower (Gulzar and Khan, 2001).

Seed vigor refers to the overall capacity of the seed to germinate and emerge after sowing and retain this potential during postharvest storage (Wang *et al.*, 2018). In present study, seed vigor of non-halophytic species was influenced both by biological characteristics and saline conditions.

For *P. taeda* and *P. elliottii* seeds, germinability was not affected when salt stress was imposed upto 50 mM NaCl, which may enable them to establish habitats under light-saline conditions. However, at 50 mM NaCl, the germination index and vigour index of seeds of both species were subjected to significant inhibition ($P < 0.01$), indicating the lagged initiation of germination. Most highly salt-tolerant halophytes could withstand high tissue salt concentrations (Gorham, 1996) owing much to osmotic adjustment (Waisel, 1991; McKersie and Leshem, 1994; Gucci *et al.*, 1997). Sodium is typically sequestered in vacuoles, hence the cytoplasm and chloroplasts are exposed to salinity in a lesser level (Shannon *et al.*, 1994; Larcher, 2003) but in some plants, osmotic adjustment results from the synthesis of compatible organic solutes in the cytoplasm. For non-halophytic species, before the external osmotic threshold is reached, the possibility of water uptake could be maintained by the seeds (Tobe *et al.*, 2001). However, within an external osmotic threshold, it becomes time-consuming for seeds to operate the intracellular osmotic adjustment and thus initiate germination, along with the increased salinity. At this time, the osmotic barrier, not the ion toxicity, is the main factor that delays the germination process (Pujol *et al.*, 2000).

Overall, we confirmed that seed vigor would be lowered by the increased salinity, which meant that the speed of germination and the seedling emergence were inhibited. Seed lots with similar total germination often vary in the rate of seedling emergence and the rate of growth (Caseiro *et al.*, 2004). In this study, we concluded that the increased salt stress impaired seed vigor more severely than germinability. Therefore, for *P. taeda* and *P. elliottii*, although survival could be achieved, the growth potential of the germinants may be reduced under the salinity regime of 50 mM NaCl.

Surface substrate contains higher salinities than the deeper soil along the coast of China (Tian *et al.*, 2008; Liu *et al.*, 2010; Zhang *et al.*, 2010; Jia *et al.*, 2011),

which makes successful germination and seedling survival much more important. We believed that *P. taeda* seeds had better salt tolerance than *P. elliotii* seeds under 50 mM NaCl (Fig. 4 and 5). The *P. elliotii* seeds were more adaptive to saline conditions when compared with *P. taeda* seeds when the degree of salt stress raised from 50 to 100 mM. However, at higher salinity level (200 mM NaCl), the exact order of salt tolerance among the four species could not be clearly identified as the *P. taeda* seeds exhibited the highest germinability (Fig. 4) while the RVI told a different story (Fig. 5). This might be explained as the adaptive strategy for *P. taeda* seeds under extreme salinity, where seeds trade off their growing power in order to gain the possibility of survival in the early stress stage. The *F. chinensis* seeds performed better than the *P. elliotii* seeds in respect of salt tolerance when it exposed to 150 mM NaCl level. In general, we could clarify that *L. indica* again maintained the worst salt tolerance under all salt regimes. Salt tolerance could not be clearly identified from each species when NaCl levels were 50 and 200 mM. This might be explained by the fact that the salt tolerance of germinating tree seeds was affected by both biological characteristics and saline conditions.

Acknowledgments

This work is supported by A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). We wish to thank the tree seeds and seedlings station of Ji'an, Jiangxi Province for providing seed material. We also thank the individuals of Minghui Du, Minjie Zhang, Kai Huang for their help with data collection, and we thank Haomin Yao, Shaoxuan Liu, Lei Sun, Jie Zhang, Georges Moreau for their kind assistance with the language.

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(Received 28 March 2018; Accepted 18 July 2018)

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