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Effect of Salt Stress on Seed Germination and Seedling Growth of Three Salinity Plants

Yan Li
Department of Biology, Dezhou University, Dezhou 253023, Shandong Province, People's Republic of China

Abstract: Seeds of three halophyte species included *Limonium sinense* Kuntze, *Glycine soja* sieb. and *Sorghum sudanense* Stapf. were used to investigate the effects of different salinity concentrations on their germination percentage, germination energy, germination index, relative germination rate, relative salt-injury rate, radicle length and hypocotyl length. Results showed that seeds of *G. soja* can germinate well and rapidly at lower level of salt (<200 mmol L^{-1}), 50 mmol L^{-1} salt concentrations is better to *S. sudanense*, suitable salt concentrations was beneficial to germinate of *S. sudanense* seeds. Seeds of *L. sinense* and *S. sudanense* can germinate at higher level of salt (400 mmol L^{-1}). Three plant seeds had different physiological mechanism for germination under salt stress, radicle hypocotyl ratio of *L. sinense* and *S. sudanense* increased with increasing salt suggested that they had the higher tolerance in shoot growth under NaCl stress, which is perhaps the reason for their wide utilisation for saline soil rehabilitation.

Key words: Salinity stress, germination percentage, radicle length, hypocoty length

INTRODUCTION

Soil salinity is a major factor limiting plant productivity, affecting about 95 million hectares worldwide (Szabolcs, 1994). The UNEP (United Nations Environment Program) estimates that 20% of the agricultural land and 50% of the cropland in the world is salt-stressed (Flowers and Yeo, 1995). Salinity imposes serious environmental problems that affect grassland cover and the availability of animal feed in arid and semi-arid regions (El-Kharbotly *et al*., 2003). Greenway and Munns (1998) reported that some crops are moderately tolerant of saline conditions; many crops are negatively affected by even low levels of salt. Salt stress unfavorably affected plant growth and productivity during all developmental stages (Epstein *et al*., 1980). For example Epstein *et al*. (1980) reported that salinity decreases seed germination, retards plant development and reduces crop yield.

Seed germination is a crucial stage in the life history of plants and salt tolerance during germination is critical for the stand establishment of plants and grow in saline soils (Khan *et al*., 2000). Several investigations of seed germination under salinity stress have indicated that seeds of most species attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seedling phases of development (Gulzar *et al*., 2003; Ghoulam and Fares, 2001). In many plant species, seed germination and early seedling growth are the most sensitive stages to salinity stress. Seeds of most halophytes attain there maximum germination in the absence of NaCl and are very sensitive to elevated salinity at the germinarion and early establishment phases (Khan *et al*., 2000). Seed germination is defined as the emergence of the radical through the seed coat (Copeland and Mcdonald, 1995). Othman (2005) reported that seed germination can be initiated by water imbibitions and any shortage in water supply will let seed under stress. Shokohifard *et al*. (1989) reported that salt stress negatively affected seed germination; either osmotically through reduced water absorption or ionically through the accumulation of Na^{+} and Cl^{-} causing an imbalance in nutrient uptake and toxicity effect.

L. sinense, *G. soja* and *S. sudanense* are salt-living protective plants with special ecological value in nature and with singular use in traditional Chinese medicine. They are crowned as a pioneering plant in saline land due to the function of removing salt from salt-soil and of improving the structure of soil. *L. sinense*, which is widely existed safeguard plants excreting salts. It has many effectual components such as flavone and glucoside of waxberry bark. So it can be used for not only medicine but also the improvement of the salt soils. For these, it is complimented as the pioneer plant. It is a folk medicine popularly used as a remedy for bleeding, piles, fever, hepatitis, diarrhea, bronchitis and other disorders. The plant is mainly distributed along seashores and salts marshes in Southern China, Ryukyus (Japan) and Western Taiwan. As reported by Lin and Chou (2000), the major constituents found in the leaves and the roots of *L. sinense* were flavonoids. *G. soja* distribute China, Far East region of Russia, Japan, Korea and Taiwan. Soybean is considered to have been domesticated from *G. soja*. in Northeast China. *G. soja* is therefore considered to be an important gene source of soybean. *S. sudanense* is used as forage for ruminants, it can be grazed or utilized in cut-and-carry forages, as hay or silage for ruminants, it is intolerant of waterlogging but has reasonable tolerance of salinity and alkaline, It is one of wild plants in Northeast China. There were many researches on them. However, reports about their seeds germination and seedling grow are very few. Thus, the objectives of this study were to screen three salinity plants for salt tolerance during germination, to study the ability of seed to germinate after exposure to different salt stress.

MATERIALS AND METHODS

Seeds of *L. sinense*, *G. soja* and *S. sudanense* were used in this study, The seeds were collected in 2007 from a natural grassland located in the east of Dezhou of Shandong province of China. The trial was conducted at Biology Department, Dezhou University, Dezhou, Shandong, China, in 2007. After being surface sterilised with 0.01% $HgCl_2$ solution for 3 min and rinsed in tap water, Seeds were germinated in covered, sterilized, disposable Petri dishes containing Whatman filter paper moistened with either distilled water (control), or different treatment solutions (50, 100, 200, 400 and 800 mmol L^{-1}). Germination was assessed using three replicates of 50 seeds in a factorial laid out in Completely Randomized Design (CRD) testing combinations of six levels of salinity on seeds of the three salinity plants cultivars in 9 cm diameter Petri dishes.

All Petri dishes were placed in a growth chamber at 25°C/13 h and 15°C/11 h, Seeds were considered germinated with the emergence of the radicle. Germination was scored when a 2 mm radicle had emerged from the seed coat. Every three days, the germinated seeds were removed from the Petri dishes. The seeds were allowed to germinate in each replicate and retained for measurements of radicle and hypocotyls lengths at the end of the experiment. After 240 h, final germination percentages were recorded. To determine the impact of the treatments on seed germination, all seedlings were separated from the remaining seeds.

In order to maintain adequate moisture, 5 mL of the original salt solutions were added to each Petri dish every three days. Germination percentage, germination gngrgy, germination index, relative germination rate, relative salt-injury rate were determined by the following formula:

Germination percentage	=	a/b
Germination energy	=	c/b
Germination index	=	Σ Gt/Dt
Relative germination rate	=	d/e
Relative salt-injury rate	=	(e-d)/e

where, a is germinated seeds total in NaCl concentration every day, b is total number of seeds for germination, c is germinated seeds total in NaCl concentration in three days. Gt is germinated seeds in t days, Dt is the number of germination days corresponding. d is germination percentage in NaCl concentration, e is germination percentage of control. Five seedlings were selected randomly from each Petri dish for radicle and hypocotyl length measurement 10 days after the salt exposed experiment. Data were analyzed by one-way using Analysis of Variance (ANOVA) and Duncan's multiple range test (for comparison of means) and using SAS software version 6.12 (SAS, 1996).

RESULTS AND DISCUSSION

Analysis of variance revealed significant differences among plant species and salinity levels for germination percentage, germination energy and index, relative germination rate, relative salt injury rate.

Increasing concentration of NaCl reduced germination percentage of *L. sinense* and *G. soja*. *L. sinense* and *S. sudanense* could germinate at the concentration of 400 mmol L^{-1} NaCl (Table 1), but *G. soja* only could germinate at the lower concentration of NaCl (<200 mmol L^{-1}), it suggest that seeds of *L. sinense* and *S. sudanense* could tolerance higher salt concentration, especially *S. sudanense*. But the germination percentage of *G. soja* is higher than *L. sinense* and *S. sudanense* at same NaCl concentration, It suggest that seeds of *G. soja* could germinate well at the lower concentration of NaCl (<200 mmol L^{-1}), despite biomass of *G. soja* decreased with increasing concentration of NaCl (Kao *et al*., 2006). The biomass of *L. sinense* was highest at the concentration of 100 mmol L^{-1} NaCl then decreased with increasing concentration of NaCl (Li, 2007), despite germination percentage decreased with increasing concentration of NaCl (Table 1). The germination percentage of *S. sudanense* reached highest at the concentration of 50 mmol L^{-1} NaCl (Table 1), 50 mmol L^{-1} NaCl is better to *S. sudanense* seeds, it is different from *L. sinense* and *G. soja*.

Table 1: Effect of NaCl on germination percentage of *L. sinense*, *G. soja* and *S. sudanense*

Species	Salt concentration (mmol L^{-1})	Germination (%)	Germination energy	Germination index	Relative germination rate	Relative salt damage rate
L. sinense	CK	0.95 ± 0.02^{a}	0.95 ± 0.02^{a}	74.34 ± 1.94^{a}	1.00	0.00
	50	0.92 ± 0.01^{a}	0.92 ± 0.05^{a}	58.34 ± 1.42^{a}	0.97	0.03
	100	0.83 ± 0.03^{b}	0.81 ± 0.02^{b}	47.23 ± 0.26^{b}	0.86	0.14
	200	0.22 ± 0.05^{c}	0.22 ± 0.03^{c}	9.18 ± 0.34^{d}	0.23	0.77
	400	0.10 ± 0.02^{c}	0.10 ± 0.02^{c}	3.42 ± 0.96^{d}	0.11	0.89
	600	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00	1.00
G. soja	CK	0.97 ± 0.03^{a}	0.94 ± 0.01^{a}	32.11 ± 1.51^{b}	1.00	0.00
	50	0.96 ± 0.01^{a}	0.95 ± 0.03^{a}	21.69 ± 2.56^{c}	0.99	0.01
	100	0.93 ± 0.02^{a}	0.78 ± 0.11^{b}	19.12 ± 1.06^{c}	0.95	0.05
	200	0.90 ± 0.05^{b}	0.65 ± 0.13^{b}	16.06 ± 1.55^{c}	0.93	0.06
	400	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00	1.00
	600	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00	1.00
S. sudanense	CK	0.95 ± 0.03^{a}	0.88 ± 0.00^{a}	15.33 ± 0.16^{c}	1.00	0.00
	50	0.96 ± 0.01^{a}	1.00 ± 0.08^{a}	17.21 ± 0.38^{c}	1.01	-0.99
	100	0.89 ± 0.04^{b}	0.84 ± 0.01^{a}	14.63 ± 2.32^{c}	0.94	0.06
	200	0.47 ± 0.03^{c}	0.37 ± 0.00^{c}	6.25 ± 2.29^{d}	0.49	0.51
	400	0.24 ± 0.00^{c}	0.20 ± 0.00^{c}	2.34 ± 0.57^{d}	0.25	0.75
	600	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00	1.00

Means with similar letter(s) in each trait is not significantly different at 5% probability level according to Duncan's Multiple Range Test. Obtained from nine replicates after seeds germinated ten days

Germination energy and germination index of *L. sinense* and *G. soja* decreased significantly than control, they decreased significantly with increasing concentration of NaCl also (Table 1), It suggest that seeds germination time of *L. sinense* and *G. soja* was elongated under salt stress. Germination energy and germination index of *S. sudanense* was highest at the concentration of 50 mmol L^{-1} NaCl (Table 1), It suggest that seeds germination time of *S. sudanense* was shorten at the concentration of 50 mmol L^{-1} NaCl, but higher level NaCl concentration (>100 mmol L^{-1}) elongated the germination time of *S. sudanense* seeds.

These results were in agreement with Xue *et al.* (2004) who found that high levels of salinity can significantly inhibit seed germination. Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity (Huang and Redmann, 1995). Several authors have been reported that salinity stress affects seed germination either by decreasing the rate of water uptake (osmotic effect) and or by facilitating the intake of ions, which may change certain enzymatic or hormonal activities inside the seed (ion toxicity) (Huang and Redmann, 1995).

Increasing concentrations of NaCl reduced germination rate of *L. sinense*, but the germination rate was not significantly different among CK, 50 and 100 mmol L^{-1} salt concentration, the starting time of seeds germination was same at CK, 50 and 100 mmol L^{-1} salt concentration also (Fig. A), Then the germination rate decreased and starting time of seeds germination elongated with increasing concentration of NaCl, It suggested that NaCl decreased seed vigor, higher salt concentration inhibition seeds germination of *L. sinense* strongly. Seeds of *G. soja* Didn't germinate at the higher

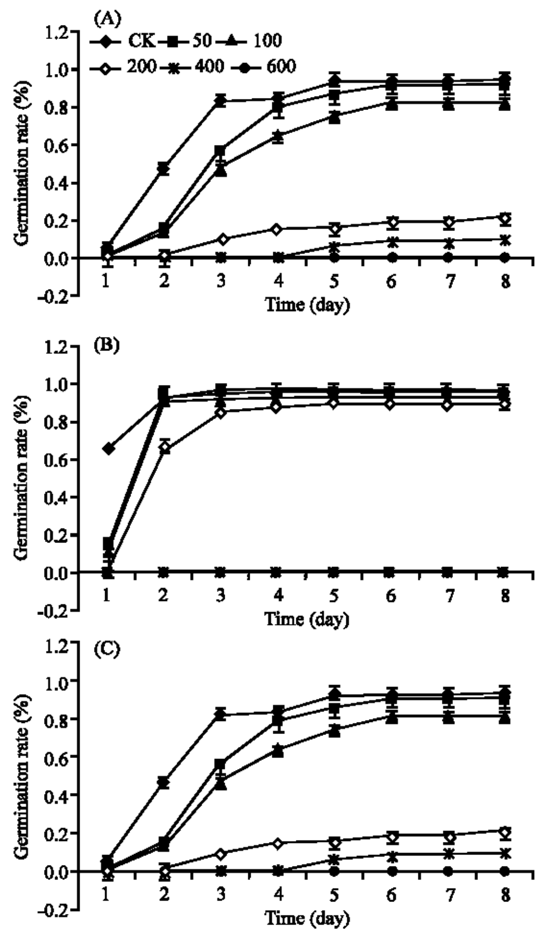

Fig. 1: Effect of different NaCl concentrations on germination rate of *L. sinense* (A), *G. soja* (B) and *S. sudanense* (C). Means with similar letter(s) in each trait is not significantly different at 5% probability level according to Duncan's multiple range test. Obtained from three replicates

Table 2: Effect of NaCl on radicle length, hypocoty length and radicle hypocoty ratio of *L. sinense, G. soja* and *S. sudanense*

Species	Parameters	CK	50 mmol L^{-1}	100 mmol L^{-1}	200 mmol L^{-1}	400 mmol L^{-1}	600 mmol L^{-1}
L. sinense	Radicle length	7.100±0.04	6.700±0.02	6.300±0.06	5.170±0.07	3.78±0.05	0.00±0.00
	Hypocoty length	15.900±0.42	4.100±0.44	2.960±0.34	2.130±0.55	0.72±0.01	0.00±0.00
	Radicle hypocoty ratio	0.447	1.634	2.128	2.427	5.250	
G. soja	Radicle length	5.067±0.15	3.980±0.14	2.960±0.05	0.813±0.01	0.00±0.00	0.00±0.00
	Hypocoty length	2.767±0.01	3.447±0.02	2.742±0.05	0.998±0.08	0.00±0.00	0.00±0.00
	Radicle hypocoty ratio	1.831	1.155	1.080	0.815		
S. sudanense	Radicle length	6.32±0.84	6.430±2.28	4.620±0.02	0.890±0.22	0.06±0.00	0.00±0.00
	Hypocoty length	10.50±2.71	6.670±0.56	4.130±0.04	0.330±0.11	0.04±0.01	0.00±0.00
	Radicle hypocoty ratio	0.602	0.964	1.119	2.697	1.500	

Means with similar letter(s) in each trait is not significantly different at 5% probability level according to Duncan's Multiple Range Test. Obtained from fifteen replicates after seeds germinated ten days

level of salt (>200 mmol L^{-1}), germination rate and starting time of seeds germination was not significantly different at lower level of salt (Fig. 1B), it suggested that Seeds of *G. soja* could germinate well under lower salt concentration (<200 mmol L^{-1}). The germination rate of *S. sudanense* seeds was highest at 50 mmol L^{-1} salt concentrations, the starting time of seeds germination was same at CK, 50 and 100 mmol L^{-1} salt concentration (Fig. 1C), higher salt concentration inhibition seeds germination of *S. sudanense* strongly.

Significant differences were found in radicle lengths depending on species and salinity levels. Increasing salt concentrations severely affected radicle elongation of *L. sinense*. and *G. soja* (Table 2). Concentration of 200 mmol L^{-1} NaCl treatment reduced radicle lengths in *G. soja* from 5.067 to 0.813 mm. Bewley and Black (1994) suggested that the inhibition of radicle growth under salt stress may be due to the reduction in the turgor of the radicle cells. Radicle lengths of *S. sudanense* reached longest at 50 mmol L^{-1} salt concentrations, then radicle lengths decreased with increasing concentration of NaCl, the result was consistent with germination percentage, germination energy and index of *S. sudanense*.

Increasing salt concentrations severely affected the lengths of hypocotyl of *L. sinense* and *S. sudanense* (Table 2). Concentration of 50 mmol L^{-1} NaCl treatment reduced hypocotyl lengths in *L. sinense* from 15.9 to 4.1 mm while 100 mmol L^{-1} NaCl treatment reduced hypocotyl lengths in *S. sudanense* from 10.50 to 4.13 mm. It suggested that salt stress damaged hypocotyls of *L. sinense* and *S. sudanense* first, then made some effects to radicle. The lengths of hypocotyl of *G. soja* reached longest at 50 mmol L^{-1} salt concentrations (Table 2), it suggested that salt stress damaged radicle mainly, *G. soja* toleranced salt stress through protection roots with special mechanism. Salinity can induce a rapid reduction in root and shoot growth (Neumann, 1997), radicle length of *G. soja* decreases proportionally more than hypocotyl length, causing a reduction in the radicle/hypocotyl ratio (Table 2), but hypocotyl length of *L. sinense* and *S. sudanense* decreases proportionally more than radicle length, causing a increasing in the radicle/hypocotyl ratio (Table 2).

CONCLUSION

The germination pattern varied among species under salt stress in this experiment. The above results suggest that seeds of *G. soja* can germinate well and rapidly at lower level of salt (<200 mmol L^{-1}), but *G. soja* seeds couldn't germinate at higher level of salt (>200 mmol L^{-1}). 50 mmol L^{-1} salt concentrations is better to *S. sudanense*, germination percentage, germination energy and index reached highest at 50 mmol L^{-1} salt concentrations, suitable salt concentrations was beneficial to germinate of *S. sudanense* seeds. Germination percentage, germination energy and index of *L. sinense* seeds decreased with increasing concentration of NaCl, but seeds of *L. sinense* and *S. sudanense* can germinate at higher level of salt (400 mmol L^{-1}), germination rate of *L. sinense* and *S. sudanense* seeds had not obviously different at lower level of salt (<100 mmol L^{-1}).

The radicle growth for *G. soja* and *L. sinense* under salt stress were depressed with increasing salt, except for *S. sudanense*. The hypocotyl growth for *L. sinense* and *S. sudanense* under salt stress were depressed with increasing salt, except for *G. soja*. Three plant seeds had different physiological mechanism for germination under salt stress. Radicle hypocoty ratio of *L. sinense* and *S. sudanense* increased with increasing salt, it suggested that *L. sinense* and *S. sudanense* had the higher tolerance in shoot growth under NaCl stress, which is perhaps the reason for their wide utilisation for saline soil rehabilitation.

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