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Influence of NaCl and $NaHCO_3$ upon Salix Sungkianica Seed Germination and Seedling Growth

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Abstract The present study used the seeds and newborn seedings of *Salix sungkianica* as material, examining the influence of NaCl and $NaHCO_3$ solutions of different concentrations on the seed germination and anatomical structure of the roots and lamina of the seedings. The results indicate such facts: (1) The presents of NaCl and $NaHCO_3$ have a depressant effect on the seed germination, while a light concentration of $NaHCO_3$ could accelerate the sprout. (2) *S. sungkianica* seedings show strong endurance under the stress of 50 mmon/L NaCl and 10 mmon/L $NaHCO_3$ solutions, the seedings growth are better in 10 mmon/L $NaHCO_3$. (3) Under the stress of such solutions of high concentration, certain degrees of endurance also present in the roots and lamina, with the anatomical results like the thicken of root endodermis, the enlargement of vascular bundle in diameter, the vanish of aerenchyma tissures and the closure of lamina stoma.

Keywords *Salix sungkianica*; Saline–alkali stress; Seed germination; Seeding growth; Anatomical structure

Introduction

Salix sungkianica is a species of Salicaceae Salix deciduous shrub. It is native only to China, where it occurs throughout the country`s northern provinces like Heilongjiang. A sun species which usually inhabit in moisture environment like wet sand of the river bank. The plant primarily reproduction modes are through seed and cuttaging. The tree has a soft texture, a gentle and slender branch, which could be used as weaving material. A graceful tree form and strong resistance make it suitable for landscaping (Zhang, 2009). It`s deep-rootness and flood enduring character makes it the optimized species for dyke strengthening and bank protection (Cao et al., 2013). In all, *S. sungkianica* has a excellent application prospect.

Salix linearistipularis is among the few ligneous plants originally inhibited in the saline-alkali soil (SAL) of Songnen plain (Liu, 2006). Therefore, it proces a high degree of saline-alkaline tolerance. Whether *S. sungkianica* as a sibling species of *S. linearistipularis*, also process some degrees of tolerance towards SAL and, whether it coult be planted in such an environment are still unknown. To answer such questions, this study used *S. sungkianica* seeds and newborn seedings as material, by simulating natural conditions, to investigate the stress effects of NaCl and $NaHCO_3$ upon seed germination and the structure of lamina and roots of newborn seedings and; to examine the germination capacity, the growth of seedings and the structural changes of *S. sungkianica* roots and lamina in adverse situation like SAL. Therefore, to dertermine the adaptability of *S. sungkianica* towards SAL. The research itself will generate a theoretical significance on the development and utilization of wild *S. sungkianica* resources and, ecologically speaking, contribute to the enrichment of plant population in SAL.

1 Result and Discussion

1.1 Influence of NaCl on germination of *S. sungkianica* seeds and sapling structure

1.1.1 Influence of NaCl on the germination of *S. sungkianica* seeds

The germination rate and potential, germination index (GI), vital index (VI) and seeding length of *S.*

sungkianica seeds are on the declining curve as the concentration of NaCl rise, due to the stress effects (Table 1). A low concentration of NaCl suppress seed germination. The germination rate was 55.50±2.81% when NaCl=50 mmol/L, far lower than the control group (0 mmol/L, 77.00±2.12%) (P<0.01), the same with other indicators. And, germination happened on the second day when NaCl≤200 mmol/L; the third day when NaCl=250 mmol/L at a rate of 7.00±0.12%, with no growth of radicle though the cotyledon were unfolded. It is thus clear that the present of NaCl will significantly postpone seed germination and suppress the growth of sapling. Linear regression analysis was adopted to analyze the different NaCl concentrations and relative germination rates. The linear regression equation is y=−0.319x+94.174, R^2=0.974. The salt-tolerance limit, semi-lethal salt concentration, and salt concentration of *S. sungkianica* seeds are 216.84, 138.47, and 60.11 mmol/L, respectively. Most seeds treated with NaCl had gone dark-brown. In the re-germination test, those which color had changed showed no sign of re-germination while the unchanged ones did. And the re-germination rate dropped as the concentration rose. Therefore a deduction can be made that the stress effects of NaCl could be the combination of infiltration effect and ion effect (Andre et al., 2014; Zeng et al., 2006).

Table 1 Effect of NaCl on the growth of the seedlings of *S.sungkianica*

Item	Concentration of NaCl (mmol/L)					
	0	50	100	150	200	250
Germination percentage (%)	77.00±2.12a	55.50±2.81b	38.50±1.01c	31.00±0.45d	23.00±0.41e	7.00±0.12f
Seeding length(mm)	5.03±0.34a	3.84±0.21b	3.11±0.29c	2.64±0.18d	2.52±0.16e	2.32±0.12e
Germination potential (%)	74.50±2.08a	49.80±1.84b	36.50±0.72c	29.00±0.46d	20.50±0.38e	0.00±0.00f
Germination index	56.50±4.02a	38.63±2.71b	19.93±1.21c	12.33±0.87d	8.00±0.78e	5.25±0.64f
Vigor index	79.66±5.99a	45.19±5.52b	16.34±1.02c	7.76±0.81d	2.78±0.280e	0.39±0.02f

1.1.2 Influence of NaCl upon anatomical structure of *S. sungkianica* root

Root plays a significant role in the vegetation process of plants. When the environmental condition becomes worse, the external form and internal structure would be modified to accommodate the change (Wang et al., 1997). Figure 1-A shows the anatomical structure of *S. sungkianica* root. From out to core, the cross section is divided by epidermis, cortex and vascular bundle. The cortex contains exodermis, cortex, parenchymatous tissue and endodermis. The exodermis contains one or two layers of tightly aligned parenchymal cell. When the epidermis is destroyed, the exodermis become the defensive tissue, then the cortex and so on. Parenchymal cell is small and less regular shaped, some of them go rupture and becomes the aerenchyma, which is a peculiar structure of hydrophyte and hygrophyte. The *S. sungkianica*, which always grow along the river or in wetland, also process the aerenchyma. In recent years, research on halophyte has gaining some progress. Plants grow on saline-alkali land have difficulty in obtaining oxygen from the hardening soil, which also happens on hydrophyte and hygrophyte. To counter this adversity, the parenchymal cell go rupture, or separate from middle lamella, and the intercellular space among the biggish cell becomes connected, thus a clear aerenchyma comes into shape which ensures a normal respiratory metabolism (Werner et al., 1990; Wang, 2008). Therefore the *S. sungkianica* process a degree of salt-resistance. Endodermis contains several layers of small parenchymal cells. Vascular bundle includes primary xylem and primary phloem. When NaCl=50 mmon/L (Figure 1B), no significant difference is observed compare with the control group, the root maintains it's structure integrity with no obvious injury, only the endodermis has the tendency to intensified. Hence the *S. sungkianica* illustrates a strong salt-resistance when the concentration of NaCl is below 50 mmol/L. When NaCl=100 mmon/L (Figure 1C), the epidermis and exodermis vanished and the parenchymal cell of the cortex becomes the protective tissue. Together with the disappear of aerenchyma, a intensification of endodermis through the thickening of radial wall and inner-tangential wall of endodermis cells. Since the mould solute is absorbed by the root and transformed crosswise into the vessels in stele then the overground part.This structure will prevent toxic and pernicious ions into the plants, thus alleviate the harmful effects enforced by the salt-contained soil (Poppet et al., 1993).

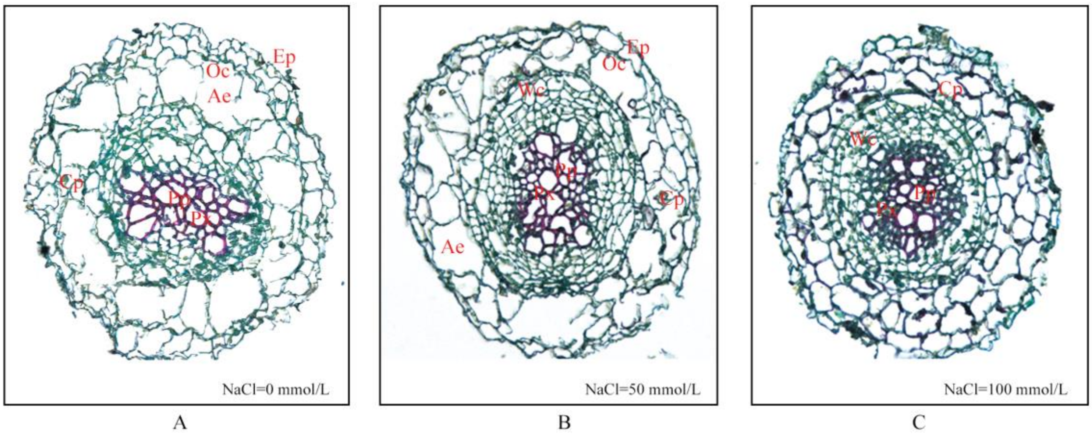

Figure 1 The effect of different concentrations of NaCl stress on root structure of *S. sungkianica* ×40

Note: Ep: Epidermis; Oc: Outer cortex; Cp: Cortical parenchyma; Ae: Aerenchyma; Wc: Within cortex; Px: Primary xylem; Pp: Primary phloem

1.1.3 Influence of NaCl upon anatomical structure of seeding lamina

Lamina is the place where photosynthesis and respiration happens, and the morphosis of lamina will be altered under salt or alkali environments (Bai et al., 2013). Figure 2-A shows the anatomical structure of *S. sungkianica* lamina of the control group, of which includes the upper epidermis, mesophyll tissue, lower epidermis, with no epidermal hair but covered with cuticle. The epidermis are made by monolayer cell, with most of its stomata open and concentrate in a particular area. The number of stoma is highly related with the conduction of water and air, and have an effect on photosynthesis and respiration. When the environment is suitable, the high density of stoma is good for photosynthesis; when the concentration of salt or alkali increase, some of the stomata closed in order to prevent water from over evaporation (Yang et al., 2011). After one month of growth, the mesophyll tissue (palisade and spongy parenchym) of the seedings have not completed it's differentiation. When NaCl=50 mmol/L (Figure 2-B), the lamina cells line up accordingly, and show a deepen of its color when dying with fast green, which means the cell walls have been thickened, the same goes with the control group (Wang, 2008). That all indicate a low concentration of NaCl presents no significant influence on lamina growth. When NaCl=100 mmol/L (Figure 2-C), the structure of blade is damaged, with no clear vision of mesophyll cell and a shrinkage of upper and lower epidermis cell; but resistance like the thicken of cuticle is well observed, which reduce water evaporation as well as providing structural support when the blade is dehydrated and wilting; and most of the stomata are closed to prevent water from over-evaporation (Yang et al., 2011).

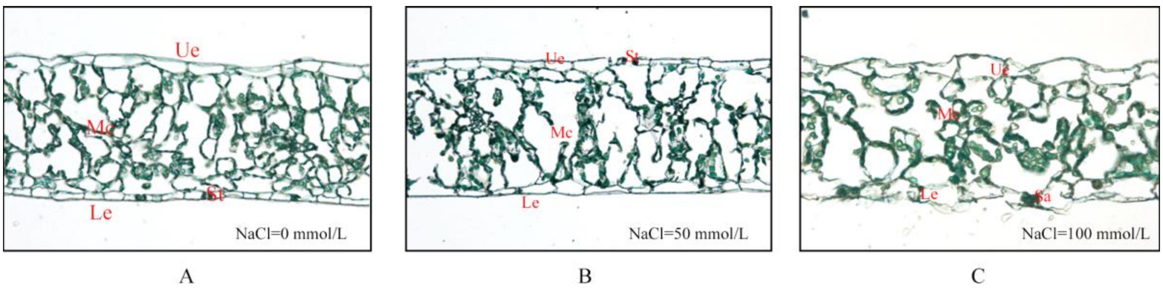

Figure 2 The effect of different concentrations of NaCl stress on leaf structure of *S. sungkianica* ×40

Note: Up: Upper epidermis; Lp: Lower epidermal; Mc: Mesophyll cells; St: Stoma

1.2 Influence of $NaHCO_3$ upon *S. sungkianica* seeds germination and seeding growth

1.2.1 Influence of $NaHCO_3$ upon seed germination

All *S. sungkianica* seeds germinated on the second day regardless the difference of $NaHCO_3$ concentration, which means the initial germination has no relation with it.For most plant species, seed germination rate is highest when it happens in pure water; and drops with the increase of $NaHCO_3$. In a low $NaHCO_3$ environment, however, some alkali-resistant seeds show a higher germination rate than they are in pure water (Zeng et al., 2014; A.El-Keblawy et al., 2005). Table 2 shows the *S. sungkianica* germination rate first rises then falls with the increase of $NaHCO_3$, when $NaHCO_3$=10 mmol/L, the number is 80.50±3.14%, which is higher than the control group (0 mmol/L, 77.00±2.12%), and shows no signs of significant differences (P>0.05). Other indexes like seeding length, germination potential, GI and VI all reach their highest points when $NaHCO_3$ =10 mmol/L, while the numbers are much lower in the control group (P<0.01). When $NaHCO_3$=30 mmol/L, the germination rate, thus 62.50 ± 1.99%, much lower than the control group; nonetheless, the rate was still as high as 18.00±0.83% when $NaHCO_3$=50 mmol/L. The linear regression equation is $y=-1.298x+113.159$, $R^2=0.954$, The alkali-tolerance limit, semi-lethal alkali concentration, and alkali concentration of *S. sungkianica* seeds are 67.92, 48.66, and 29.39 mmol/L, respectively. In the re-germination experiment, which all the un-germinated seeds were put into pure water, result shows that seeds under stress treatment mostly turn dark-brown and lose its germination ability; those color remain unchanged, however, can re-germinate. And the rate of dark-brown seed rose with the increase of $NaHCO_3$ concentration.

Table 2 Effect of $NaHCO_3$ on the growth of the seedlings of *S. sungkianica*

Item	Concentration of $NaHCO_3$ (mmol/L)					
	0	10	20	30	40	50
Germination percentage (%)	77.00±2.12ab	80.50±3.14a	70.00±2.23b	62.50±1.99c	54.00±1.58d	18.00±0.83e
Seeding length (mm)	5.03±0.34b	7.92±0.42a	7.76±0.39ab	5.01±0.19c	2.75±0.12d	2.64±0.11e
Germination potential (%)	74.50±2.08a	77.50±2.96a	67.00±2.19b	61.00±1.78c	52.00±1.45d	10.50±0.77e
Germination index	56.50±4.02a	58.88±3.89a	51.00±3.44b	46.13±3.65c	39.50±1.24d	19.50±1.22e
Vigor index	79.66±5.99b	128.94±5.32a	85.17±4.45b	52.12±3.11c	29.63±1.59d	5.52±2.03e

1.2.2 Influence of $NaHCO_3$ upon anatomical structure of seeding roots

Figure 3-A is the anatomical structure of *S. sungkianica* root. From out to core, the cross section is divided by epidermis, cortex and vascular bundle, with the presence of aerenchyma. The *S. sungkianica* showed a strong resistance when $NaHCO_3$ = 10 mmol/L (Figure 3B), the anatomical structure is still intact, with no significant differences with that of the control group. When $NaHCO_3$=30 mmol/L (Figure 3C), the epidermis and exodermis all disappear while the parenchymatous become the protective tissue, and the aerenchyma is also disappear; the radial and tangential walls of the cortical cell show a tendency of thicken, as for vascular bundle, the number of vessels in primary xylem decrease but enlarged in diameter, which is good for the water transportation and the physiological metabolism of plants (Wang, 2012).

1.2.3 Influence of $NaHCO_3$ upon the anatomical structure of seeding lamina

Figure 4-A shows the anatomical structure of *S. sungkianica* lamina of the control group, of which includes upper epidermis, mesophyll tissue and lower epidermis, with no epidermal hair but covered with cuticle. When $NaHCO_3$=10 mmol/L (Figure 4B), compare to control group, the lamina structure remains intact, lamina and both upper and lower epidermis cells are thickened, which means the lamina grew better in this condition (Werner et al., 1990); when $NaHCO_3$ = 30 mmol/L (Figure 4C), both upper and lower epidermis cells become shrunk and the

mesophyll cells are damaged, while most stomas are closed and section condition, it shows that concentration of $NaHCO_3$ presents significant wreck influence on lamina structure.

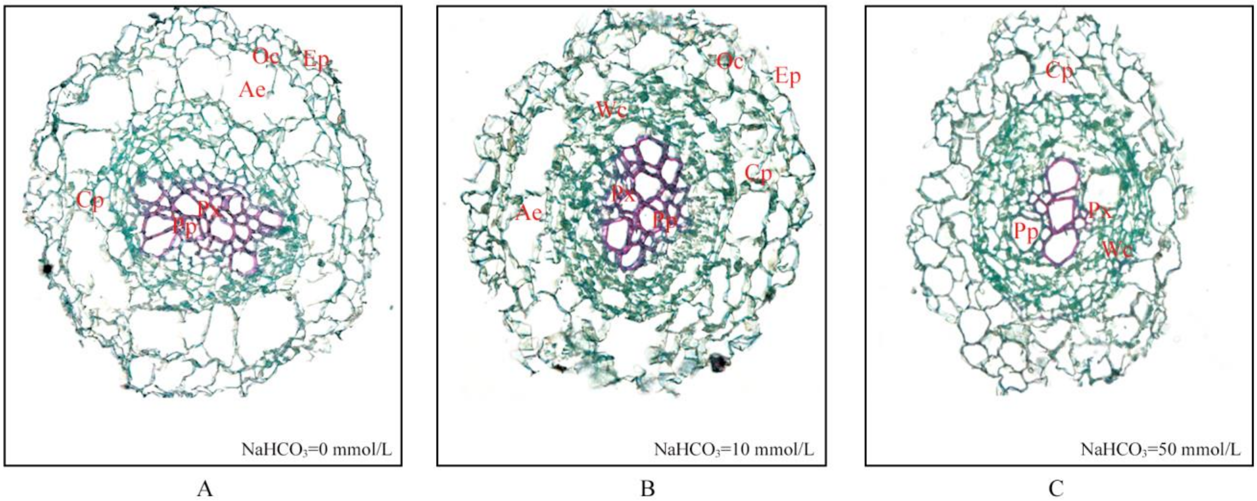

Figure 3 The effect of different concentrations of $NaHCO_3$ stress on root structure of *S. sungkianica* ×40

Note: Ep: Epidermis; Oc: Outer cortex; Cp: Cortical parenchyma; Ae: Aerenchyma; Wc: Within cortex; Px: Primary xylem; Pp: Primary phloem

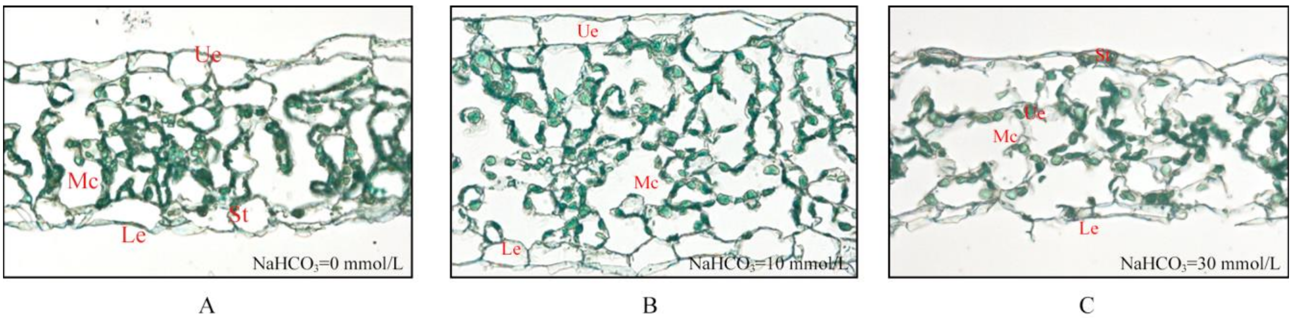

Figure 4 The effect of different concentrations of $NaHCO_3$ stress on leaf structure of *S. sungkianica* ×40

Note: Up: Upper epidermis; Lp: Lower epidermal; Mc: Mesophyll cells; St: Stoma

In conclusion, *S. sungkianica* seeds process a certain degree of salt-alkali resistance; when the concentration of NaCl is below 50 mmon/L or, $NaHCO_3$ under 10 mmon/L, the saplings are highly adaptable and a low concentration of $NaHCO_3$ could accelerate seeds germination and seedings development; under the suppress of a high concentration of salt or alkali, the anatomic results show that the roots and lamina of *S. sungkianica* all present a certain degree of resistance. Hence the thought of using *S. sungkianica* as reproducing tress species on salt-alkali soil is reasonable, of which should take full advantage of wild *S. sungkianica* resources and improves the quality of salt-alkali soil.

2 Materials and methods

2.1 Acquisition and preservation of seeds

The *S. sungkianica* seeds were collected in late May, 2013, from *S. sungkianica* trees along the bank of Songhuajiang river of Harbin city. Only the most voluptuous and non-verminous were selected and preserved in −80 degree for further test.

2.2 Seed germination test

NaCl concentration was set at 0, 50, 100, 150, 200, and 250 mmol/L, and $NaHCO_3$ concentration was divided into six gradients, namely, 0, 10, 20, 30, 40, and 50 mmol/L.Put 50 seeds from each of these concentrations on a culture dish, which is 10cm in diameter and filled only with 0.8% of agar. All the dishes were placed in the ZPQ−400 intelligent climatron.The illumination time was set as 12 h/d, intensity 5 grade, humidity 19−23 % and temperature 25℃. Four dishes were prepared for each concentration. A seed was germinated when 1mm of the radicle was observed breaking out of seed coat. Phenomena was recorded every day. All stress treatments were removed on the fourth day when 20 saplings were randomly selected from each dishes for the measuring and calculating of their length, raw weight, germination rate (%), germination potential (%), germination index (GI), vital index (VI) and salt-resistance. Non-germinating seeds were placed in a container with distilled water and subjected to germination experiment, and germinative number was then recorded (Guan et al., 2009).

2.3 Seeding cultivation

Based on the experiments of seed germination, the seeding cultivation test was conducted in five groups, the control group 0 (CK), two groups with NaCl was 50 and 100 mmon/L, and another two groups which contend 10 and 30 mmon/L $NaHCO_3$ .Each plant pot was filled with a mixture of compost and vermiculite (1:1), the seedings were planted uniformly and covered with a thin layer of soil, while all pots were covered with plastic wrap to prevent water from over-evaporation. Then each pot was irrigated at fixed intervals with specific treating fluid, maintaining the soil`s water content at 100%. Each group with six samples. After 30 days, the seedings were picked up and cleaned, then treated with FAA stationary liquid for 48 h for the final preparation of paraffin section.

2.4 Anatomical observation of root and lamina

The samples were made from taproot and central lamina, paraffin section were prepared with general method, staining with sarranine-fast green (counter-dying). All sections were observed with Olympus optical microscope (BX41) and photoed by camera (Wang et al., 2010).

2.5 Data analysis method

All data were plotted and analyzed with Excel 2003, significance of difference and correlation analysis are examined with SPSS 19.0. If analysis of variance was significance, then doing multiple comparative studies via Duncan method and equation of linear regression (Yan et al., 2013).

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