# Effect of Salinities Stress on Seed Germination of *Glycine. Soja*

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**Abstract**: Wild soybeans (*Glycine soja*), the ancestors of cultivated soybean (*Glycine max*), are important sources of major genes for resistance to pests, diseases and environmental stresses. The study of salt stress on the seed germination is invaluable for efficient utilization of saline-alkali soil resources and wild soybeans. Wild soybean, collected from Dezhou, Shandong province, were treated with NaCl, $Na_2SO_4$, $Na_2CO_3$ and the mixture of three solutions. Seed germination percentage, viability, index and the growth of radicle and hypocotyl of annual wild soybean were measured and analyzed. Results showed seed germination percentage, germination viability, germination index and the growth of radicle and hypocotyl annual wild soybean reduced with increasing of salt concentrations of $Na_2CO_3$ and the mixture. Low concentrations of $Na_2SO_4$ (10-50 mmol·$L^{-1}$), $Na_2CO_3$ (0-10 mmol·$L^{-1}$) promoted germination of seeds, while high concentrations of NaCl ( >200 mmol·$L^{-1}$), $Na_2SO_4$ (≥200 mmol·$L^{-1}$) and $Na_2CO_3$ (≥75 mmol·$L^{-1}$) restrained it. Under low concentration of salt solution (10 mmol·$L^{-1}$ $Na_2SO_4$), the radicel length of *Glycine soja* was longer than that of the control (distilled water). Results suggest that lower concentration of salt solution simulated the growth of radicle and hypocotyl of wild soybean, and the radicle is more sensitive to salt stress than that of hypocotyl.

**Key words**: Annual wild soybean; Salt stress; Germination rate; Germination viability

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# 不同盐分胁迫对野生大豆种子发芽的影响

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**摘 要**:一年生野生大豆是栽培大豆的野生近缘种和基因资源,具有耐盐碱、抗寒、抗病等优良性状。研究其种子萌发的耐盐性,为利用盐碱地资源和野大豆的引种驯化供依据。以山东德州一年生野大豆为材料,研究 NaCl、$Na_2SO_4$、$Na_2CO_3$及三者的混合盐的胁迫对野大豆种子的发芽率、发芽势、发芽指数及胚生长的影响。随盐溶液浓度的增加,野大豆种子的发芽率、发芽速度、发芽指数均呈下降趋势,而低浓度的 $Na_2SO_4$(10~50 mmol·$L^{-1}$),$Na_2CO_3$(0~10 mmol·$L^{-1}$)促进种子萌发,高浓度的 NaCl( >200 mmol·$L^{-1}$)、$Na_2SO_4$(≥200 mmol·$L^{-1}$)、$Na_2CO_3$(≥75 mmol·$L^{-1}$)抑制种子萌发;10 mmol·$L^{-1}$ $Na_2SO_4$处理下,其胚根胚轴长度都大于未经盐处理的,低浓度的盐分促进了胚根和胚轴的生长。结果表明低浓度的盐分促进一年生野生大豆胚的生长,野生大豆的胚根比胚轴对盐分更敏感。

**关键词**: 野生大豆;盐胁迫;发芽率;发芽势

Germination is a key part of plant life histories in saline environments as it determines whether or not the plants can establish successfully in certain areas[1]. In general, non-halophyte and halophyte respond to salinity in a similar way during the germination stage; the initial germination process is delayed under salt stress[2]. Seed of halophyte under natural condition are subjected to saline stress and usually it is dominated by NaCl. However, other sulfate and carbonate salt and their interaction plays a significant role in affecting seed ger-

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mination[3]. Salt can affected seed germination either by restricting the supply of water (osmotic affect) or causing specific injury through ions to the metabolic machinery (ionic effect). The mechanism for how salinity inhibits seed germination of halophytes in a highly saline environment has received a lot of attention in recent years. For example Bajji et al. [4] reported that the inhibitory effect of NaCl stress on seed germination is mainly due to an osmotic effect, rather than specific ion toxicity, in halophytes of *A. halimus*. However, others reported both osmotic and ionic effects[5].

In general, Authors have concluded that salinity is inhibitory to the germination in following ways: either causing a complete inhibition of the germination process at salinities beyond the tolerance limits of a species, or delaying the germination of seeds, at salinities that cause some stress to seeds but do not prevent germination, causing the loss of seeds viability by high salinity [6-7].

Wild soybean (*Glycine soja* Sieb. et Zucc) is an annual herb, it is a slender twiner and grows in fields, along roadsides and riverbanks[8]. The plant has trifoliate leaves. Seeds are oval-oblong. Flower color is pink, lilac or purple. Wild soybean has been thought to be the wild progenitor of the domesticated soybean (*G. max* [L.] Merr.) which was thought to have originated in China [9] and is grown in China and adjacent countries or regions. Some wild soybeans have been used as a genetic resource for improvement on the cultivars.

Annual wild soybeans (*Glycine soja*) are distributed throughout East Asia, including Korea, Japan and the far eastern regions of Russia, and are centered in China[10]. The geographical distribution of wild soybeans in China is very wide. The horizontal distribution is from Tahe County, Heilongjiang (latitude: 53) in the north to Xiangzhou County, Guangxi (latitude: 24) in the south and from Raohe County, Heilongjiang (longitude: 134), in the east to Chayu County, Tibet (longitude: 97), in the west. The vertical distribution is from 1.8 to 2650 m above sea level[11].

Annual wild soybean, the ancestors of cultivated soybeans (*G. max*), is important sources of major genes for resistance to pests, diseases and environmental stresses. It can be used for improving the soil organic matter. In addition, it is a good source of food and forage, it's grain, stem and leaf can be used as feedstuffs. In the current, salt tolerance of cultivated soybeans (*G. max*) is limited and the genetic basis of more and more narrow circumstances, wild soybeans is therefore considered to be an important gene source of soybean[12]. Because soil mainly contain NaCl, $Na_2SO_4$ and $Na_2CO_3$, NaCl is the most abundant salt in many salinized lacotions in the North China. We used them in our experiment. The present report describes the effect of various concentrations of sodium chloride, sodium sulfate, sodium carbonate and the mixture of three solutions on seed germination, seedling growth of annual wild soybean.

## 1 Materials and Methods

Annual wild soybeans were collected from a natural grassland located in the east of Dezhou, Shandong province (China) (38°0′N, 117°34′E) in October 2006. Dry seeds were stored in a refrigerator at $<4$℃ for 6 months before being used. Because the seed coat was hard, the seed coat was break by mechanical scarification. The trial was conducted at Biology Department, Dezhou University, Dezhou, Shandong, China, in April 2007. After being surface sterilized with 0.1% $KMnO_4$ solution for 20 min and rinsed in tap water. 40 seeds of annual wild soybean were placed on two layers of filter paper in a 1 200 mm Petri dishes. The filter paper was moistened with about 10 mL distilled water (control) or solution of salt. Each experiment was replicated three times. Seeds were considered to have germinated when the emerging radicle was at least 5 mm.

Germination tests were conducted in incubators with a regime of 13 h light at 25℃ and 11 h darkness at 20℃. The filter paper was moistened with distilled water for the controls, or with aqueous solution of NaCl, $Na_2SO_4$, $Na_2CO_4$ and the mixture of three solutions. To determine the effect of different salt on germination, solution of four salts were used, containing increasing levels of NaCl, $Na_2SO_4$, $Na_2CO_4$ and the mixture of three solutions (Table 1). In the course of the experiment, the Petri dishes were covered with lids and kept in a controlled environment. About two-thirds of volume of the water or solution in each Petri dish was replaced daily to remain the filter paper under the same concentration. The number of germinated seeds was counted daily, and the final percentage germination was recorded 6 d after incubation when the number of germination is constant. The lengths of radicel and hypocotyl were measured 15 times in each repeat when seed germination ended on the sixth day.

Table 1 The composition, concentraion( mmol · $L^{-1}$ ) and pH value of a set of salt treatment

| No. of Treatment | NaCl | pH | $Na_2SO_4$ | pH | $Na_2CO_3$ | pH | Mixed salt | | | | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $Na^+$ | $Cl^-$ | $SO_4^{2-}$ | $CO_3^{2-}$ | |
| CK | 0 | 7 | 0 | 7 | 0 | 7 | 0 | 0 | 0 | 0 | 7 |
| 1 | 50 | 7.06 | 10 | 7.32 | 10 | 9.38 | 90 | 50 | 10 | 10 | 9.26 |
| 2 | 200 | 6.68 | 50 | 7.46 | 50 | 10.21 | 400 | 200 | 50 | 50 | 10.01 |
| 3 | 400 | 6.26 | 150 | 7.60 | 75 | 10.41 | 850 | 400 | 150 | 75 | 10.12 |
| 4 | 600 | 6.02 | 200 | 7.80 | 150 | 10.63 | 1300 | 600 | 200 | 150 | 10.26 |
| 5 | 800 | 5.80 | 400 | 8.50 | 200 | 10.73 | 2000 | 800 | 400 | 200 | 10.34 |

The germination percentage, germination viability, germination index and relative germination percentages, were determined by the following formulae, respectively:

Germination percentage(%) = [a/b]100,

Germination viability(%) = [d/b]100,

Germination index $= \sum Gt/Dt$,

Relative germination percentages = f/e,

Where a is the total number of seeds germinated on each day, b is the total number of seeds, d is the total number of seeds germinated on second day, $G_t$ is the number of seeds germinated on t days, $D_t$ is the number of germination days corresponding, f is germination percentage in salts solution, e is germination percentage of control.

Statistical analyses were carried out using SPSS 13.0. A one-way ANOVA was carried out to determine the difference among treatment group means for germination percent, germination viability, germination index and relative percentages, and significance is indicated in the form of superscripted letters in the tables.

## 2 Results and discussion

### 2.1 Effects of salt stress on seed germination

The germination pattern varied under different salts in this experiment. Seed germination was affected by different concentrations of NaCl, $Na_2SO_4$, $Na_2CO_3$ and the mixture of three solutions. An obvious decrease was observed in annual wild soybean when the concentration of NaCl, $Na_2SO_4$, $Na_2CO_3$ and the mixture of three solutions was increased. But the germination percentage was not significantly different among 0, 50 mmol · $L^{-1}$ NaCl, 10, 50 mmol · $L^{-1}$ $Na_2SO_4$ and 10 mmol · $L^{-1}$ $Na_2CO_3$. In distilled water, germination percentage of annual wild soybean was 96.67%. Low concentrations of $Na_2SO_4$ (10 − 50 mmol · $L^{-1}$) and $Na_2CO_3$ (0 − 10 mmol · $L^{-1}$) promoted the germination percentage of seeds, while high concentrations of NaCl (>200 mmol · $L^{-1}$), $Na_2SO_4$ (≥200 mmol · $L^{-1}$), $Na_2CO_3$ (>75 mmol · $L^{-1}$) and mixture salt 2 restrained it. There were 90.00% seeds germinated at 200 mmol · $L^{-1}$ NaCl treatment, 59.17% seeds germinated at 200 mmol · $L^{-1}$ $Na_2SO_4$ treatment, 84.17% seeds germinated at 75 mmol · $L^{-1}$ $Na_2CO_3$ treatment and 94.17% seeds germinated at mixture salt 1 treatment. At the higher level of salinity (≥400 mmol · $L^{-1}$ NaCl and $Na_2SO_4$ concentration, ≥150 mmol · $L^{-1}$ $Na_2CO_3$ and mixture 2) no seed germinated. The high salt stress inhibits the seed germination completely (Table 2). The germination viability, index of annual wild soybeans decreased significantly with increasing of salt concentration of NaCl, $Na_2SO_4$, $Na_2CO_3$ and the mixture of three solutions, and have significant difference with the control.

Table 2 The effect of different salt stress on germination percentage, viability, index and relative germination rate

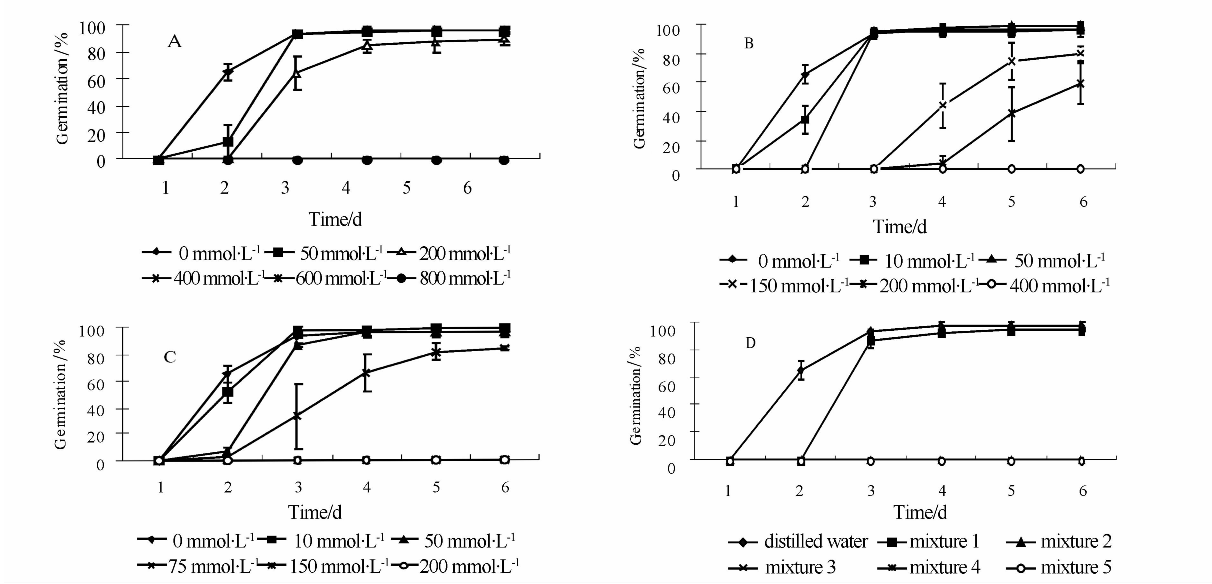
| Salt sort | Concentration/ mmol · $L^{-1}$ | Germination percentage/% | Germination viability | Germination index | Relative germination percentage |
|---|---|---|---|---|---|
| NaCl | 0 | 96.67 ±2.89a | 65.00 ±6.61a | 32.11 ±1.51a | 100.00 ±2.99a |
| | 50 | 95.83 ±1.44a | 13.33 ±11.81b | 21.69 ±2.56a | 99.14 ±1.49a |
| | 200 | 90.00 ±5.00b | 0c | 16.06 ±1.55b | 93.10 ±5.17b |
| | 400 | 0c | 0c | 0c | 0c |
| | 600 | 0c | 0c | 0c | 0c |
| | 800 | 0c | 0c | 0c | 0c |
| $Na_2SO_4$ | 0 | 96.67 ±2.89a | 65.00 ±6.61a | 32.11 ±1.51a | 100.00 ±2.99a |
| | 10 | 95.83 ±5.20a | 34.17 ±10.10b | 25.84 ±2.33a | 99.14 ±5.38a |
| | 50 | 98.33 ±1.44a | 0c | 19.42 ±0.36a | 101.72 ±1.40a |
| | 150 | 80.00 ±5.00b | 0c | 9.36 ±1.16b | 82.76 ±5.17b |
| | 200 | 59.17 ±14.22c | 0c | 5.64 ±1.36b | 61.21 ±14.71c |
| | 400 | 0d | 0c | 0b | 0d |
| $Na_2CO_3$ | 0 | 96.67 ±2.89a | 65.00 ±6.61a | 32.11 ±1.51a | 100.00 ±2.99a |
| | 10 | 99.17 ±1.44a | 51.67 ±8.04b | 30.08 ±1.38a | 102.59 ±1.49a |
| | 50 | 96.67 ±3.82a | 7.50 ±2.50c | 20.14 ±0.13a | 100.00 ±3.95a |
| | 75 | 84.17 ±1.44b | 2.50 ±2.50c | 13.28 ±2.77b | 87.07 ±1.49b |
| | 150 | 0c | 0c | 0c | 0c |
| | 200 | 0c | 0c | 0c | 0c |
| Mixture salt | 0 | 96.67 ±2.89a | 65.00 ±6.61a | 32.11 ±1.51a | 100.00 ±2.99a |
| | Mixture 1 | 94.17 ±1.44b | 0b | 18.22 ±0.57b | 97.41 ±1.49b |
| | Mixture 2 | 0c | 0b | 0c | 0c |
| | Mixture 3 | 0c | 0b | 0c | 0c |
| | Mixture 4 | 0c | 0b | 0c | 0c |
| | Mixture 5 | 0c | 0b | 0c | 0c |

Optimal germination for most halophyte has been reported in non-saline conditions [13-14]. Seeds of annual wild soybeans germinated better in non-saline controls and any increase in salinity progressively inhibited seed germination and no seed germinated beyond the 400 mmol · $L^{-1}$ NaCl, $Na_2SO_4$, 150 mmol · $L^{-1}$ $Na_2CO_3$ and the mixture 2 salt treatment. An increase in salinity can induce reduction in the percentage of seeds germinated, a delay in the initiation of the germination process, and dormancy of seeds [6,15]. Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity[16]. 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 of the seed (ion toxicity)[16]. Our data clear indicate that seed germination percentage increased with the increase of salt concentrations in suitable range of salt concentration (Table 2).

Not only germination percentage but also the speed of germination was affected by salt. In distilled water and the low concentration of salt solution, seed germinated on second day, in 200 mmol · $L^{-1}$ NaCl, 50 mmol · $L^{-1}$ $Na_2SO_4$, 75 mmol · $L^{-1}$ $Na_2CO_3$ and the mixture 2 seed germination on third day. Higher concentration of salt delay in the initiation of the germination process. Salinity beyond the tolerance limits can cause complete inhibition of the germination process, even lead to loss of seeds viability (Fig. 1A, Fig. 1B, Fig. 1C, Fig. 1D).

## 2.2 Effects of salinity on radicle and hypocotyl growth

The radicle growth for annual wild soybeans under salt stress were depressed with increasing of salt concentration. For annual wild soybeans, significant differences were found in radicle and hypocotyle lengths depending on salinity levels. Elongation of radicle and hypocotyle were affected by increasing salt concentration severely (Table 3). Concentration of 200 mmol · $L^{-1}$ NaCl treatment reduced radicle in annual wild soybeans from 5.07 to 0.81 cm. Concentration of 75 mmol · $L^{-1}$ $Na_2CO_3$ treatment reduced radicle in annual wild soybeans from 5.07 to 0.59 cm. The inhibition of radicle growth under salt stress may be due to the reduction in the turgor of the radicle cells[17].




A: NaCl A; B: $Na_2SO_4$; C: $Na_2CO_3$; D: Mixture of three solutions

Fig. 1 Effect of different salt on germination rate of annual wild soybean

Under salt stress, the length of radicle and hypocotyl decreased as salt concentrations increased. But the radicle length was longer than those of control at 10 mmol · $L^{-1}$ $Na_2SO_4$, the proper lower concentration stimulated the growth of radicle. The hypocotyle length was longer than those of control at 50 mmol · $L^{-1}$ NaCl, 10 mmol · $L^{-1}$ $Na_2SO_4$ and 10 mmol · $L^{-1}$ $Na_2CO_3$, the proper lower concentration stimulated the growth of hypocotyl. Radicle and hypocotyl length depended on the type and concentration of salts, our result indicate the radicle is more sensitive to salinity than hypocotyl (Table 3). The sensitivity of radicle and hypocotyl of annual wild soybeans to different kind and gradient salt stress were different.

Table 3 Effect of different salt stress on radicle length, hypocotyl length of annual wild soybean

| Salt sort | Concentration /mmol · $L^{-1}$ | Radicle length /cm | Hypocotyl length /cm | Salt sort | Concentration /mmol · $L^{-1}$ | Radicle length /cm | Hypocotyl length /cm |
|---|---|---|---|---|---|---|---|
| NaCl | 0 | 5.07 ±1.42 | 2.77 ±0.47 | $Na_2CO_3$ | 0 | 5.07 ±1.42 | 2.77 ±0.47 |
| | 50 | 3.98 ±0.92 | 3.45 ±0.40 | | 10 | 2.73 ±1.06 | 3.10 ±0.71 |
| | 200 | 0.81 ±0.23 | 1.00 ±0.20 | | 50 | 0.59 ±0.16 | 1.11 ±0.36 |
| | 400 | 0 | 0 | | 75 | 0.59 ±0.18 | 0.73 ±0.05 |
| | 600 | 0 | 0 | | 150 | 0.00 ±0.00 | 0.00 ±0.00 |
| | 800 | 0 | 0 | | 200 | 0.00 ±0.00 | 0.00 ±0.00 |
| $Na_2SO_4$ | 0 | 5.07 ±1.42 | 2.77 ±0.47 | Mixture salt | 0 | 5.07 ±1.42 | 2.77 ±0.47 |
| | 10 | 5.23 ±1.11 | 4.32 ±1.56 | | mixture 1 | 0.96 ±0.47 | 1.44 ±0.38 |
| | 50 | 2.24 ±0.47 | 2.71 ±0.98 | | mixture 2 | 0 | 0 |
| | 150 | 0.33 ±0.07 | 0.72 ±0.26 | | mixture 3 | 0 | 0 |
| | 200 | 0.43 ±0.09 | 0.05 ±0.01 | | mixture 4 | 0 | 0 |
| | 400 | 0 | 0 | | mixture 5 | 0 | 0 |

What constitutes the favorable level of salinity might, however, vary greatly with species due to the differences in their physiological mechanism of resisting salt stress. Euhalophytes have great salt-tolerance than recretohalophytes. Liu et al[18] reported that *Limonium bicolor* (a recretohalophtye) had a lower range of favorable salinity than *S. salsa* (an euhalophtye). In many other halophytes, most small seedlings survived and radicle continued to elongate at salinities that allowed seeds to germinate. Appropriate salinity in soil is necessary for the growth of most halophytes[19].

## 3 Conclusion

All salts significantly inhibited seed germination of annual wild soybean. The magnitude of inhibition of different salts was in the order: mixture salt > NaCl > $Na_2CO_3$ > $Na_2SO_4$.

Low salinity (10 – 50 mmol · $L^{-1}$ $Na_2SO_4$ and 0 – 10 mmol · $L^{-1}$ $Na_2CO_3$) promoted seed germination and high salinity inhibited seed germination. The high salt stress inhibits the seed germination completely. The germination viability and germination index of annual wild soybeans decreased with increasing concentration of NaCl, $Na_2SO_4$, $Na_2CO_3$ and the mixture of three solutions.

The radicle and hypocotyl had different response to the salts; the radicle is more sensitive than that of hypocotyl.

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