

Full Length Research Paper

# ***Sophora alopecuroides* L. response to salt stress at germination and seedling growth stages**

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***Sophora alopecuroides* L. is a perennial herb that has been widely used in pharmaceutical industry due to the high alkaloid level in its seeds which can be considered for medical usages. In this study, response of *S. alopecuroides* to five NaCl concentrations (0, 50, 100, 150 and 200 mM) was investigated. The study was evaluated in two steps, seed germination and seedling growth. The results showed that sodium chloride induced salt stress significantly affected seed germination and seedling growth of *S. alopecuroides* in a concentration-dependent manner; generally, low concentrations enhanced seed germination and seedling growth while high concentrations limited plant growth. Seed germination was more sensitive to salt stress than seedling growth, and root growth was more sensitive to salt stress than shoot growth in seedling growth stage. In conclusion, *S. alopecuroides* were resistant 100 mM NaCl and in higher concentrations of NaCl, seed germination and seedling growth parameters reduced.**

**Key words:** *Sophora alopecuroides* L., salt stress, seed germination, seedling growth.

## INTRODUCTION

Salinity is one of the most important abiotic stresses reducing the yield of wide variety of crops all over the world, high concentrations of salts in soils account for large decreases in crop production. Soil contaminated salts (ECe > 4 dS m<sup>-1</sup> or 40 mM NaCl or osmotic potential < 0.117 MPa) are defined as salinity land, which directly affects plant growth and development in vegetative growth prior to reproductive stage, especially crop species (Allakhverdiev et al., 2000; Sairam and Tyagi, 2004; Chinnusamy et al., 2005; Ashraf et al., 2008;

more than 770 000 km<sup>2</sup> of land is salt-affected by secondary salinization: 20% of irrigated land, and about Ashraf, 2009; Suriyan and Chalernpol, 2009). Globally, 2% of dry land agricultural land (FAO, 2006). Salt stress leads to suppression of plant growth and development at all growth stages, however, depending upon plant species, certain stages such as germination, seedling or flowering stage could be the most critical stages for salt stress. Seed germination is first critical and the most sensitive stage in the life cycles of plants (Ashraf and Mehmood, 1990; Ahmad et al., 2009) and the seeds exposed to unfavorable environment conditions like salt stress may have to compromise the seedlings establishment (Albuquerque and Carvalho, 2003). Seed germination, proteins and proline could be used as physiological criteria of early selection for salt tolerant leguminous plants (Taffouo et al., 2009). For economical production in saline soils, it is important that many commercially released crop species require to be tested for salinity using a rapid reliable screening method. As a result, in terms of either development of salt tolerant plants or determination of suitable salt tolerant crops for

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**Abbreviations:** PI, promptness index; GSI, germination stress tolerance index; PHSI, plant height stress tolerance index; RLSI, root length stress tolerance index; DMSI, dry matter stress tolerance index; LSD, least significant difference.

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a region, selection and evaluation of salt tolerance of plants has a preliminary importance. Looking for species or tolerant varieties able to develop in saline medium is one of the possible ways to solve salt stress problem. The plant *S. alopecuroides* L. (family Leguminosae) is widely distributed in desert and semi-desert area of northwest China. It grown at the edges of fields, banks and less frequently on sand dunes, from sea level to 1000 m.

The seeds of *S. alopecuroides* are commonly used in traditional Chinese medicine. *S. alopecuroides* are known to contain quinolizidine alkaloids as their principal bioactive constituents, which have been shown to exhibit sedative, analgesic, antipyretic, anti-inflammatory, anti-tumour and notable antiviral activities (Atta-ur-Rahman et al., 2000; Ding et al., 2006; Guo et al., 2005; Kinghorn and Balandrin, 1984; Wang et al., 2003). Quinolizidine alkaloids also play a chemical defensive role against herbivores and pathogen micro-organisms (Wink, 1988, 1992). Besides, *S. alopecuroides* is fine sand-fixation plants and important forage plant resources. However, in some areas, *S. alopecuroides* production faces salinity problem. Therefore, the aim of this study was to evaluate effect of different NaCl levels on seed germination and seedling growth, further to determine the tolerance range of *S. alopecuroides* to salt.

## MATERIALS AND METHODS

Experiment was carried out in the laboratory of the department of pharmaceutical, Ningxia Medical University. *S. alopecuroides* seeds were supplied by the local farmers. Sodium chloride was used as a salt stimulator and five salt stress levels of zero (control), 50, 100, 150, and 200 mM were developed by dissolving 3, 6, 9 and 12 g of NaCl per 1000 mL distilled water.

### Seed germination

The seeds of *S. alopecuroides* were surface sterilized in 0.1% H<sub>2</sub>O<sub>2</sub> for 20 min, rinsed 5 times with distilled water and imbibed in deionized water for 24 h at 25°C. Thirty seeds were sown in Petri dishes (9 cm dia) lined with two layers of filter paper. Five mL of designated treatment solution was added to each Petri dish and distilled water (5 mL) was used as control. All Petri dishes were covered with lids and placed in incubator at 20°C for 18 days. Added periodically to maintain the filter wet during the course of the experiment. Only seeds with 1 mm emerged radicle were considered germinated. The numbers of germinated seeds were recorded daily.

### Seedling growth

Twenty five pre-germinated seeds were placed on filter papers in Petri dish (9 cm dia) containing 5 ml of salt solution or distilled water (control). The Petri dishes were placed in an incubator 12/12 h light, darkness at 20°C. The fresh and dry weights root and shoot length, and the number of secondary roots was measured after 10 days. Plant dry weight determined after drying at 60°C to a constant weight. From these measurements, the promptness index (PI), germination stress tolerance index (GSI), plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI)

and dry matter stress tolerance index (DMSI) were calculated using the following formulae given by Ashraf et al. (2006).  $PI = nd2 (1.00) + nd4 (0.75) + nd6 (0.5) + nd8 (0.25)$ , where n is the number of seeds germinated at day d,  $GSI (\%) = [PI \text{ of stressed seeds} / PI \text{ control seeds}] \times 100$ ,  $PHSI = (\text{Plant height of stressed plant} / \text{Plant height of control plants}) \times 100$ ,  $RLSI = (\text{Root length stressed plant} / \text{Root length of control plants}) \times 100$ ,  $DMSI = (\text{Dry matter of stressed plant} / \text{Dry matter of control plants}) \times 100$ . The experiment was in a completely randomized design with 4 replicates for each experimental unit. All experimental data were subjected to analysis of variance (ANOVA) using SPSS and the treatment means were tested using least significant difference (LSD) at  $P < 0.05$  level.

## RESULTS AND DISCUSSION

### Seed germination

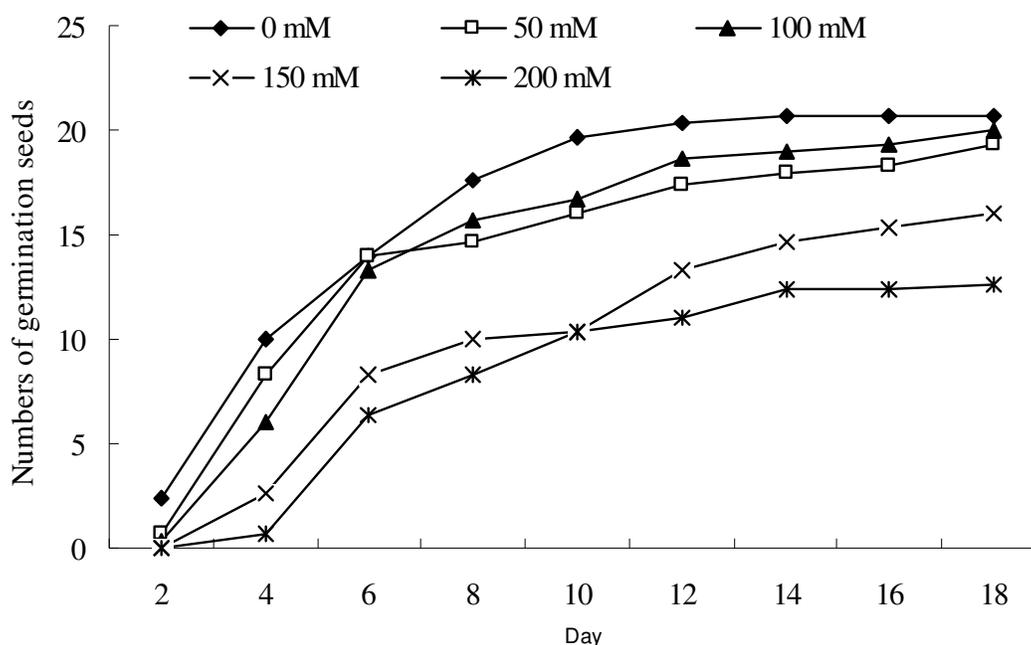
Seeds of *S. alopecuroides* germinated best in the absence of salt. The response of germination rate, germination energy, germination index and vigor index to salt stress induced by sodium chloride was different depending on concentrations. The salt stress has no remarkable effect on germination rate and germination energy at low concentration of 100 mM or below ( $P < 0.05$ ) (Table 1). At 150 mM or higher of salt stress, however, germination rate and germination energy was significantly decreased compared to the control. As shown in Table 1, the salt stress at all concentrations significantly decreased the germination index and vigor index, with a greater degree of inhibition by a higher concentration, and only between the 50 and 100 mM concentration was there no significant difference. The NaCl solutions at the 150 mM concentration delayed the seed germination till 4<sup>th</sup> day (Figure 1). According to Asraf et al. (2003), NaCl has an inhibitory effect on sunflower seed germination. Increasing salinity levels caused delay in seedling emergence as a result of reducing cell division and plant growth metabolism (Maas and Nieman, 1978). At the highest NaCl concentration, seedling emergence was inhibited and some of seeds did not emerge at all (Turhan and Ayaz, 2004). Taffouo et al. (2009) reported that the increase of NaCl concentration affected negatively the seeds germination rates of five leguminous species. The results of these previous studies are in line with results of our present study in *S. alopecuroides*.

### Seedling growth

As shown in Table 2, applying NaCl at a rate of 50 mM significantly increased the plant height, root length, number of secondary roots and root dry weight at the measured stage. However, not only these parameter were not further increased by the application of a higher levels of NaCl, but also the plant height and root length were significantly decreased at high concentrations of 150 mM or above, and the number of secondary roots and root dry weight were significantly decreased at high concentrations of 100 mM or above, and this decrease was greater at higher concentration. The shoot dry weight

**Table 1.** Effect of different NaCl concentrations on germination parameters of *S. alopecuroides*.

NaCl concentration (mM)	Germination rate %	Germination energy %	Germination index	Vigor index
0	68.89a	46.67a	27.77a	4.31a
50	64.44a	46.66a	23.22b	3.69b
100	66.67a	44.45a	22.69b	3.41b
150	53.33b	27.78b	14.44c	1.84c
200	42.22c	21.11b	11.25c	0.94d

**Figure 1.** Effect of different NaCl concentrations on number of *S. alopecuroides* seeds germination. Each point represents the mean of four replicate experiments**Table 2.** Effect of different NaCl concentrations on seedling growth parameters of *S. alopecuroides*.

NaCl concentration (mM)	Plant height (cm)	Root length (cm)	Secondary roots (number/plant)	Shoot dry weight (g/10 plants)	Root dry weight (g/10 plants)	Root/shoot
0	4.17b	3.75b	1.43c	0.0875c	0.0215b	0.2461a
50	5.61a	4.44a	3.30a	0.0927c	0.0244a	0.2639a
100	5.63a	3.99ab	2.30b	0.1046b	0.0193b	0.1848b
150	2.95c	2.58c	0.97cd	0.1254a	0.0152c	0.1214c
200	1.63d	1.64d	0.38d	0.1043b	0.0108d	0.1029c

a, b and c difference between concentrations in the same parameter, identical letters mean no significant differences.

was not significantly different between NaCl treatment at the 50 mM concentration compared to the control, but the shoot dry weight was significantly increased at high concentrations of 100 mM or above, and this increase was the greatest at 150 mM. Delgado and Sanchez

(1999) also found that sunflower seedling growth was affected from NaCl treatments. The effect of salinity concentration on plant growth has been studied in different leguminous species. Taffouo et al. (2004) found that the increase in salt concentration significantly

**Table 3.** Effect of different NaCl concentrations on germination stress tolerance index (GSI), plant height stress tolerance index (PHSI), root length stress tolerance index and dry matter stress tolerance index (DMSI) of *S. alopecuroides*.

NaCl concentration (mM)	GSI%	PHSI	RLSI	DMSI
50	87.20a	118.40a	135.09a	127.16a
100	80.36a	106.49a	134.45a	126.70a
150	47.32b	68.71b	70.74b	122.78a
200	33.63b	43.64c	39.01c	92.35b

a b and c difference between concentrations in the same parameter, identical letters mean no significant differences.

reduced the production of dry weight at 50 mM of NaCl in *Glycine max* and *Phaseolus vulgaris* (sensitive glycophytes). In *Vigna unguiculata* (moderately tolerant glycophyte) and *Mucuna poggei* (facultative halophyte) effect of salinity was observed for at least 100 mM of NaCl. In the natural halophyte (*Phaseolus adenanthus*) the presence of salt did not significantly affect the leaves production of dry matter. The leaves dry weights of *Gossypium hirsutum* (tolerant glycophyte) were also reduced significantly in plants irrigated with saline nutrient solution in contrast with control plants (Taffouo et al., 2006, 2009).

These results indicating that the effect of salt stress on plant depending on the salt concentration and plant species.

### Ratio of root to shoot biomass

Sodium chloride application at a rate of 50 mM significantly increased the ratio of root biomass to above ground biomass. The increase root growth with NaCl application at the low concentration would have the potential to enhance root's absorptive capacity and nutrient uptake in natural and agricultural ecosystems. However, the ratio of root biomass to above ground biomass was significantly decreased by the application of a higher level of NaCl, and this decrease was greater at the higher concentration. This suggesting that the effect of NaCl stress on ratio of root to shoot biomass of *S. alopecuroides* in a concentration-dependent manner.

### Stress indices

The seed germination and seedling growth responded differently to the sodium chloride induced salt stress. NaCl induced salt stress significantly reduced the GSI (Table 3), the minimum GSI values (33.63) was recorded at the highest 200 mM NaCl which was maximum (87.20) under 50 mM NaCl in *S. alopecuroides*. PHSI, RLSI and DMSI were decreased with the increase in NaCl concentrations and were minimum (43.64, 39.01 and 92.35, respectively) at the highest concentration (200 mM) of NaCl.

Above results indicating that seed germination was

more sensitive to NaCl stress than seedling growth, and root length was more sensitive to NaCl stress than plant height.

### Conclusions

These results indicated that *S. alopecuroides* plants are resistant to 100 mM NaCl and in higher concentrations of NaCl, seed germination and seedling growth parameters reduced. Thus *S. alopecuroides* is the salt tolerant specie.

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