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GERMINATION RESPONSE TO DIFFERING SALINITY LEVELS FOR 18 GRASS SPECIES FROM THE SALINE-ALKALINE GRASSLANDS OF THE SONGNEN PLAIN, CHINA

HONGYUAN MA[1,2*], BINGSHENG LV[1,2], XIAOWEI LI[1,2] AND ZHENGWEI LIANG[1,2*]

[1]*Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, Jilin 130012, China*
[2]*Da'an Sodic Land Experiment Station of China, Da'an, Jilin, 131317 China*
[*]*Corresponding authors e-mail: mahongyuan1620@gmail.com; liangzhengwei@neigae.ac.cn*

Abstract

Seed germination responses to salinity differ among grass species. The germination of halophytes in saline substrates is a useful criterion for selecting tolerant plants for restoring saline environments. The salt tolerance of germinating seeds from 18 native grass species that greow in the saline-alkali grasslands of the Songnen Plain in Northeastern China. Was studied The effects of different concentrations of sodium chloride (NaCl) (0-500mM) on the germination of seeds were tested at 28°C using a 12-h photoperiod. The 18 species showed different levels of salt tolerance and were classified into 4 groups. *Artemisia scoparia* was the most tolerant of high salt concentrations, with high germination rates even at 300mM NaCl (91.1%). This species also had the shortest germination time (T_{50}=0.5 d). *Echinochloa crusgalli* and *Cynanchum chinense* were moderately salt tolerant, showing high germination rates (84.7%-100%) at150-200mM NaCl. *Medicago sativa*, *Suaeda salsa*, *Herba taraxaci*, and *Plantago asiatica* were the next most tolerant, with germination rates of 56.7%-95.0% at 25-150mM NaCl. The remaining 11 species did not tolerate saline conditions or had generally low germination rates regardless of saline concentration. Because of their high salt tolerance, *A. scoparia*, *E. crusgalli*, and *C. chinense* have the greatest potential and can be used in vegetative restoration of saline soils in the arid and semiarid regions of the Songnen Plain.

Introduction

There are about one billion hectares of salt-affected land worldwide, and these regions are affected by different degrees of salinity (Flowers, 2004). Salinity is an environmental factor that has a critical influence on seed germination and plant establishment (Katembe *et al*., 1998; Zhang *et al*., 2011; Abbas *et al*., 2013). In recent decades, vegetative bioremediation has been found to be an efficient, inexpensive, and environmentally acceptable strategy to ameliorate saline soils (Qadir *et al*., 2006; Rajput *et al*., 2013). To become established in a saline environment, plants must first adapt to the saline conditions at the germination stage; seed germination responses to environmental parameters determine their distribution in saline environments (Ungar, 2001; Tobe *et al*., 2000). Species restricted to harsh environments typically have distinctive seed germination properties that enable them to cope with severe disturbances and unpredictable conditions (Leyer & Pross, 2009; Anwar-ul-Haq *et al*., 2013). To some extent, ecological restoration in those areas will depend on the availability of naturally dispersed seeds from suitable plants (Wolters *et al*., 2008; Jiang *et al*., 2010). However, some places may require seeds to be intentionally introduced. Thus, it is necessary and important to know the salt tolerances of different species seeds that grow in saline and alkaline conditions.

Seed germination of species in saline substrates is a legitimate criterion in selecting halophytes for saline environments (Sosa *et al*., 2005), and developing vegetation restoration projects (Zheng *et al*., 2005). However, halophyte species vary in their tolerance to salinity during seed germination. Baskin & Baskin (1998) listed 65 halophytes in which germination was found to be reduced by salinity. Germination rates are typically highest in fresh water, and they rapidly decline as salinity increases. However, for some species, low concentrations of NaCl do not inhibit germination; they may even enhance it (Croser *et al*., 2001; Huang *et al*., 2003; Muhammad & Hussain, 2012). However, previous studies have primarily focused on seed germination responses for only 1 or 2 specific species. Few studies have assessed the salt tolerances of germinating seeds taken from numerous plant species within a region (Bayuelo-Jiménez *et al*., 2002; Easton & Kleindorfer, 2009; Al-Hawija *et al*., 2012). In general, the information available on the germination of halophytic seeds is far from complete (Khan & Gul, 2006): athough there are roughly 2400 known halophytic species, the availability of data regarding germination is patchy. A better understanding of interspecific variation to salinity stress would be constructive from both basic and applied perspectives, and would be especially important for identifying plant species for specific restoration and remediation projects (Easton & Kleindorfer 2009).

The Songnen grassland (42°30′-51°20 N′, 121°40′-128°30′E), in western Jilin province, southwestern Heilongjiang province, and parts of Inner Mongolia, China, is one of the most important grazing and pasture areas in the region, but it is also one of the world's largest areas of saline soil. The grassland has been degraded as a result of overgrazing and human development, which have increased steadily since the late 1970s (Jiang *et al*., 2010). In this region, most studies are focused on *Leymus chinensis* (Ma *et al*., 2008), *Lathyrus quinquenervius*, and *Medicago sativa* (Gao *et al*., 2011), and little is known about the salt tolerance of many other species native to this region. In this study, we collected 18 species native to this region and investigated the salt tolerance of their seeds.

In order to better restore the vegetation, studies focus on that screening for native salt-tolerant species for their salt tolerances are needed. The aims of our study were to (1) compare the salt tolerance thresholds for germination of 18 species native to the Songnen Plain, China and (2) select halophytes that can be successfully be used for vegetation restoration in this area.

Materials and Methods

Experimental site description: The Songnen grassland is 30500 km^2 is located at the eastern end of the Eurasian steppe belt. We collected seeds at the Da'an Sodic Land Experiment Station (45°35′58″–45°36′28″N, 123°50′27″–123°51′31″E), in the western part of the Songnen grassland. The climate of this area is semi arid to arid. The mean annual air temperature is 5.93°C, and the average temperatures range from -14.93°C in January (minimum) to 23.60°C in July (maxium). Based on 30 years of data recording (1971-2000), the mean annual rainfall in this area is 350-450 mm, of which 70%-80% falls from July to September. Overall, the mean annual evaporation (1600-800 mm) greatly exceeds mean annual precipitation. In this grassland, the vegetation is heterogeneous across different degradational and successional stages. For example, in lightly degraded areas, *L. chinensis* remains the dominant species although the height and density have decreased since the 1970s. In moderately degraded areas, monodominant communities of *Chloris virgata* are present, and in severely degraded areas, *Suaeda salsa* communities occur. Where degradation is extreme, there bare saline-alkaline patches that do not contain any plants (Ma *et al*., 2012). Chernozem is the main type of soil in the grassland and it contains 2.0% soil organic carbon, 1.4% soil humus, and 0.15% total nitrogen (N) (Qu & Guo 2003). In this region, salt affected soils contain 0.5 to 1.0% total salt (Chi *et al*., 2012).

Seed collection: Seeds (including true seeds and diaspores) of 18 plant species were collected from different communities of the study site from June to October in 2011. Seeds of the same species were collected from at least 5 plants and mixed together. After air-drying, seeds were put into paper bags and stored at room temperature until the germination experiments were initiated in February 2012.

Seed germination under different salinities: The effects of different sodium chloride (NaCl) concentrations (0, 25, 50, 75, 100, 150, 200, 300, 400, and 500mM NaCl) on seed germination were tested. Four replicates were tested for each NaCl concentration, and each replicate contained 20-50 seeds (depending on the number of seeds available for each species). Seeds were sown in 9-cm diameter Petri dishes containing two layers of filter paper saturated with 10 mL of a salt solution. Seed-containing Petri dishes incubated in an artificial climate incubator (Haerbin, China) at 28°C under a 12-h photoperiod consisting of fluorescent and incandescent white light of 54 µmol m^{-2}s^{-1}. Before placing them in incubators, Petri dishes were first sealed with Parafilm to decrease the evaporation of water. Subsequently, they were randomly distributed in incubators and their positions were changed daily. Seed germination was counted daily until no germination occurred for 3 consecutive days. The time required for the germination percentage to reach 50% of final germination (T_{50}), which gave an estimate of germination speed, was calculated according to the following formula (Farooq *et al*., 2005): $T_{50}=t_i+(N/2-n_i)(t_j-t_i)/(n_j-n_i)$, where N is the final number of seeds that underwent germination, and n_i and n_j are the total number of seeds that had germinated (by adjacent counts) at times t_i and t_j, when n_i□<N/2□<n_j. Salt tolerance indices were calculated as described by Li *et al*., (2010): t%= □ /G_0×100, where □ is the average number of seeds that germinated under a specific salt solution (from 25mM to 500mM NaCl), and G_0 is the number of seeds that germinated in the distilled water control (0mM NaCl).

Statistical analysis: Data analysis was carried out using SPSS version 16 (SPSS, Chicago, IL, USA). Germination data were normalized using the arcsine transformation. Data in figures are presented as arithmetic means□±□SE. The means were tested using one-way analysis of variance (ANOVA). Duncan's tests were used for multiple comparisons between treatments. A cluster analysis on the salt tolerance of the 18 species tested was based on the seed germination profiles at different salt levels. Results were considered statistically significant at α□=□0.05.

Results

At the control condition (0mM NaCl), the rates of seed germination varied considerably among the 18 grass species. A total of 5 species, *S. salsa*, *Echinochloa crusgalli*, *Cynanchum chinense*, *Artemisia scoparia*, *Plantago asiatica*, and *Ixeris sonchifolia*, had germination rates >90%; 3 species, *Medicago sativa*, *Amaranthus retroflexus*, and *Herba taraxaci*, had germination rates between 50% and 70%; and the remaining 9 species, *Chloris virgata*, *Atriplex centralasiatica*, *Melilotus officinalis*, *Artemisia capillaries*, *Chenopodium acuminatum*, *Hordeum brevisubulatum*, *Allium chrysanthum*, *Leonurus japonicus*, and *S. brachotus* had germination rates <50%, and in this group, no germination was observed for *M. officinalis* (Fig. 1).

Each species' threshold value of NaCl concentration, which was identified as the greatest NaCl concentration that did not significantly inhibit germination, varied among species (Fig. 1). The threshold value was ≤300mM NaCl for *A. scoparia*; ≤ 200mM for *E. crusgalli* and *A. centralasiatica*; ≤150mM for *C. chinense*, *H. taraxaci*, and *A. chrysanthum*; ≤100mM for *M. officinalis*, *C. virgata*, and *L. japonicus*; and ≤25mM for *P. asiatica*, *A. retroflexus*, *S. salsa*, *C. acuminatum*, and *A. capillaries*. However, even 25mM NaCl significantly inhibited the germination of *I. sonchifolia*, *S. brachotus*, and *H. brevisubulatum* seeds. One-way ANOVA verified that seed germination in all the species tested was significantly inhibited by the saline conditions with p<0.01, except for

A. chrysanthum, for which $p<0.05$ (Table 1).

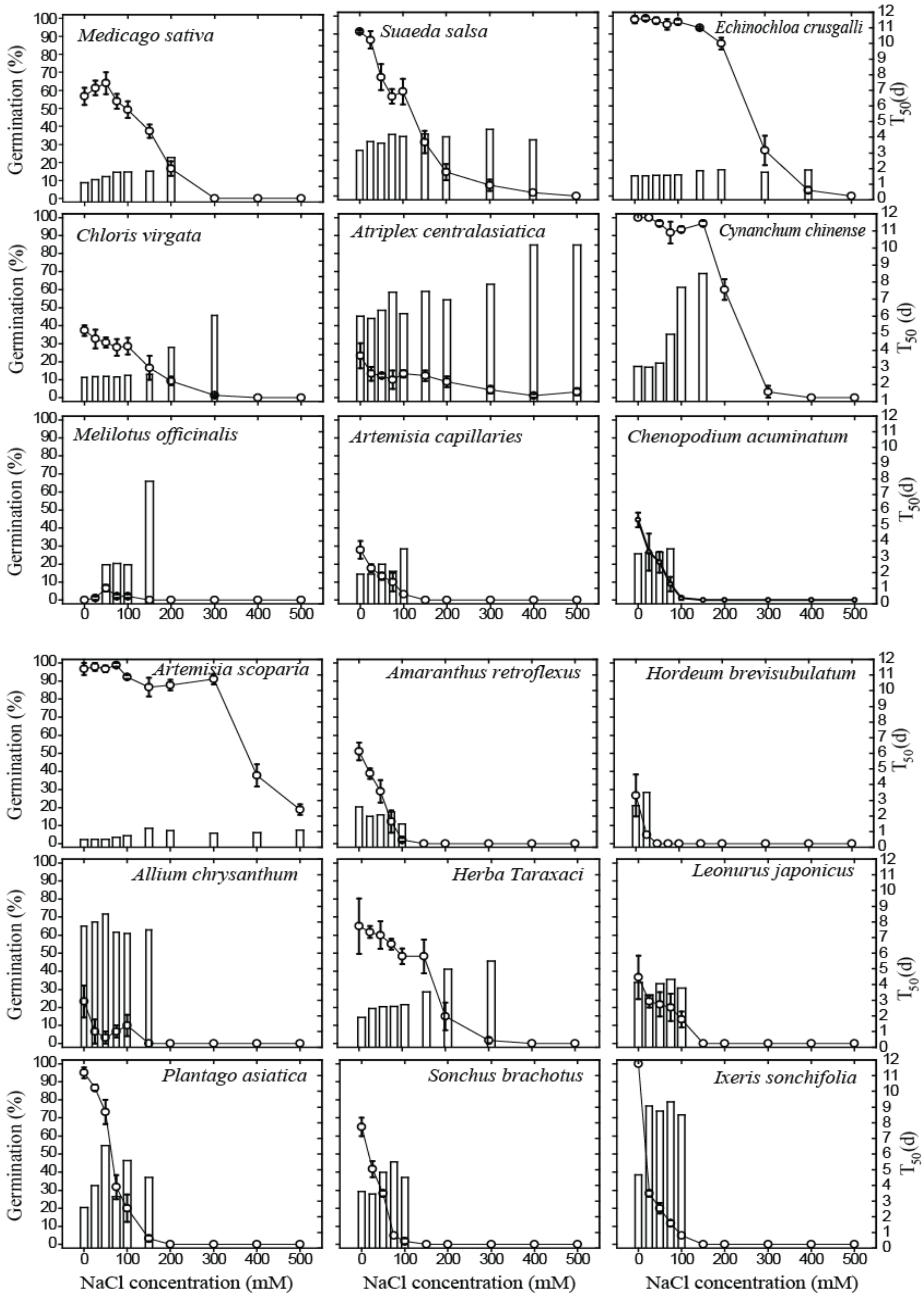

Fig. 1. Effects of sodium chloride (NaCl) concentration on seed germination (lines) and T_{50} (bars) of 18 halophyte species. Concentrations of NaCl ranged from 0 mM to 500 mM. Values are Means±SE. At some NaCl concentrations where there was no seed germination, no T_{50} data was available.

Table 1. One-way ANOVA for effects of NaCl concentration on seed germination of the 18 species.

Species	df	SS	MS	F-value	P-value
Medicago sativa	9	19726.5	2191.8	49.966	0.000
Suaeda salsa	9	33154.8	3683.9	59.290	0.000
Echinochloa crusgalli	9	47398.5	5266.5	174.003	0.000
Chloris virgata	9	5890.8	654.5	15.835	0.000
Atriplex centralasiatica	9	1149.9	127.8	3.584	0.008
Cynanchum chinense	9	54720.8	6080.1	228.0	0.000
Melilotus officinalis	9	133.3	14.8	4.967	0.001
Artemisia capillaries	9	2567.6	285.3	16.330	0.000
Chenopodium acuminatum	9	6506.6	723.0	14.012	0.000
Artemisia scoparia	9	21374.9	2375.0	33.713	0.000
Amaranthus retroflexus	9	10003.9	1111.5	33.713	0.000
Hordeum brevisubulatum	9	1907.5	211.9	5.190	0.000
Allium chrysanthum	9	1413.3	157.0	2.771	0.028
Herba Taraxaci	9	20303.3	2255.9	17.579	0.000
Leonurus japonicas	9	4840.8	537.9	6.454	0.000
Plantago asiatica	9	41153.3	4572.6	94.605	0.000
Sonchus brachotus	9	14354.2	1594.9	106.327	0.000
Ixeris sonchifolia	9	25924.2	2880.5	691.311	0.000

The T_{50} values also differed depending on the species and the salinity. As shown in Fig. 1, the T_{50} values found in this study ranged from 0.5 d to 7.7 d at the control condition, 0mM NaCl. *A. scoparia* had the shortest T_{50} of 0.5 d, and the longest T_{50} (7.7 d) was found for *A. chrysanthum*. As the NaCl concentration increased, the T_{50} values for some species tended to increase (as in *I. sonchifolia* and *P. asiatica*), but for some other species, such as *L. chinensis*, *E. crusgalli*, and *L. japanicus*, T_{50} values did not significantly change at low NaCl concentrations.

Based on the germination rates of seeds at different salinity levels, a cluster analysis was used to classify each of the 18 species into one of 4 groups (Fig. 2). Group 1 included *A. scoparia*, which had the highest salt tolerance; Group 2 included *E. crusgalli* and *C. chinense,* which had moderate salt tolerances; Group 3 included *M. sativa*, *S. salsa*, *H. taraxaci*, and *P. asiatica*, which had low salt tolerances; and Group 4 consisted of the remaining 11 species, which were either salt sensitive at all salinity levels or had low germination rates overall.

Discussion

Our results confirmed that halophytes have the ability to germinate under saline conditions, but the level of salt tolerance varied across species, which has been shown by several other studies (e.g., Ashraf & Harris, 2004; Easton & Kleindorfer, 2009). In our study, the germination rate of *A. scoparia* seeds at 300 mM NaCl did not differ from the control, as was the case for *E. crusgalli* and *A. centralasiatica* seeds in 200 mM NaCl. This level of tolerance has been identified for other halophytes, such as *Haloxylon ammodendron* (200 mM, Huang *et al*., 2003), *Salsola affinis* (400 mM; Wei *et al*., 2008), and *Bromus inermis* (20 mM; Yang *et al*., 2009). However, we also found that seed germination in other species (*I. sonchifolia*) decreased linearly with an increase in salinity (Fig. 1). Some other reports have shown that low salt concentrations can stimulate germination in some species (Croser *et al*., 2001), and this was observed for *M. sativa* in our study, in which the germination rate increased slightly at the 25 mM NaCl compared to the control (0 mM NaCl) (Fig. 1).

Seed germination, even for highly salt tolerant halophytes, is sensitive to soil salinity (Debez *et al*., 2004; Vicente *et al*., 2004). As the salinity increased in our study, the threshold concentration, at which seed germination ceased, differed between species. We found that most species would not germinate at 300 mM NaCl, but *A. scoparia* was still able to germinate at 500 mM NaCl (18.7%) (Fig. 1). Yildirim *et al*., (2011) found that *Physalis peruviana* and *Physalis ixocarpa* seeds germinated only when the NaCl concentration was lower than 90 mM. *Halocnemum strobilaceum* and *Kalidium capsicum* germinated when NaCl concentrations were lower than 500 and 400 mM, respectively (Qu *et al*., 2008). However, even being exposed to 1000 mM NaCl solution, *Arthrocnemum macrostachyum* still showed 10% germination success (Khan & Gul, 1998).

In our study, some species showed delayed seed germination in response to elevated NaCl concentrations (Fig. 1), which has been reported for several other halophytes, such as *Chenopodium quinoa* (Koyro & Eisa, 2008), *Leymus chinensis* (Ma *et al*., 2008), and *Acacia longifolia* (Morais *et al*., 2012). Bayuelo-Jiménez *et al*., (2002) also reported that the T_{50} of *Phaseolus leptostachyus* increased by 6 days with the addition of 180 mM NaCl. Rapid seed germination is frequently observed in halophytes, and they may use it as be an adaptive strategy to utilize soil water with reduced salinity levels (Easton & Kleindorfer, 2009). For halophytes, which tend to grow in high-salinity environments, rapid germination in soil water with reduced salinity, such as after the salinity has been diluted by rainfall, may be important for survival.

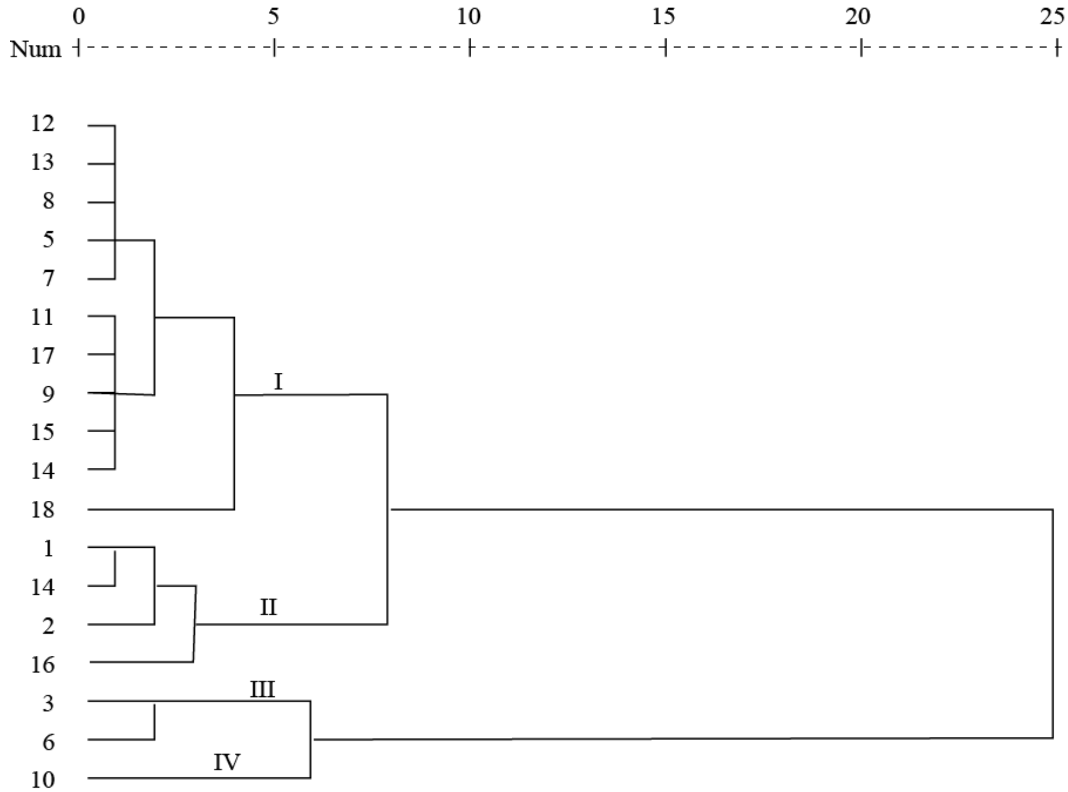

Fig. 2 Cluster analysis of the 18 species tested based on their seed germination at various NaCl concentrations. The numbers 1, 2, 3, ……, 18 represent the species of *M.sativa*, *S. salsa*, *E. crusgalli*, *C. virgata*, *A. centralasiatica*, *C. chinense*, *M. officinalis*, *A. capillaries*, *C. acuminatum*, *A. scoparia*, *A. retroflexus*, *H. brevisubulatum*, *A. chrysanthum*, *H. taraxaci*, *L. japonicus*, *P. asiatica*, *S. brachotus*, *I. sonchifolia* respectively. I, II, III and IVrepresent four classifications with different salt tolerance.

Several halophytic plant species have been used in the reclamation and vegetation restoration of salt-affected soils (Tanner & Parham, 2010). Quantifying the germination of halophytes in saline substrates is a legitimate way to assess their tolerance in saline environments (Dasgan *et al*., 2002; Ghaloo *et al*., 2011; Sosa *et al*., 2005; Khan & Gul, 2006). In our study, the 18 species tested were classified into groups according to their germination responses to salt stress. *A. scoparia*, *E. crusgalli*, and *C. chinense* were the most salt tolerant species, and *M. sativa*, *S. salsa*, *H. taraxaci* and *P. asiatica* showed moderate salt tolerance. These halophytes might be good candidates to use as primer plants for phyto-remediation of the secondary salinized soils in the Songnen grasslands.

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