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Salinity effect and seed priming treatments on the germination of *Suaeda salsa* in the tidal marsh of the Yellow River estuary

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The effects of salinity and seed priming treatments (hydropriming, water, KNO_3 and KH_2PO_3) on the germination of the euhalophyte *Suaeda salsa* in intertidal zone of the Yellow River estuary were investigated. Results show that the seed germination percentage decreased with increasing NaCl concentration, and at the high NaCl level (800 mM), the lowest germination percentage was recorded. At the low NaCl levels, the highest germination rate was observed on day two and the seedling length was promoted slightly. In contrast, the germination delayed and the seedling length decreased at the high salinity. According to the survival functions, we also found that, at the low salinity, the seeds germinated quickly at the initial days and then the germination rate decreased, while few seeds germinated at the initial days at the high salinity. From the results of germination percentage and seedling length, we found that the effect of Yellow river water on germination was similar to the 400 mM NaCl. For priming treatments, the hydropriming has no promotion to the seeds germination, but it promoted the seedling growth at the river water and 400 mM NaCl. Seeds primed with KNO_3 could improve the germination at the low salinity, while priming with KH_2PO_4 could improve the seedling growth at the high salinity, indicating that seed priming with proper nutrient (N, P) solutions could improve the germination or seedling growth as the nutrient (N, P) availability in the soil of *S. salsa* marsh was very limited.

Key words: *Suaeda salsa*, germination, salinity, priming, Yellow River estuary.

INTRODUCTION

About 9.5 hundred million hectares of the world's soil are saline soil, not including large area of secondarily salinized soil in cultivated land (Zhao et al., 2002). Soil salinization results from salt water intrusion, run off from salt used during road deicing, brine contamination associated with the extraction of oil and gas products, or a buildup of inorganic ions in irrigated regions (Keiffer and Ungar, 2001). Plant germination and growth (especially

germination) is detrimentally impacted by salinity because germination is a key stage in the life cycle of plants in saline environment and it determines whether or not the plants can establish successfully in certain areas.

One important effect of salts on the initial growth of plants is toxicity. High intracellular concentrations of Na^+ and/or Cl^- are toxic, since they inhibit the activity of many enzymatic systems and some cellular processes, such as protein synthesis or mRNA processing (Serrano, 1996; Yeo, 1998; Zhu, 2001; Forment et al., 2002). Another inhibitory effect of salinity is mainly due to osmotic effect. The osmotic component results from dehydration and

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loss of turgor induced by external solutes (Greenway and Munns, 1980; Serrano and Gaxiola, 1994). Some studies have shown that the inhibitory effect of NaCl on seed germination was more severe than that of iso-osmotic polyethyleneglycol (PEG). Katembe et al., (1998) found that higher concentrations of NaCl (~1.0 MPa) more inhibitory to the germination and seedling root elongation of *Atriplex prostrata* and *Atriplex patula* than iso-osmotic PEG solutions. Duan et al., (2004) also found that at the ~1.8 MPa and ~2.7 MPa PEG-6000 solution, there was no *Glaucum L.* seed germination, while at the ~1.8 MPa NaCl solution, there was also 2.5% seeds germination. However, some studies also found that the inhibition in germination at equivalent water potential of NaCl and PEG was mainly due to an osmotic effect rather than salt toxicity. Demir and Mavi (2008) discovered that both NaCl and PEG inhibited germination and seedling growth in both the varieties, but the effects of NaCl compared to PEG was less on germination and seedling growth. Similar results were reported by Kaya et al., (2006), Yagmur and Kaydan (2008), and Gunjan (2010) when they studied *Phaseolus mungo*, sunflower and triticale. The effects of salinity on seed germination were mainly attributed to osmotic effect and/or ion toxicity, and these different mechanisms might be related to the plant species (Song et al., 2005).

There are many strategies to overcome the negative effects of salinity. An alternative strategy for the possibilities to overcome salt stress is by seed treatments with hydropriming or other treatments (Yagmur and Kaydan, 2008). Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many plants. Korkmaz and Pill (2003) found that among all the priming agents (K_3PO_4 , KH_2PO_4 or $NH_4H_2PO_4$ and PEG 8000), only priming with KH_2PO_4 improved the germination synchrony of low vigor cultivar 'Greenlakes'. Kaya et al. (2006) applied 500 ppm KNO_3 and distilled water to immerse the sunflower (*Helianthus annuus L.*) seeds, and showed that both seed treatments gave better performance than control under salt and drought stresses with clear effectiveness of hydropriming in improving the germination percentage at low water potential. Some other studies also indicated that priming with deionized water (Casenave and Toselli, 2007; Toselli and Casenave, 2005; Ghassemi-Golezani et al., 2008), KNO_3 (Amjad et al., 2007; Demir and Mavi, 2004) and KH_2PO_3 (Nerson and Govers, 1986) are effective to the germination of seeds.

The Yellow River is well known as a sediment-laden river. Every year, approximately 1.05×10^7 tons of sediment is carried to the estuary (Cui et al., 2009) and deposited in the delta where the flow rate slows down resulting in vast area of floodplain and special wetland landscape (Xu et al., 2002; Wang et al., 2004). Yellow River delta is the important back-up land resource of China; however, about half of the area (44.3×10^4 hm²) distributes salinized soil (Zhang et al., 2002). *Suaeda*

salsa (L.) Pall. (Chenopodiaceae) is the most important succulent halophytic herb, which can grow in both saline soils and intertidal zone, and has strong adaptations to the environmental stresses, such as high salinity, flooding, and sediment burial. In the growing season, the leaves and stems of *S. salsa* are generally red-violet due to the accumulation of Na^+ , Cl^- and betacyanin (Zhang and Zhao, 1998), and compartmentalization of these ions in the vacuole to their lower osmotic potential under high saline conditions (Zhao et al., 2003). Due to the ratio of evaporation and rainfall being 3.5 in this region and the freshwater supply from the Yellow River was significantly decreased in recent years, the salinity of marsh soil is very high ($>8g\ kg^{-1}$) and approximately 70% area of salt marsh is differently salinized (Guan et al., 2001). Therefore, exploring an effective seed priming technique, to a great extent, directly determined the restoration effect of the degraded *S. salsa* marsh.

In this study, the distilled water, (hydropriming) KNO_3 and KH_2PO_3 are selected to treat *S. salsa* seeds due to two main reasons. Firstly, many current studies have proved that KNO_3 and KH_2PO_3 had significant effects on seed germination (Korkmaz and Pill, 2003; Kaya et al., 2006; Yagmur and Kaydan, 2008). Secondly, the nutrient status of *S. salsa* marsh is also an important factor. In general, N and P are the essential nutrients to the growth of marsh plants (Mistch and Gosselin, 2000), and the N/P ratio is an effective indicator to estimate nutrient limitation and N saturation (Tessier and Raynal, 2003). Koerselman and Meuleman (1996) found that marsh plant growth was limited by N if N/P ratio was less than 14. If N/P ratio was between 14 and 16, growth was limited by both N and P. If N/P ratio was more than 16, growth was limited by P. Mou (2010) applied the relationships to study the nutrient limitation of *S. salsa*, and found that the plant was limited by N in the low salinity status, while in high salinity status, the plant was limited by both N and P, and the limitation degree of P was very high, but the P limitation was more significant.

The purposes of this paper were: (i) to investigate the germination of *S. salsa* seeds at different salinity levels, and identify the actual effect of Yellow River water to the germination of *S. salsa* seeds; (ii) to assess the various treatments (hydropriming, KNO_3 and KH_2PO_3) as a pre-sowing seed treatment; (iii) to examine the effects of salinity levels on the inhibition of germination and seedling damage.

MATERIALS AND METHODS

Study region and seeds sampling

Seeds of *S. salsa* were collected in October 2009 from multiple individuals at an experimental plot in *S. salsa* marsh wetland (37°45'57.0"N, 119°09'40.7"E) in intertidal zone of the Yellow River estuary, located in the Nature Reserve of Yellow River Delta (37°35'N to 38°12'N and 118°33'E to 119°20'E) in Dongying City, Shandong Province, China. Seeds were cleaned, dried at room

temperature for two to three weeks, then stored at 8°C under dry and dark conditions. The nature reserve is of typical continental monsoon climate with distinctive seasons; summer is warm and rainy while winter is cold. The annual average temperature is 12.1°C, the frost-free period is 196 days, and the effective accumulative temperature is about 4300°C. Annual evaporation is 1962 mm and annual precipitation is 551.6 mm, with about 70% of precipitation occurring between June and August. The soils in the study area are dominated by intrazonal tide soil and salt soil (Tian et al., 2005). Compared with the intrazonal tide soil, the dissolubility salt content in the upper layer (0 to 20 cm) of salt soil is much higher (>8g kg⁻¹), and its grain composition is dominated by sand and silt (50 to 80%). The main vegetations include *Phragmites australis*, *S. salsa*, *Triarrhena sacchariflora*, *Myriophyllum spicatum*, *Tamarix chinensis* and *Limonium sinense*. *S. salsa*, an annual C₃ plant, is one of the most important halophytes in the Yellow River estuary and can tolerate coastal seawater salinity and salinity fluctuations resulting from water evaporation and tidal inundation (Han et al., 2005). It generally germinates in late April, blooms in July, matures in late September and completely dies in late November (Gu, 1998).

Determination of seed germination and seedling growth

The experiment was carried out at the Laboratory of Coastal Wetland Ecology in Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences in October 2010. The experimental design was two factors factorial (4 × 7) arranged in a completely randomized design; with three replications and 50 seeds per replicate. The first factor was seed treatments (control, hydropriming, KNO₃ and KH₂PO₃), the second factor was osmotic potential levels (deionized water (control), river water (sampling date: 31 September 2010), and 100, 200, 400, 600 and 800 mM NaCl solutions). The treated and untreated seeds were then transferred to Petri dishes (50 seeds per Petri dish with three replications) containing one filter paper moistened with 4 ml of control solution or the same solution added with NaCl. In order to avoid water losses, Petri dishes were sealed with an impermeable colourless parafilm. Germination was carried out in a germination chamber with a regime of 12 h light at 25°C and 12 h darkness at 20°C. The number of germinated seeds was counted every day during 10 days from the start of the test. Seeds were considered to have germinated when the emerging radicle was at least 1 mm. At the end of the experiment, the length of seedlings in each petri dish was measured. Mean value of length in each Petri dish was used as one replicate. Three replicates were used for each treatment.

Priming techniques

Three priming medium were used such as deionized water, 500 ppm KNO₃ and KH₂PO₃ solutions. For hydropriming, *S. salsa* seeds were immersed in deionized water at room temperature for 6 h. For KNO₃ and KH₂PO₃ treatments, the seeds were immersed in 500 ppm KNO₃ and KH₂PO₃ solution at room temperature for 2 h. Since the KNO₃ and KH₂PO₃ solutions have the same concentration, we can compare their effects on germination. The treated seeds were removed from priming media at the same time, and dried back to their original moisture content. The control treatment consisted of untreated seed.

Germination rate

The germination rate for *S. salsa* seed was estimated using a modified Timson Index:

$$\text{Germination rate} = \sum \left[\left(\frac{G^1}{t} \right) + \left(\frac{G^2}{t} \right) \dots + \left(\frac{G^t}{t} \right) \right],$$

Where, *G* is the percentage of seed germination at two-day intervals, and *t* is the days of the germination period. A greater value of *G* indicates a faster germination rate.

Germination function

Due to their peculiar characteristics, seed germination and emergence assays may pose problems for data analysis, due to non-normal error distribution and serial correlation between the numbers of seeds counted on different dates from the same experimental unit (Petri dish, pot, and plot). Furthermore, it is necessary to consider viable seeds that have not germinated/emerged at the end of an experiment (censored observations), as well as late germination/emergence flushes, that relate to genotypic differences within natural occurring seed populations. Traditional methods of data analysis may not be optimal for dealing with these problems. Therefore, survival analysis may represent an appropriate alternative. In this analysis, the time course of germination/emergence is described by using a non-parametric step function ('germination function') (Onofri et al., 2010). Like survival probability, the 'germination/emergence probability' can be estimated non-parametrically by using the Kaplan–Meyer method (Venables and Ripley, 2003):

$$S(t) = \prod_{j=1}^s \left(1 - \frac{d_j}{n_j} \right),$$

Where, *d_j* is the number of seeds that germinate in a given interval of time *j*, *n_j* is the number of seeds 'at risk' (this term stems from the epidemiological field) of germination in the same interval (number of non-germinated seeds entering the interval, minus one half of the number of lost seeds during the same interval).

Statistical analysis

Analysis of variance (ANOVA) was conducted between different treatments. Significant differences between treatments were evaluated by one-way ANOVA, and the data are expressed as the mean values ± S.E. of at least three replicates.

RESULTS

Germination

Seed germination percentage generally decreased with increasing NaCl concentration, but the low salinity levels (control, 100 and 200 mM) had no significant influence on it (*p*>0.05) (Figure 1). As NaCl concentration increased over 600 mM, the germination time delayed and the germination percentage drastically declined. The lowest germination percentage was observed at the highest NaCl concentration (800 mM). Considering seed treatments, KNO₃ gave the highest germination percentage at

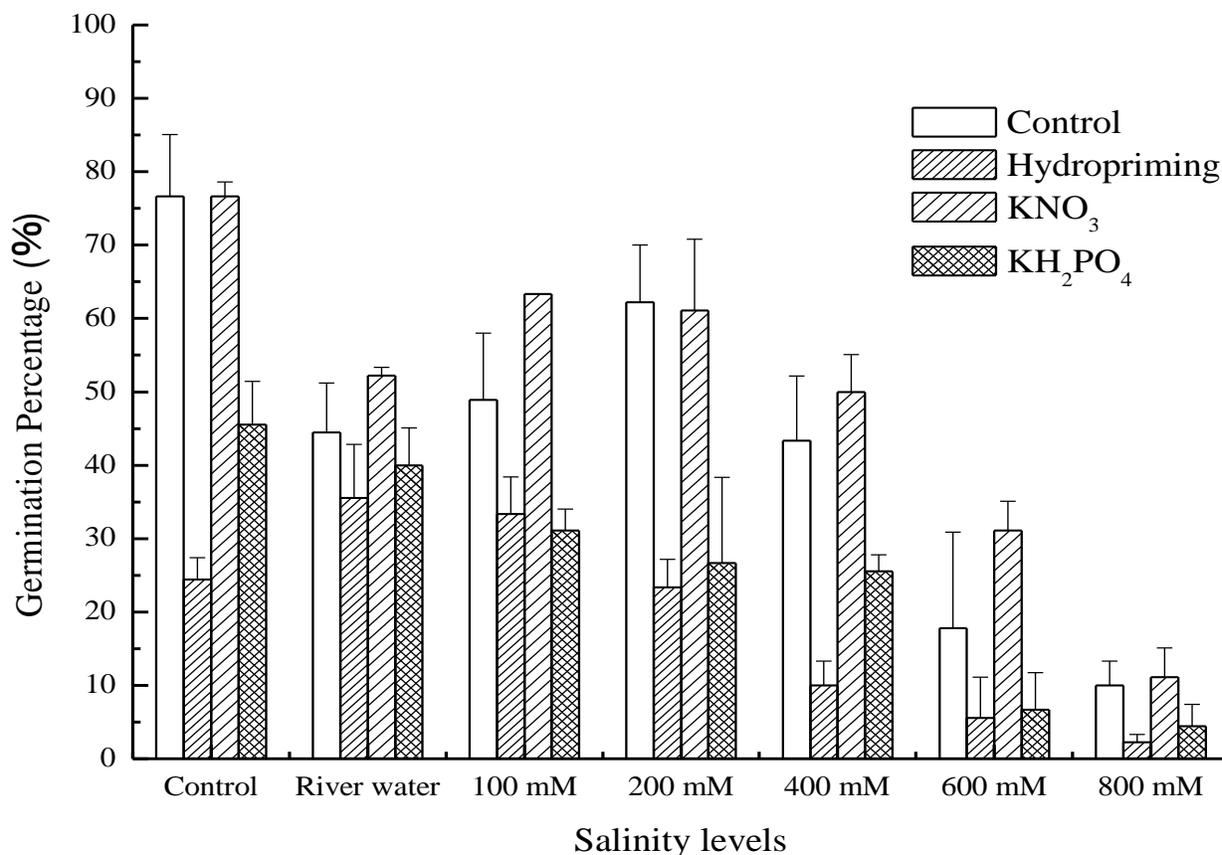


Figure 1. Germination percentage of seeds treated with water, KNO₃, KH₂PO₃ and control at different NaCl concentrations. Values are means (\pm S.E, n = 3).

different NaCl levels (except 200 mM), indicating that the KNO₃ had improved germination, and the promotion became more significant at the high salinity. Compared with untreated seeds, seeds primed with KH₂PO₃ and deionized water generally had lower germination percentage (Figure 1).

Germination rates

Germination rates were calculated by a modified Timson Index at days two, four, six and eight per salinity level. For all treatments, the highest germination rate was observed on day two at the low salinity levels (control: River water, 100 and 200 mM) (Figure 2), while at the high salinity levels (400 and 600 mM), the value was occurred on day four except for the water priming treatment (Figure 2b), indicating that high salinity generally delayed germination. As the salinity reached 800Mm, there were still few seeds germinated. Considering seed treatments, seeds treated with KNO₃ gave the highest germination rate at the high salinity levels, and the germination rate generally decreased in the order of KNO₃ > Control > KH₂PO₃ > Hydropriming.

Seedling growth

The length of *S. salsa* was not significantly affected by salinity (Figure 3), and the low NaCl concentrations (100 and 200 mM) slightly promoted the seedling growth. Comparatively, the seedling length of the three seed treatments was different. At the low NaCl concentrations (control and 100 mM), seeds primed with KNO₃ promoted the seedling growth, while at the high concentrations (200, 600 and 800 mM), the promotion effect of KH₂PO₃ was more significant (Figure 3). For hydropriming, the seedlings had the longest length at the treatments of river water and 400 mM NaCl concentration.

Germination function

Survival functions provide the information on the salinity of the seed emergence trends over time (Figure 4). The emergence trends were generally different between the low salinity and high salinity as the seeds primed with KNO₃ and KH₂PO₃, and the survival function of seeds primed with water differed from other priming techniques. At the low salinity status (Control, River water, 100 and

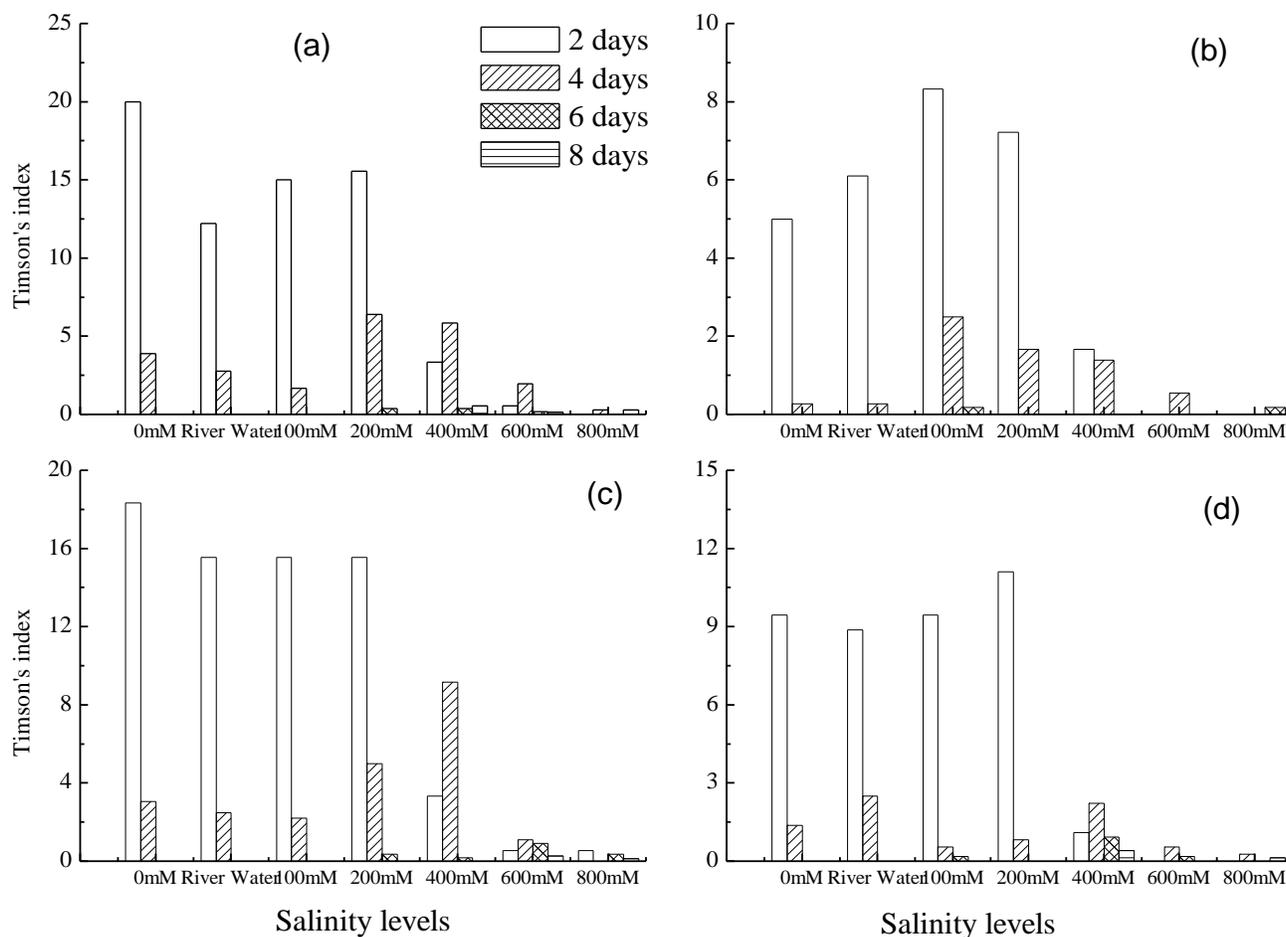


Figure 2. Germination rates of seeds treated with control (a), water (hydropriming) (b), KNO_3 (c) and KH_2PO_3 (d) at different NaCl concentrations.

200 mM), the seeds germinated quickly at the initial days and then the germination rate declined, while at the high salinity status (600 and 800 mM), few seeds germinated at the initial days. The survival function of 400 mM was different at the different priming techniques. The survival function tended to the low salinity status as the seeds primed with KNO_3 , while primed with KH_2PO_3 and control, the survival function tended to the high salinity status, which indicated that the seeds primed with KNO_3 increased the tolerance of salinity.

DISCUSSION

Effects salinity on seed germination

This study showed that the germination of *S. salsa* seeds was significantly affected by salinity, and the germination percentage and germination rate generally decreased with increasing salinity. There were several probable reasons. Firstly, NaCl had inhibitory effects on the water uptake of *S. salsa* seed. Water generally plays the most

important role in the process of seed germination, but high salinity prevents the seeds from absorbing enough water. Secondly, the increase of salinity in medium resulted in the decrease of α -amylase and protease activities, decrease in concentration of reducing and non-reducing sugars, and slower mobilization of reserve protein and reduced amino acids levels during seeds germination (Yasin Ashraf et al., 2002). Thirdly, with increasing salinity, K^+ content decreased while Na^+ content increased (Khan et al., 2000). The current study has showed that more than 50 enzymes were activated by K^+ , and Na^+ could not substitute in this role (Bhandal and Malik, 1998). Owing to the K^+ requirement for the binding of tRNA to ribosome, protein synthesis requires high concentrations of K^+ (Blanha et al., 2000). However, the disruption of protein synthesis by high concentrations of Na^+ appears to be an important cause of damage (Tester and Davenport, 2003).

Different species have dissimilar ability to tolerant salinity. Halophytes such as *Salicornia stricta* and *Salicornia ramosissima* could germinate in as high as

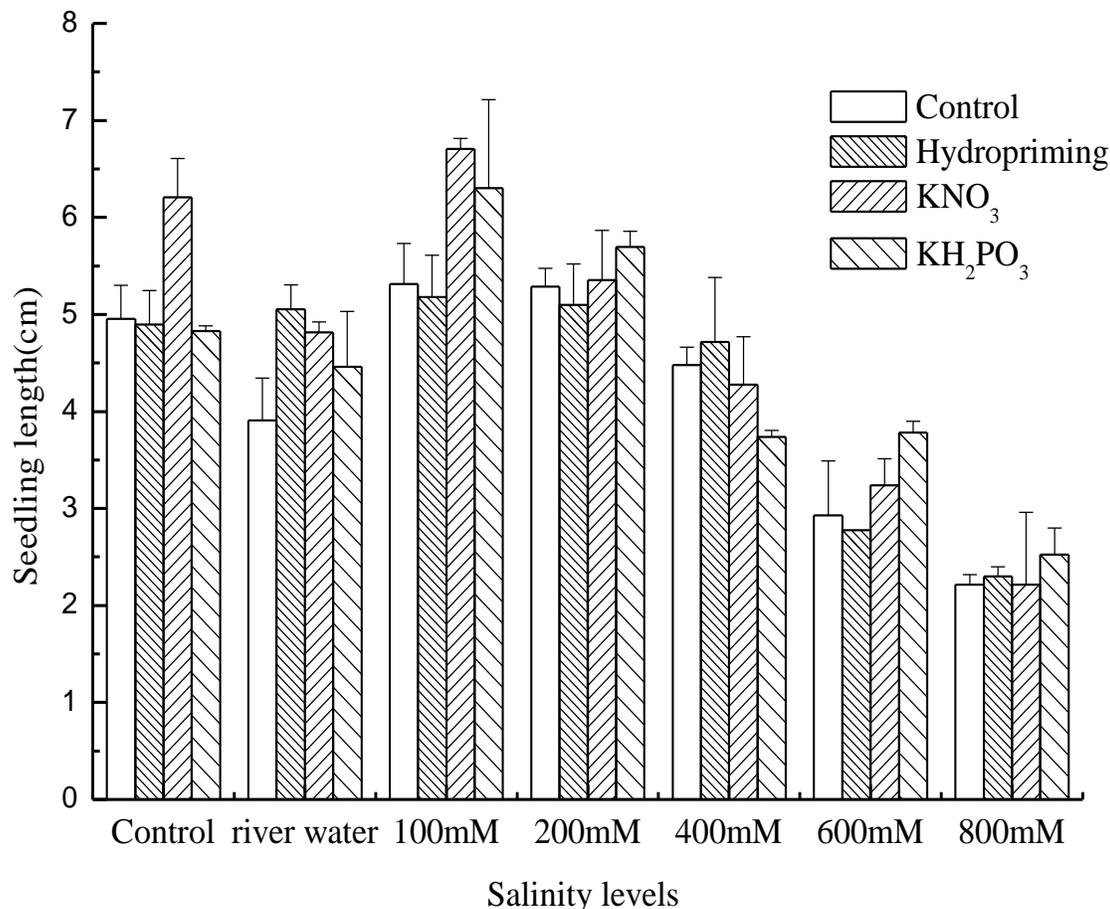


Figure 3. Seedling length of seeds treated with water, KNO₃, KH₂PO₃ and control at different NaCl concentrations. Values are means (\pm S.E, n=3).

4.0% NaCl, while other halophytes (including *Triglochin maritima*, *Plantago maritima*, *Aster tripolium*, *Spergularia marginata*, *Puccinellia distans*, *Atriplex littorale*, and *Anubias hastatum*) could not germinate in salinities above 1.5% NaCl (Ungar, 1978). In this study, we found that there were still few seeds which germinated at 800 mM (~4.47%), indicating that *S. salsa* could tolerate high salinity, which might be related to its physiological characteristic. *S. salsa* is a succulent halophytic herb, and succulence is an anatomical adaptation which, by increasing the vacuolar volume, permits the accumulation of large amounts of water (and dissolved ions) in the leaves (Vicente et al., 2004). One of the important attributes of halophyte seeds and possibly the principal characteristic distinguishing them from glycophytes could be their seeds' ability to remain viable for long periods under extremely high salinity stress and then germinate at a later time when soil water potentials were raised (Ungar, 1978).

This study also showed that the highest germination rate of *S. salsa* was occurred on day two at the low salinity levels, while at the high salinity levels (400 and 600 mM), the value was observed on day 4, but there

were no seeds which germinated at the initial day at the high salinity (800 mM), indicating that the *S. salsa* seemingly developed a strategy to delay germination at the high salinity. This result is similar to the report by Song et al., (2005) who found that the seeds of *Haloxylon ammodendron* and *Suaeda physophora* germinated rapidly at low NaCl concentration but remained ungerminated at high NaCl concentration during the initial stage. Easton and Kleindorfer (2009) indicated that the germination rates of *Frankenia cordata* decreased as salinity levels increased. The highest germination rate was recorded on day four at 0% salinity, on day six at 10% salinity, and on day eight at 20 and 30% salinity. The special strategy taken by the halophytic species might produce a persistent seed bank of viable seeds in salt habitat that would maintain the populations over time.

One of the most critical stages in the life cycle of halophytes is the period of germination and establishment. Once the bottleneck of seed germination is overcome, the salt tolerance of *S. salsa* increases progressively with the growth of plants. In this paper, we found that the effects of salinity on germination were more significant than those on seedling length (Figures 1

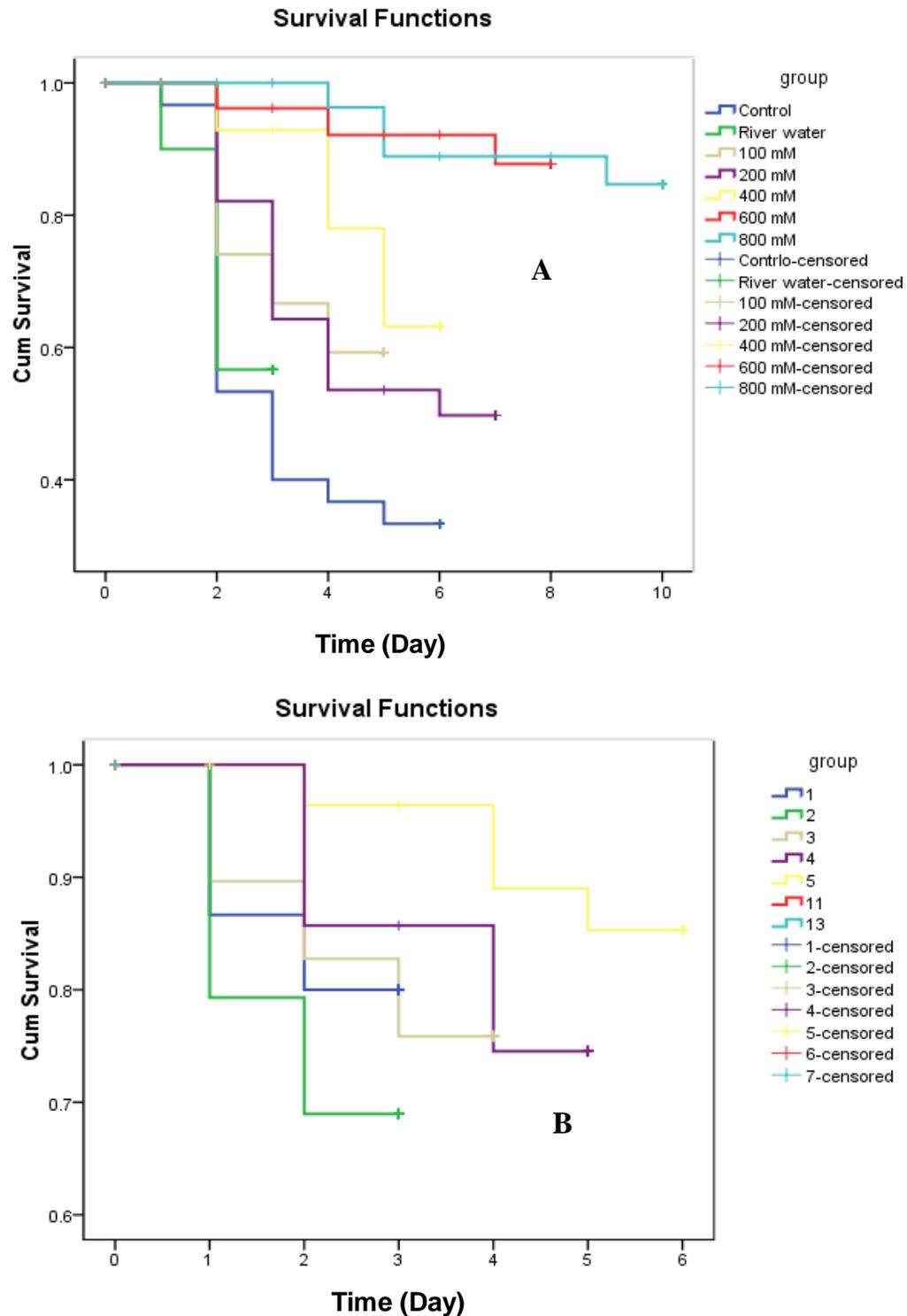


Figure 4. A, Survival function of seeds treated with control at different NaCl concentrations; B, survival function of seeds treated with water at different NaCl concentrations; C, survival function of seeds treated with KH_2PO_3 at different NaCl concentrations; D, survival function of seeds treated with KNO_3 at different NaCl concentrations.

and 3). Similar result was reported by Vicente et al. (2004) who found that seedlings of *Plantago crassifolia*

survived NaCl concentrations of 200 to 300 mM, which completely inhibited germination, showing little or no

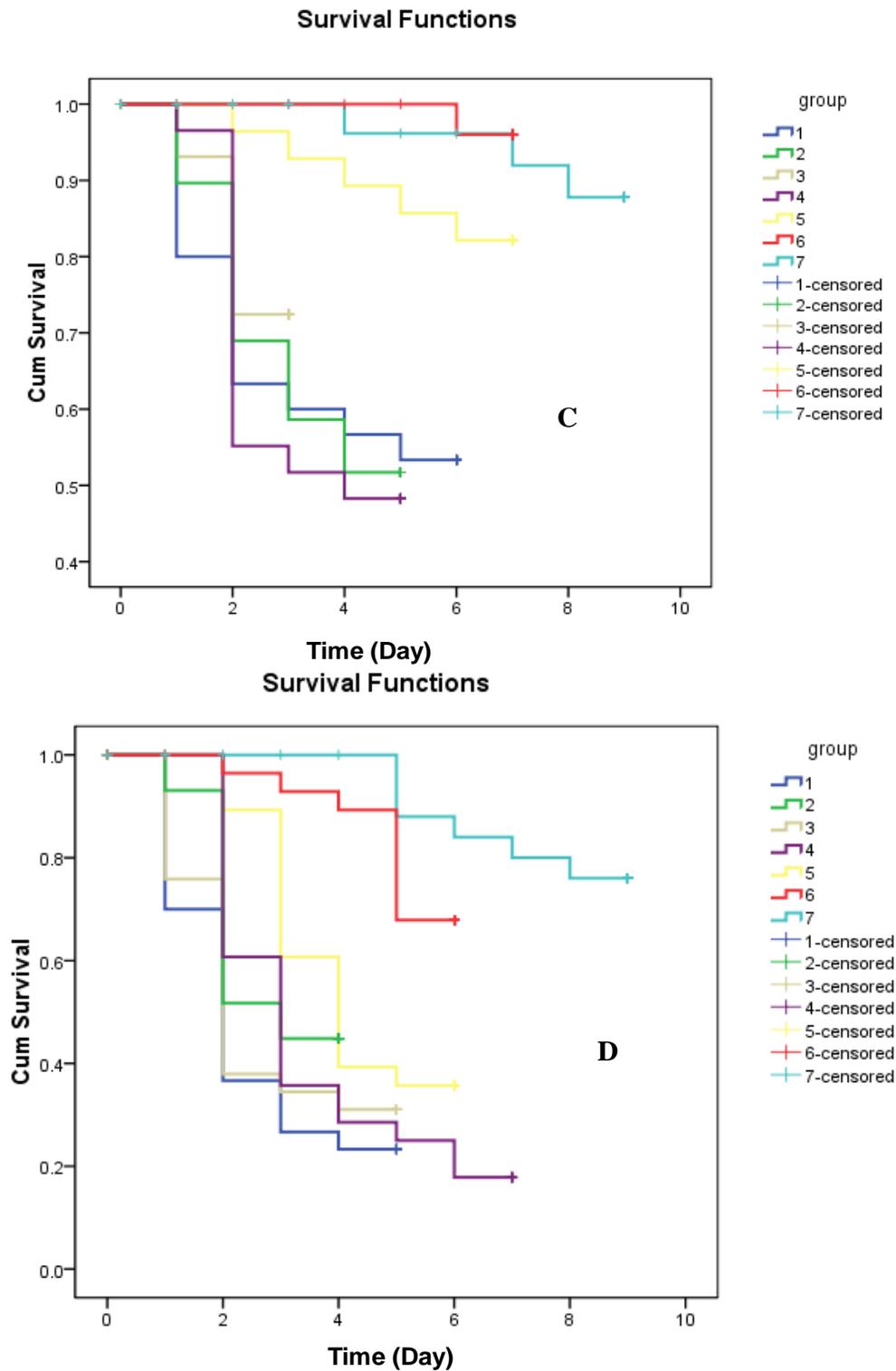


Figure 4. Contd.

damage, although growth was slower than in non-treated controls. Slow growth is a general adaptive feature for plants survival under different environmental stresses, allowing redirection of cell resources (such as energy and

metabolic precursors) towards the defense reactions against stress (Zhu, 2001). In this study, the seedling length of *S. salsa* decreased as the NaCl concentrations were high (400, 600 and 800 mM), which was mainly due

to the shoot growth. Hsiao and Xu (2000), Munns and Sharp (1993) reported that shoot growth was often more reduced than root growth by salinity. There are two probable causes for growth reduction under stress. One cause is inadequate photosynthesis owing to stomatal closure and consequently limited carbon dioxide uptake. Another one is that the stress might inhibit cell division and expansion directly (Zhu, 2001). This study also showed that the low NaCl concentrations (100 and 200 mM) slightly promoted the seedling growth, which might be related to the reason why *S. salsa* was not responsive enough and run the risk of dying by continuous growth as stress is already serious. Fine-tuning the response could potentially increase the productivity of some plants under salt stress, while other plants might show response to stress that they 'panic' and almost cease growing as only stress occurs (Zhu, 2001). According to the studies of germination percentage and seedling length, we found that the effects of Yellow river water were similar to the 400 mM NaCl solution when the seeds were primed with deionized water, indicating that the actual salinity of Yellow water might approximate 400 mM. In addition, since the river water sampled from the Yellow river reflected the actual salinity of water in *S. salsa* marsh, and at the salinity of river water, the hydropriming had the most promotion to the seedling growth compared with the other treatments (Figure 3), indicating that the combination of hydropriming technique and appropriate amount of Yellow River water imported into the degraded *S. salsa* marsh in seed germination period might be an advantage to the restoration of *S. salsa*.

Effects of seeds priming

This study showed that hydropriming generally had no significant effects on seeds germination and seedling growth (Figures 1 and 3), which was not related to two reasons: (1) priming time; time is very important for seeds priming, and it generally varies from species. If time is not well controlled, it will have adverse effect on germination. In this paper, the priming time was set as 6 h, which was probably the perfect priming time for *S. salsa* seeds according to the analysis of seeds germination and seedling growth. (2) Environmental factors; environmental factors also influence the priming effect greatly. Subedi and Ma (2005) found that seeds soaking with water for 16 h significantly reduced percentage emergence and final plant stand; seeds soaking with 2.5% KCl and 20 ppm gibberellic acid (GA3) solution for 16 h significantly reduced plant stand and grain yield under the 150 kg N ha⁻¹ treatment, and the possible causes were due to more wet and cool conditions during seedling establishment stage and higher soil nitrate nitrogen availability. They also concluded that in a humid growing environment such as eastern Ontario, seed priming in corn had limited or no benefits on crop development, N response, and grain yield (Subedi and

Ma, 2005). Some studies also showed that there were no or limited benefits of seeds priming. For example, Giri and Schillinger (2003) indicated that none of the seed-priming media used (water, KCl, and polyethylene glycol) improved field emergence and subsequent grain yield in deep-planted winter wheat.

Under soil conditions of low nutrient availability, seed dressing or coating with the limiting nutrient element has been shown to be more economical and more convenient than either soil or foliar applications (Savithri et al., 1999; Ajouri et al., 2004). Under conditions of P and Zn deficiency, Ajouri et al. (2004) used the solutions containing 5 to 500 mM P, Zn and P + Zn to soak the seeds of traditional barley cultivar *Arabi aswad*, and found that the priming of barley seeds for 12 h in 10 mM Zn and 50 mM P solution increased the content of these nutrients in the seeds and on the seed coat by up to 200 and 300 mg P (kg seed⁻¹). In this paper, we found that seeds primed with KNO₃ promoted the seedling growth at the low salinity, while at the high salinity; the promotion effect of KH₂PO₃ was more significant (Figure 3), which was probably related to the nutrient status of *S. salsa* wetland. In intertidal zone of the Yellow River estuary, the salinity generally increased from high tidal marsh to bare flat. Mou (2010) found that the N/P ratio (9.87 ± 1.23) of *S. salsa* in the middle tidal marsh was less than 14, indicating that plant growth was limited by N. Comparatively, the N/P ratio (15.73 ± 1.77) of *S. salsa* in the low tidal marsh was less than 16, indicating that the growth was limited by both N and P, but the P limitation was more significant. Therefore, at the low salinity, N is the limited nutrient, and seeds primed with KNO₃ had prominent effect. Differently, at the high salinity, P was the most limited nutrient, and seeds primed with KH₂PO₃ had prominent influence. Presently, the nutrient (N, P) import amount of the Yellow River estuary is increasing due to frequent human activities, and approximately 4650 tons of nutrient is carried to the estuary every year (Bulletin of Shandong Environmental Status, 2009). Therefore, appropriate amount of Yellow River water imported into the degraded *S. salsa* marsh in seed germination period can improve the nutrient and salinity status of *S. salsa* marsh, which might be propitious to the seeds germination and seedling growth of *S. salsa*. However, the definite import amount of the Yellow River water is still needed to be determined in the following studies.

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