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Research Article

Effects of Salinity and Water Depth on Germination of *Phragmites australis* in Coastal Wetland of the Yellow River Delta

Phragmites australis, a perennial herb and a widespread species, is widely distributed in coastal wetland in the Yellow River Delta. Laboratory experiments were carried out to assess the effects of water and salinity on seed germination of *P. australis* and recovery response after seed transferred to distilled water. The germination responses of seeds were determined over a wide range of salinities of 0, 0.5, 1, 2, 2.5, and 3% and water depths of 0, 2, 4, 6, 8, 10, and 15 cm. Final percent germination and germination speed except for at 0.5% salinity treatment were decreased with increasing salinities. Lower salinity (0.5%) could stimulate germination of *P. australis*, while seed germination was nearly inhibited completely at salinity 3%. *P. australis* percent germination was decreased with water depth increasing except 2 cm water depth treatment. Percent germination and speed were great and fast at lower water depth. After end of the recovery period, the final percent germination of *P. australis* after high salinity treatments (2, 2.5, and 3%) was up to 80%, while those after low salinity treatments were lower than 1%, and percent germination after different water depth treatments was zero. The results indicate that it is possible to accelerate the degraded coastal wetland restoration process of *P. australis* population by sexual ways in the Yellow River Delta.

Keywords: Germination; Hydrological relationship; *Phragmites australis*; Seawater intrusion; Wetland restoration process

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1 Introduction

In wetland habitats, several environmental constraints may influence plant growth and physiological attributes [1–6]. Light [5, 7–9], temperature [5, 7, 8, 10, 11], water supply [12–14], and salinity [4, 5, 8, 11, 13, 14] have been studied extensively. Among them, salinity and water supply are two key factors in coastal wetlands [14]. Determination of species salt and waterlogging tolerance will contribute to an understanding of common patterns of colonization of the plants [15].

Phragmites australis (*P. australis*) is a widespread species, whose typical habitats are fresh or brackish water areas of swamps, riversides, and lakesides. It is often the key species that propagates both by asexual and sexual ways, develops from vegetative colonization is much more common than seeds in wetland ecosystems [7, 10, 16–18]. A matter of fact, the optimum habitat conditions for germination with highly vulnerable period differ greatly the environment for the adult stage. *P. australis* can produce large quantities of small seeds which are pollinated by wind, thus the viability of seeds is from 30 to 90% [8, 13]. Several researches have reported that *P. australis* seeding can account for initial colonization of an area though with high mortality [15, 19, 20]. Therefore, the germination of *P. australis* seed plays an important role in protection and restoration of *P. australis* population, and it has an important ecological significance in coastal wetland restoration.

In the coastal wetland of the Yellow River Delta (YRD), *P. australis* is one of the most dominant plant species which covers about 25% of total area of the YRD. Its habitats support a wide variety of flora and fauna. In recent years, the dramatic water discharge reduction caused by anthropological activities and climate change has led to a great decrease in water supply of the Yellow River to the wetlands [21]. In addition, the YRD is also a famous oil production base in China. Large number of roads and oil wells are constructed. As a result, natural hydrological relationships between the river and its floodplain are seriously destroyed. The secondary salinization is severe because of sea water intrusion for lack of freshwater supply. All above returns results of *P. australis* wetland degraded. In the YRD, salinization and water supply became two key factor affecting *P. australis* growth and physiological attributes.

Several scientific management strategies have been established to control the discontinuous flow of the Yellow River since 2002 [21]. In the following years, runoff of the river stabilizes at 20 billion m^3, and it is possible to provide redundant water for wetland restoration [22]. In July 2002, a restoration project governed by local government was implemented for the region. The restoration aims were to improve

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Abbreviations: YRD, Yellow River Delta; **ANOVA**, One-way analysis of variance; ***P. australis***, *Phragmites australis*

the wetland functions and protect the natural *P. australis* habitat for rare birds, and the first step of the project was to restore degraded plants. Since then, several projects of coastal wetland restoration were carried out in the YRD [23–26]. The restoration method for all projects is freshwater diversion from the Yellow River. Based on our field investigation, there are numerous patches of *P. australis* community in the YRD. This phenomenon also is a possible supporting evidence for sexual spread of *P. australis*. Therefore, besides vegetative propagation, the sexual way of *P. australis* also needs to be considered to accelerate the restoration process of wetland. The objectives of this study are (1) to determine the effects of salinity and water depth on seed germination percentages and germination speed of *P. australis* and (2) to provide feasibility in stimulating *P. australis* population restoration through seed bank in the YRD.

2 Materials and methods

2.1 Region description

The YRD (37°35′N–38°12′N and 118°33′E–119°20′E) is located in the middle of eastern China, Shandong Province (see Fig. 1a in Yu et al. [21]). The climate of study area is a temperate semi-humid continental monsoon climate with distinctive seasons and rainy summer. The detailed information of regional sunshine hours, temperature, frost-free period, and precipitation was described in reference [27]. The descriptions of regional soil and vegetation were introduced in reference [28].

2.2 Experimental design

P. australis seeds were local genotypes obtained on October 2010 in the Yellow River Delta National Natural Reserve which established in 1990 to preserve the native coastal wetland ecosystems and habit for birds. The mature panicles and fully fledged seeds of *P. australis* were collected. All seeds were kept in an ice-box with dark and dry conditions at 4°C until the time of germination experiments starting. At beginning of experiment, full seeds with similar sizes were selected and surface sterilized with aqueous solution of 0.1% $KMnO_4$ for 10 min to avoid attacking from fungus. Subsequently, the seed germination experiments were carried out after the seed was rinsed by distilled water. Germination experiments were conducted in incubators with distilled water at 0, 2, 4, 6, 8, 10, and 15 cm water depth, respectively. Seeds were germinated in distilled water (0), and in 0.5, 1, 2, 2.5, and 3% NaCl concentration. For each treatment, 25 seeds (four replicates) were placed on two layers of Whatman grade No.1 filter paper (pH 7) in 90-mm Petri dishes with 10 mL of treatment solutions or distilled water. A germinator (LRH-250-GS II, China) was used to place dishes in. The incubation period was 20 days in germinator with an alternating diurnal regime of 12 h of light at 25°C and 12 h of dark at 15°C. The selected alternating temperature was much close to field conditions in spring. Percent germination was recorded everyday. A seed was considered germinated when coleoptiles elongated to 1 mm. The initial experiment lasted 20 days, subsequently, without germination seeds in each treatment were washed three times with distilled water, then transferred to new Petri dishes with two layers filter papers and 10 mL distilled water, and incubated for germination recovery. After 5 days experiment in fresh water, the appended germination was recorded. Distilled water equal to the mean loss of water from dishes containing only water was added to each Petri dish every 2 days to maintain salt concentration near the target levels throughout the germination period.

2.3 Statistical analyses

The software of SPSS12.0 (Lead Technologies, Inc., USA) was used for statistical analysis. A speed of germination index was used to calculate the speed of germination [29].

$$S = [N1 + (N_2 - N_1)/2 + (N_3 - N_2)/3 + \ldots\ldots + (N_n - N_{n-1})/N] \times 100 \quad (1)$$

where S is the speed of germination; N is the proportion of germinated seeds obtained at n days.

Final percent germination and germination speed and recovery potential were evaluated ($p = 0.05$) using one-way analysis of variance (ANOVA), for independent variables of salinity and surface water depth. Tukey's HSD test was used to separate factors with in these effects ($p = 0.05$).

3 Results

3.1 Effects of salinity on seed germination

Final percent germination decreased ($F = 78.80$, $p = 0.000$) in response to increasing salinity, except for at 0.5% salinity (Fig. 1A). *P. australis* had the highest percent germination (97% ± 1 SE) at 0.5% salt concentration, but failed to germination at the highest concentration (3%). The variation of germination speed with salinity ($F = 71.420$, $p = 0.000$) had a similar trend with final germination percentages (Fig. 1B). *P. australis* germination was fast

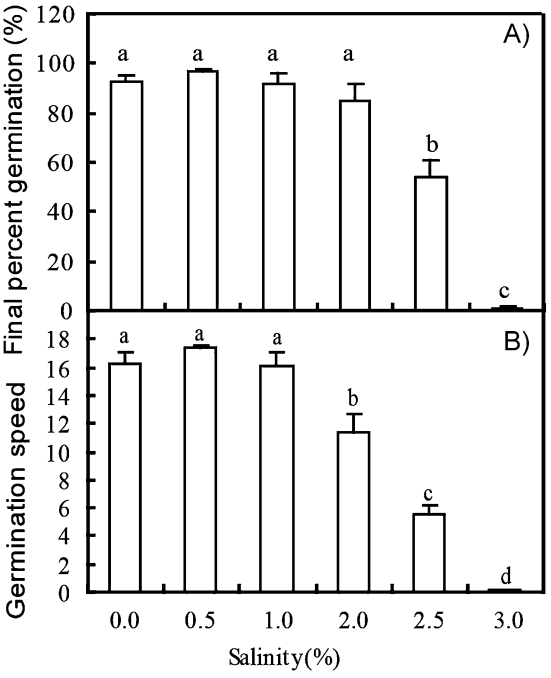

Figure 1. Final percent germination (A) and germination speed (B) (mean ± SE ($n = 4$)) subjected to six salinity treatments. Different letters indicated differences in germination percentages or germination speed under different salinity treatments.

Table 1. Shoot and root length [mean ± SE ($n = 4$)] subjected to six salinity treatments

Salinity (%)	Shoot length		Root length	
	Mean (mm)	SE	Mean (mm)	SE
0.0	9.80	0.6683	9.70	1.0472
0.5	8.00	0.2450	11.25	0.8261
1.0	7.85	0.3304	6.15	1.0966
2.0	4.10	0.2646	0.60	0.1414
2.5	2.95	0.3403	2.80	0.5228
3.0	0.25	0.2500	0.00	0.00

(17.3338 ± 0.2955 SE) at 0.5% salt concentration, while its speed was only 0.1 (± 0.1 SE) at 3% concentration. The longest root and shoot were observed at treatments of 0.5% salt concentration and 0% salt concentration, respectively. At 3% salt concentration, it had the lowest root and shoot (Tab. 1).

3.2 Effects of water depth on seed germination

The changes of both final percentage germination ($F = 39.557$, $p \leq 0.001$) and germination speed ($F = 73.12$, $p \leq 0.01$) of *P. australis* with water depth had a similar trends (Fig. 2). Except at 2 cm water depth treatment, great and fast germination of *P. australis* was observed at lower water depth treatment. Besides at 0 cm water depth, the highest final germination percentages (55% ± 8.85 SE) of and germination speed (6.40 ± 0.94 SE) were found at 4 cm water depth treatment. At 15 cm water depth treatment, germination percentages and speed of *P. australis* were relatively low, which were only 2% ± 1.15 SE and 0.17 ± 0.10 SE, respectively. The shoots in salinity treatments were much stronger than those in water depth treatments where nearly no roots could be observed visibly.

3.3 Seed germination in recovery period

Seeds were recovered in fresh water for 5 days after experiment treatments of salinity and water. In completion of the recovery period, there was a contrary trend of germination percentages of *P. australis* compared to salinity treatment period, i.e., percent germination in high salinity treatment was low, but that was high in recovery period. The final percent germination increased with increasing salinity under recovery period (Fig. 3). The highest recovery germination percentage of 91.1663 ± 2.3917 SE was at treatment of 3% salinity. The recovery germination percentages after water depth treatment were zero.

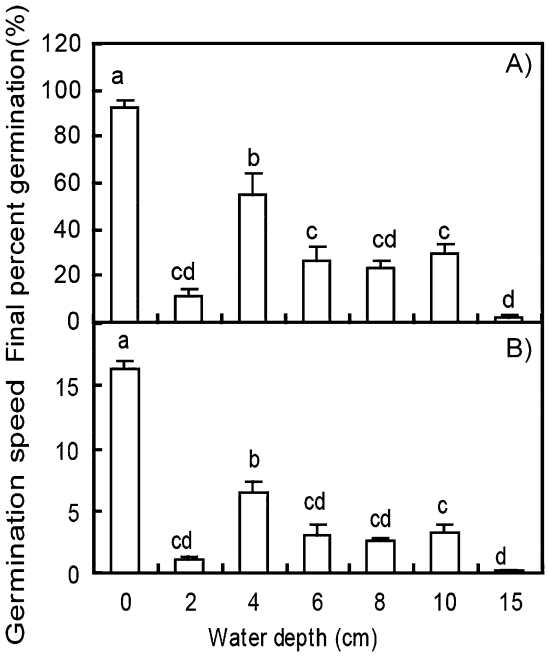

Figure 2. Final percent germination (A) and germination speed (B) [mean ± SE ($n = 4$)] subjected to seven water depth treatments. Different letters indicated differences in germination percentages or germination speed under different water depth treatments.

4 Discussion

Percent germination of *P. australis* recorded in earlier studies varied greatly [9]. Many reasons including rapid seed decomposition and the loss of viability [3] due to salinity emersion and lack of solution oxygen, could contribute to the previously reported transient seed bank. As so many *P. australis* seeds are produced, it would allow ample opportunity for colonization by sexual ways. In our study, *P. australis* were able to germinate at salinity up to 2.5%, and 0 cm water depth was the optimum for germination (Fig. 1). As salinity and water depth within the YRD varies considerably, the large range of possible habitat would be appeared. After 20 days salinity treatments, high germination of *P. australis* was occurred in fresh water and low salinity (Figs. 1 and 2), while the high germination was found in high salinity treatment in recovery period (Fig. 3).

Salinity is one of the most important factors to determine reed seeds germination [4, 5, 8, 11, 13, 14]. It is also generally accepted that the percentages and speed of germination decrease with salinity [8].

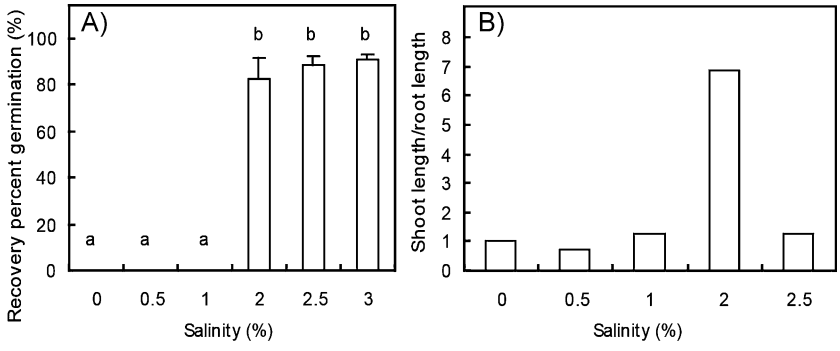

Figure 3. Final percent germination [mean ± SE ($n = 4$)] after recovery period. Different letters indicated differences in germination percentages or germination speed under different water depth treatments.

Some studies suggested that the germination did not decrease obviously even though salinity up to 3% NaCl [7], while some researches pointed that lower NaCl concentration (50 mM) had decreased germination capacity up to 16%, and reed seed was inhibited to germinate completely at 500 mM concentration [11]. In our study, up to 2.5% NaCl concentration did not decrease reed germination significantly, while up to 3%, germination capacity was decreased up to 1%. The ability of mild salinity regimes (0.5–1%) to stimulate germination of *P. australis* has been reported and there is a competitive advantage for *P. australis* germination in salt marsh conditions when receiving freshwater inputs [13]. Our study also had the similar results, and there was the highest percent germination and speed at 0.5% salt concentration (Figs. 1 and 2). Therefore, *P. australis* is thought to have competitive advantages of colonization by seeds in coastal wetland in the YRD. This also provided evidences for the *P. australis* restoration through sexual ways in the YRD.

Water depth is thought to be another most important factor determining germination which could only happen when shallow water prevails [12–14]. O_2 concentration can account for changes of germination effected by water depth [30]. It is reported that 5 cm was the optimum water level allowing reed germination [7], and 2.5% O_2 concentration was sufficient for reed germination [13]. In this paper, besides at 0 cm, reed seeds germination was highest at 4 cm water depth. Though germination percentages decreased to 2%, it could still germinate at 15 cm water depth, and this was not coincident with earlier studies [7]. This result may be the function of adaptive physiological characteristics of *P. australis* seeds in the coastal wetland of the YRD.

Usually, root is thought to be vital for plant growth and enlargement [31, 32]. Strong root is propitious to plant to extend around and get more suitable conditions (nutrients and water) to survive [32–34]. In this work, there were significant differences among roots and shoots subjected to six salinities, and there were longer roots and shoots at lower salinity treatments (Tab. 1). There was nearly no root could be observed visibly in water depth treatment, and their shoots were also much weaker than those subjected to six salinity treatments. The results not only provide evidences for expansion of stands in habitat with sufficient water, but indicate that manipulation of water depth is necessary to stimulate the germination of *P. australis* in future coastal wetland restoration in the YRD.

Reed seeds germination under natural conditions is more complex. Besides salinity and water depth, other factors such as light and temperature also pose effects on germination capacity [5, 7–9, 11]. Haslam suggested that sufficient light was necessary for maximum germination, while Greenwood and MacFarlane found no differences in the germination under light or dark conditions. Some researchers supposed that climatic conditions affect reed seed production in some extent [12–14]. High rainfalls in August had negative effects on reed seed sets, while increased seed production in September and October. They also considered that high average monthly temperature induce positive effects on reed seed production [7]. In fields experiment, Weisner and Strand [30] concluded that there were high rhizome biomass of reed with early germination. The water depth tolerance of juvenile plants could be determined by the rhizome biomass in the next year, further to increase the survival rates in the following summer. Some scientists emphasized the importance of temperature for reed germination, i.e., fluctuating and high temperature is preferred for germination [7]. Reed seeds germinated faster and greater at 10–25°C than 15–30°C [8], and nearly no germination was observed <5 and >35°C [11]. In the YRD, high rainfalls with high temperature is occurred in summer, then relatively dry and cold weather is appeared in late fall and winter. Therefore, the environmental conditions in spring were the optimum season for *P. australis* restoration process by sexual ways.

Besides above environmental factors, seed dormancy is also considered to be important for establishment following seed dispersal. Reed seeds usually require cold, moist conditions for loss of seed dormancy. If fresh seeds are unable to germinate immediately in the field, it would depend on dormancy breaking down over the winter to prepare to germinate in the following spring. Some studies observed that approximately half of all viable *P. australis* seeds produced at maturity were dormant, and would germinated at a high percentage after a cold-moist seed treatment [3]. However, contrary result was recorded in the European *P. australis* seeds, which are non-dormant at maturity [9]. According to our filed observation, the *P. australis* seeds disperse throughout the late fall and early winter in the YRD, likely with ample time to break seed dormancy because of short cold treatment required for dormancy loss. However, other conditions at the same time are not suitable for germination, leading to low seeds germination of reed in natural conditions of the YRD. Our results of *P. australis* germination in the YRD is much higher than those recorded in North American and Australia studies [13, 35], and similar to those reported in most European literature [16].

5 Conclusions

Most of coastal wetlands in the YRD are in the degradation process for seawater intrusion and human being activities from quick economic development. In order to accelerate the restoration processes of degraded *P. australis* coastal wetland according to seed germination in the YRD, our results suggested to collect seeds in the fall and winter, store them in cold-moist conditions to lose seed dormancy, then disperse the seeds artificially in the following spring to confirm seed germination and survival rates. In the restoration process, water depths and salt concentration should be manipulated by regulating water to make a benefiting condition.

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