

The Effects of Water and Soil Types on the Seed Germination and Seedling Survival of *Populus euphratica* in Arid Region in China

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Abstract

Both of water and soil are the most essential factors for the establishment and growth of plants. This study firstly investigated the sexual reproduction of two stages of Populus euphratica, seed germination and seedling growth. We focused on the various screening effects of nine combinations (three moisture gradients × three normal soil types) on seed and seedling of Populus euphratica. With the water content rising (from 10% to 20%), seeds and seedlings on all the soil substrates showed higher germination rate, germination speed or germination index and survival rate and better growth conditions, respectively. Besides, higher moisture could guarantee seeds to germinate 4-8 hours earlier and greater germination rate. In the comparison among the soil substrates, seeds and seedlings favored the lower-salinity and richer-nutrient river bank soil, which impelled a 4-hour earlier and higher germination peak period and ensured the greatest seedling quality. Therefore, the combination of '20%, river bank soil' was the most suitable condition for P. euphratica seeds and seedlings, which highly consisted with its riparian sexual regeneration phenomenon. Finally, by analyzing the unsteady river flow and river bank status, we concluded that the reason for the failure of P. euphratica sexual reproduction was that the time and quantity of water supply should be adjusted to create more suitable and stable habitat for P. euphratica seeds and seedlings.

Key words: *Populus euphratica*, water content, soil types, sexual reproduction, seed germination, Chinese Arid region

1. **Introduction**

Populus euphratica Olive. is a dominant species in the riparian forests located in the northwestern desert in China (Kürschner, 2004; Monda *et al*. 2008). The sparse and variable precipitation in arid and semi-arid regions is believed to exert strong control over the life histories, physiological characteristics, and species composition of their biota (Chesson *et al*. 2004). A seed will go through many tests (e.g. predation, freezing, drought, physical damage and water stress) and stages (e.g. pre-dispersal, dispersal, germination, seedling and sapling) until being a survived sapling (Rey and Alcántara 2000). Although seed dispersal is a key process in plant population dynamics (Harper 1997), the post-dispersed environment has a powerful screening effect on the survival probability of seed and its subsequent periods.

As an outstanding germplasm resource in Inner Mongolia region, it is being seriously threatened by the deterioration of the arid environment (Liu *et al*. 2011). P. euphratica cannot be successfully regenerated by seeds in natural conditions, but it also could regenerate by a large production of root suckers (Hukin *et al*. 2005). It's extremely urgent for us to solve the problem of seed regeneration failure because of the lower diversity leading by long-term asexual regeneration.

Knowledge of seed movement and fates of P. euphratica are essential for ecosystem restoration and conservation efforts and for the control of alien species in all biomes (Chambers and MacMahon 1994), especially in the extreme arid regions. The fates of seed are affected both by abiotic and biotic factors. Some previous researches have figured out that mother trees of P. euphratica can produce hundreds of thousands of seeds every year, however, many seedlings hardly being found in the forest. Instead, this species usually occurred as a belt along the river bank but quickly died in a large area (Cao *et al*. 2009a, b), only few ensued the least water demand for seeds and early seedlings and other recruitment limiting factors, such as soil surface, the site where seeds not only fall and germinate but also be stored. The desertification in the P. euphratica region has led soil contents, types and moisture changing and then it is shown that various P. euphratica forests primarily originated from seeds live on different soil substrates. However, there are few researches on the adaption mechanism of this species in the aspect of reproduction.

We assumed that the constantly changing and threatening environment was the key reason for sexual regeneration failure based on the fact of large production of vigorous seeds per year and temporal heterogeneity of seed rain (Zhang *et al*. 2005; Wu *et al*. 2007; Cao *et al*. 2009).

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Besides, considering that plants exhibit a variety of behaviors in response to environmental stimuli (Karban 2008), this paper focused on the influence of two important natural factors (i.e., water and common soil substrate) on the seed germination and seedling survival in P. euphratica by manipulating their water gradients or soil types. Our goal was to figure out why P. euphratica failed to regenerate naturally by seeds to some extent, and then to provide some useful measures of management for P. euphratica forest restoration and conservation in arid region of China.

2. Materials and Methods

2.1 Study site

This study was conducted in the National Natural Reserve of P. euhpratica, in Ejina Oasis at the lower reaches of Heihe River (41°30′~42°30′N, 101°03′~101°30′E, with an altitude of 900 to 1600m), in Inner Mongolia Autonomous Region, P. R. China (Fig. 1). The climate of the study area is arid continental with a mean annual potential evaporation of approximately 3700 mm per year, and the mean annual precipitation at Ejina Oasis is only 37.9 mm according to 1957-2000 climate data.

The mean annual temperature is 8.2°C, with the mean monthly maximum and minimum air temperatures are 40°C and –26°C, respectively. The vegetation is characterized by riparian P. euphratica forest. The most common soil in the forest region of the study area is shrubby meadow soil, alternatively distributing with fixed or semi-fixed sandy soil and fluvo-aquic soil.

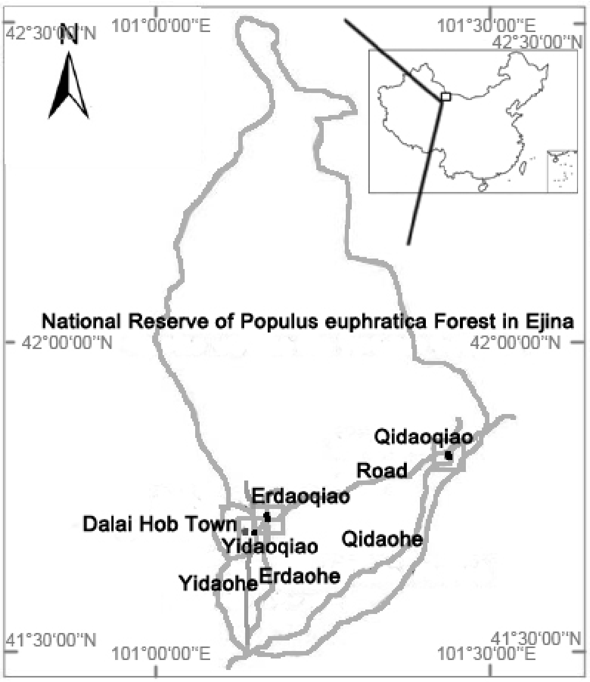


Fig. 1. Study site.

2.2 Experimental manipulation

We randomly harvested the seeds from 10 random healthy P. euphratica female trees with diameter of breast height (hereafter DBH) between 15cm and 25cm in July 2010. At the same time, 3 types of normal soils including shrubby meadow soil (SM), sandy soil (S) and river bank soil (RB) were randomly selected at Erdaoqiao (41°58′N, 101°05′E), Yidaoqiao (41°57′N, 101°04′E) and Qidaoqiao (42°00′N, 101°13′E) of P. euphratica forest region respectively (Fig. 1; Table 1). All the soils were chosen as three kinds of substrates for seed germination and seedling growth, and each was homogenized, sterilized (134°C) and sieved (5 mm). Soil chemical parameters are shown in Table 1. Moreover, 3 gradients of soil water content (i.e., 10%, 15% and 20%) were also set. The water contents were controlled twice a day (i.e., 9:00 AM and 9:00 PM) by weight ratio. To know the complete germination rate, we chose the pure water (i.e. distilled water, hereafter, CG) as an additional germination substrate.

In the germination experiment, there were 5 replicates for each group and 30 seeds in each replicate. The seeds were observed in a 4-hour-interval and the germinated ones were immediately taken into the other containers (3 seeds evenly in one container) with the same conditions for the subsequent growth experiment. The term of "germination" means that we observed a seed radicel breaks its coat. Then, we counted the survived seedlings every day. After the 45th day from the treatment of the whole experiment, we randomly chose 5 seedlings from each group and recorded their heights, leaf area and taproot lengths. We also recorded the fresh and dry weights of above- and below-ground biomass by analytical balance (MS104S, Mettler-Toledo International Inc., USA).

Table 1. Chemical parameters and water contents of shrubby meadow soil, sandy soil and river bank soil

Property of element	Shrubby meadow soil	Sandy soil	River bank soil
Site	Erdaoqiao (41°58'N, 101°05'E)	Qidaoqiao (41°00'N, 101°13'E)	Yidaoqiao (41°57'N, 101°04'E)
CO_3^{-2} (%)	0.000	0.006	0.067
HCO_3^- (%)	0.018	0.012	0.025
Cl^- (%)	0.746	0.356	0.835
K (mg/kg)	56.57	42.49	24.26
Na(mg/kg)	1340.97	483.75	145.82
Ca(mg/kg)	2507.14	420.52	119.52
Mg(mg/kg)	193.72	205.2	68.72
N (%)	0.090	0.017	0.221
P(mg/kg)	627.31	127.12	590.25
Surface water content (%)	(0, 20)	(0, 15)	(5, 30)

Note: Values are for 10 bulked and homogenized samples from the top 15cm of the soil profile following removal of current-year litter.

In total, 1500 seeds (30seeds × 5 replicates × 3 soil substrates × 3 water gradients + 30seeds × 5 replicates × 1 pure water substrate) were used for the whole experiment. The containers for seed germination test and examining seedling growth were all the plastic bowls (150mm in diameter and 100mm in height) with full of different substrates placed in a room with a constant temperature of 25°C and a 16:8 L:D light cycle with incandescent lumps.

2.3 Statistics indexes and analysis

After the whole test, we calculated the germination percentage (G) and germination index (GI, an index of germination speed) according to Liu *et al*. (2011). We also calculated the root to shoot ratio, the absolute growth rate, the absolute elongation rate of shoot and the absolute elongation rate of root by the following equations:

$R/S = DWR / DWS$,

where R/S is the root to shoot ratio, DWR the dry weight of root biomass and DWS the dry weight of shoot biomass.

$AGRS = W / D_t$,

where AGRS is the absolute growth rate of seedlings and W the dry weight of seedlings in day t.

$AERS = H / D_t$,

where AERS is the absolute elongation rate of shoot and H the height of seedlings in day t.

$AERR = \ln (L / D_t)$

where AERR is the absolute elongation rate of root and L the length of main root in day t.

SPSS 17.0 for Windows was used in one-way and two-way ANOVA with multiple comparisons, using least significant difference tests (LSD).

3. Results

3.1 Seed germination

It was found that the various normal soil types and the soil water contents had significantly different impacts on P. euphratica seed germination. Taking the germination rate (G) and the germination index (GI) into account, there were differences in the terms of water contents of the same substrate and substrates with the same water content (Fig. 2, 3).

Figure 2 shows that, to all the three soil types, the germination rates went larger with the increasing soil water contents, and the curves became sharp from 15% to 20% for both the sandy soil and the river bank soil, that is, 32.00% to 64.67% and 31.33% to 62.67%, respectively. In the comparisons among diverse substrates with the same water content, it was always showed that the germination rate of seeds was higher in the order of on shrubby meadow soil, river bank soil, and sandy soil. Namely, at the level of 20% water content, the germination rates in sandy soil and river bank soil were extremely higher than that of in shrubby meadow soil, although all of them were smaller than that on pure water, 73.33%.

The situation was a little bit different in the terms of GI (Fig. 3), that is, at all the three levels of water content, the GIs of RB soil was gradually becoming higher than those of S soil, which could easily be shown by the slopes of corresponding curves. However, the GIs of SM were still lower than those of S and RB, especially at the higher level of water content.

Considering the number of germinated seeds at each experimental time point, there still were significant variations (Fig. 4). First, the beginning time of P. euphratica seed germination on all the three substrates was obviously delayed with the decreasing water

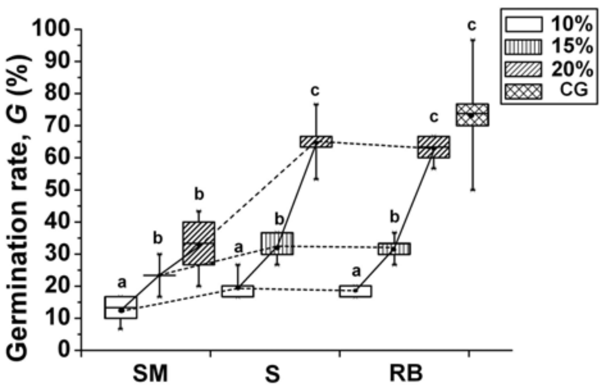


Fig. 2. Effects of three substrates with different water contents on germination rate, *G*. 10, 15 and 20 means 10%, 15% and 20% soil water contents respectively. The same below.

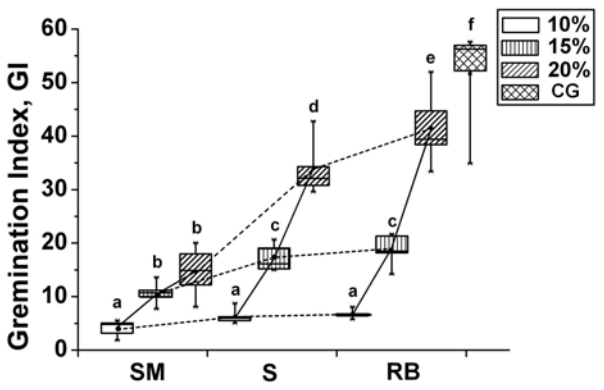
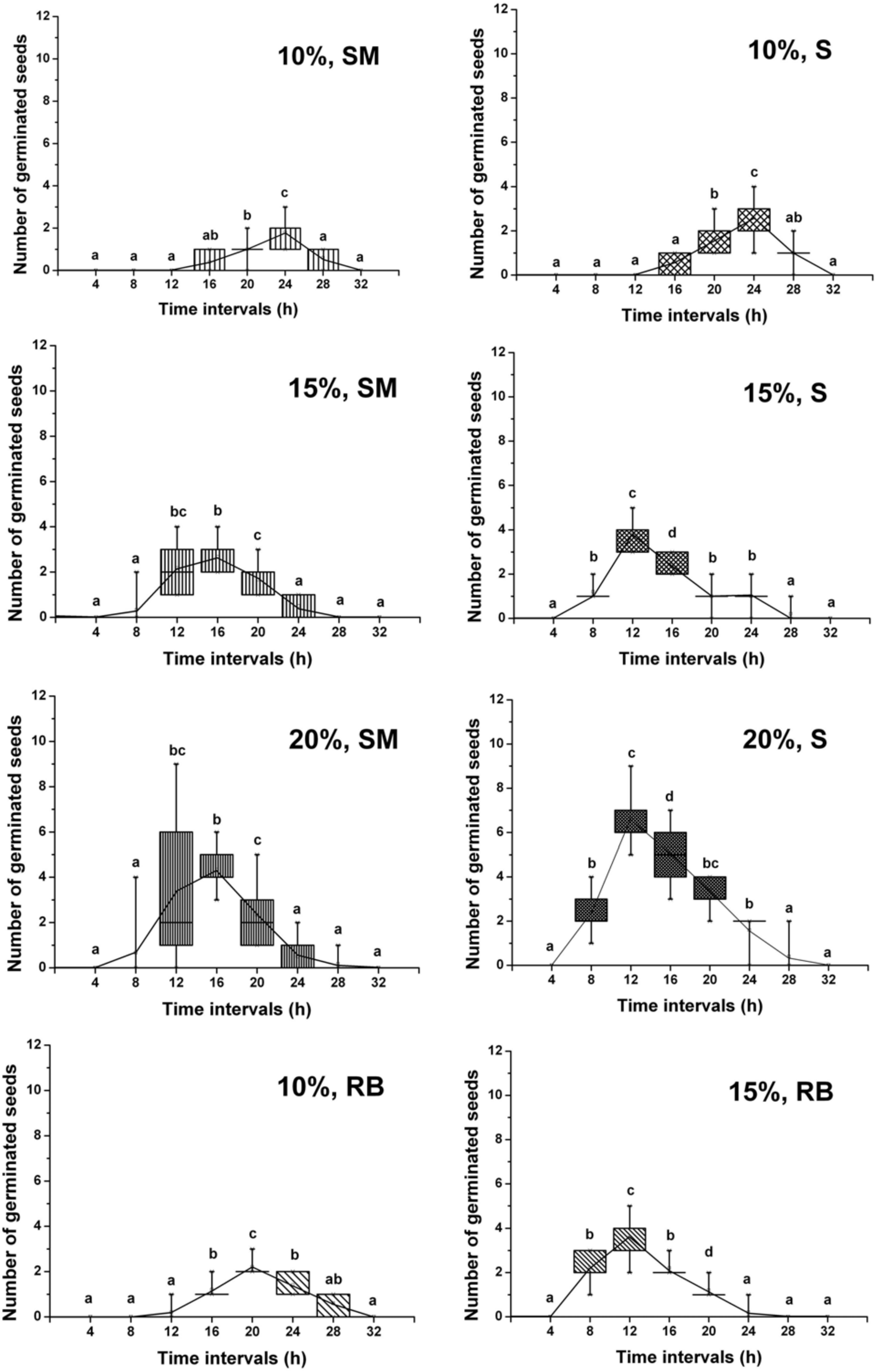


Fig. 3. Effects of three substrates with different water contents on germination index, GI.


10%, SM
10%, S
15%, SM
15%, S
20%, SM
20%, S
10%, RB
15%, RB
Number of germinated seeds
Time intervals (h)

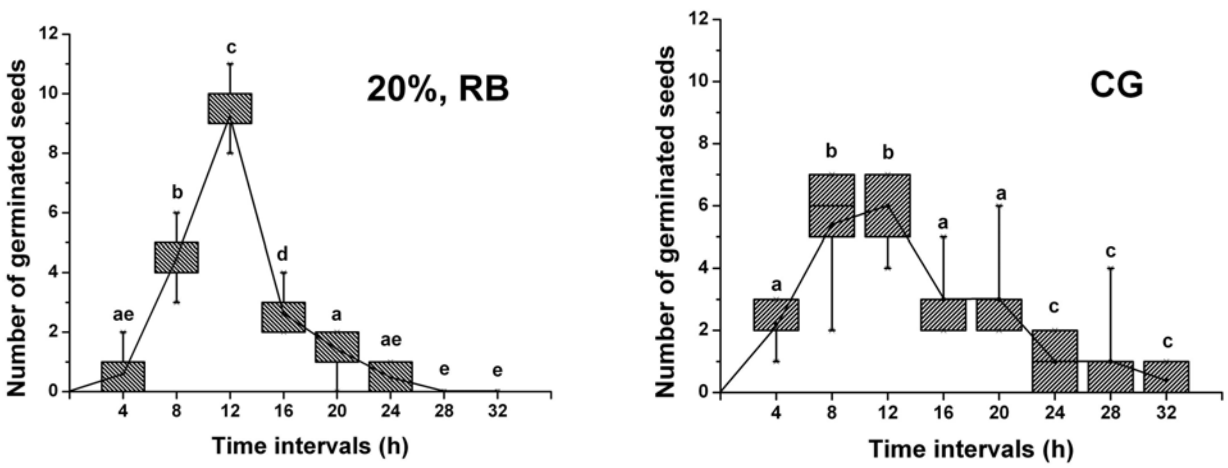


Fig. 4. Variation in number of germinated seeds at each experimental time point for seed germinated on three substrates with three water contents.

content. Most of seeds on the substrates with 10% water content were firstly found germinated in the time interval between 12 and 16h, but at the other levels, seeds began to be germinated in the intervals between 0 and 4h or 4 and 8h. Consequently the germination peak periods were correspondingly postponed. Second, the lasting time of germination was also affected by the water content: 16h at 10%, 20h at 15%, 24h at 20% and 32h at CG, which meant sufficient water could extend the time for P. euphratica seed germination. Third, in comparison with the other two soil types, the peak intervals on the RB soil occurred 4 hours earlier at the same levels of water content. Besides, the greatest number of germinated seeds was appeared in the group combined 20% water content, RB soil, about 9.4.

3.2 Seedling survival

As shown in Table 2, different types of soil had diverse screening effects on seedling survival. No matter what water content of sandy soil, P. erphratica seedlings couldn’t pass through the ‘20-day-barrier’ at all. Neither did the seedlings growing on the other two soils with 10% water content. However, at the levels of 15% and 20%, more than 10% seedlings were survived for 40 days on the SM soil and the RB soil. Moreover, the seedling survival percentages were always higher in the condition of RB than those of SM.

3.3 Seedling growth

Because the seedlings could not survive over 20 days in some conditions (Table 2), it was only necessary to test the growth indexes of seedlings on shrubby meadow soil and river bank soil with 15% and 20% water content. A two-way ANOVA was conducted to determine whether soil and water influenced the indexes of seedling growth (Table 3 and 4). The values of each index served as the response variable, with soil type, water content, and their interaction serving as fixed effects. Almost all the indexes showed that seedlings on RB significantly grew better than those on SM (Table 3; Fig. 5). As to soil types, the P values of all the indexes except R/S were less than 0.01, which means that soil types have a significant effect on the seedling growth; however, as to the water content and the interaction between soil type and water content, only the P values of TL were less than 0.01 (Table 4). Besides, it was worth being mentioned that the taproot of seedlings at the 15% level could elongate longer and faster than those at the 20% level on the same substrates (Table 3; Fig. 5).

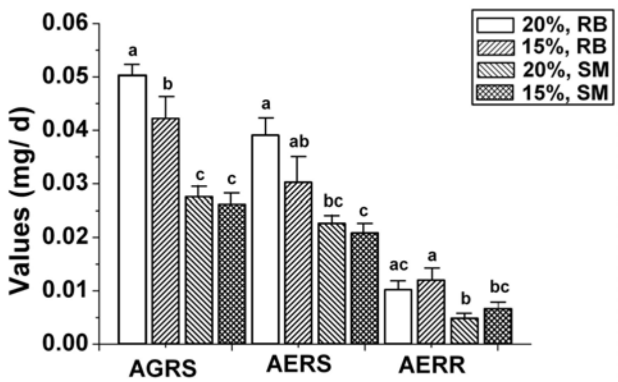


Fig. 5. Comparisons among two substrates with two water content on three seedling survival indexes.

Table 4. Results of two-way ANOVA

	TL			Height			LA			DB			DWS			DWR			R/S		
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
soil type	1	38.19	0	1	55.76	0	1	16.3	0	1	66.4	0	1	17.9	0	1	10.2	0.01	1	1.54	0.23
water content	1	18.29	0.001	1	4.274	0.06	1	1.03	0.32	1	3.87	0.07	1	3.06	0.1	1	1.13	0.3	1	2.85	0.11
soil type * water	1	11.21	0.004	1	0.865	0.37	1	0.15	0.7	1	1.97	0.18	1	1.36	0.26	1	0	1	1	0.25	0.62

4. Discussions

4.1 Suitable moisture and soil conditions

This study showed that RB soil was the most suitable soil substrate in the natural condition and higher water content (20%) for P. euphratica seed germination and seedling growth.

RB soil always consists with lower salty content and higher nutrients than S soil and SM soil in nature conditions (Table 1). Wu *et al*. (2007) have proved that lower concentration of NaCl solution has little effect on the total seed germination rate; and when the concentration reaches 90mmol/L, the rate will obviously decrease. Besides, there were another two reasons for higher germination rate in RB soil: (1) because of its more organic constituents and higher compaction, RB soil retention was greater than those of S and SM soils, for example, the seedlings growing on the S soil with 20% moisture cannot survive over 20 days, let alone with lower moisture in natural conditions (Table1, 2); (2) many arid plants, such as Sophora alopecuroides L., usually exude allelochemicals into SM soil, inhibiting other plants' seed germination or growth. Zhang *et al*. (2005) figured out that low moisture, high salt content and allelopathy chemicals, which were the characteristics of S soil and SM soil, had negative effect on germination. Therefore, we argued that soil type in nature conditions could significantly influence the seed germination and seedling growth of P. euphratica, which is consistent with the results of two-way ANOVA (Table 4).

Although higher soil moisture could effectively promote the seedling growth on the whole, less moisture availed the taproot elongation and AERR instead of the shoot height, leaf largeness or biomass accumulation of P. euphratica seedling (Table 3). However, Peters (1957) found that at a soil suction (i.e. the relative vapor pressure of the soil moisture) of 1/3 bar and a bulk density of $1.25g \cdot cm^{-2}$, corn roots elongated faster in a soil mixture with a gravimetric water content of 27% than in one with a water content of 8%, which was different with our results in P. euphratica. The actual reason for this may be the fact of different plant with different characteristics but still need further study.

All the values in the experimental group of '20%, RB' showed that this condition could guarantee the higher seed germination rate and seedling survival percentage (Fig. 2; Table 2). This condition made most seeds germinate faster and longer and seedlings grow stronger (Fig. 3, 4 and 5), which may be the results of lower salty content, higher nutrients and less allelo-chemicals as above (Table 1).

4.2 Reasons for the failure of natural seed-regeneration

Many studies have focused on P. euphraticai growth and its reliance on groundwater (Gries *et al*. 2003; Rüger *et al*. 2005; Liu *et al*. 2007; Fu *et al*. 2010), but still few on its seed and seedling stage. In natural conditions, there were no favorable conditions for P. euphratica seed germination during their short lives (Zhang *et al*. 2005), and the channeling has changed the natural hydrologic regimes and processes leading to the failure of sexual regeneration of P. euphratica (Cao *et al*. 2009b). However, we argued that the suitable habitat for its seed germination and seedling growth really existed in the nature and found that many branches of Ejina River were not cemented except the main channel (Photo 1, 2). The areas found being covered by many seed-originated seedlings are usually low and flat flood plains, which can store river water to keep high moisture and low salinity. But there were few seedlings in the other areas, such as in the sandy areas or in the main P. euphratica forests. That fact was just coincident with our experimental results.

Seed dispersal process was generally divided into two periods: Phase I, the movement of germinable seeds from the plant to a surface and Phase II, the secondary horizontal and vertical movements of seeds (Chambers and MacMahon 1994). As to the anemophilous P. ruphratica seeds, wind is the key factor in Phase I. Considering the adhesive effect of abundant vegetation in the forests on seeds, we thought river bank and the other shallow sites with sufficient water were the most suitable ones for seed germination among all of the surfaces (e.g. river, soil and plants). That may be the reason why P. ruphratica forests are always called 'riparian'. After quick germination in the water, seeds adhered to the flooded river bank as 'belt' where were suitable for seedling growth.

Although Cao *et al*. (2009b) revealed that the supplemental flows were sufficient during the seed rain period of July 14^{th} to August 28^{th}, we still found that the water could not reach the Nature Reserve in that period every year. It was proved that the most steady water supplement was during the two periods of March to April and September to October every year, but unstable in the seed rain period (Chen, 2010), which leaded to the discontinuous suitable habitats for seedlings in the preliminary key years and might be the main reason for the failure of P. euphratica sexual reproduction. Due to the huge economic effect of the hot and developing tourism of riparian P. euphratica forest from September to October, the precious and rare river flow was usually supplied to create beautiful landscape in autumn and more impossible to be got from July to August in this extreme arid region, which accelerated the vegetation deterioration.

It was reported that there was a 'biennial fruiting variation' phenomenon among many plants (Li *et al*. 1998; Borgardt and Nixon 2003; Hirayama *et al*. 2008). Based on the assumption that the whole community gets the equal energy and nutrients every year, some thought that there might also be a biennial seed production in P. euphratica which severely affected the seed quality and then the subsequent growth stage. However, the high seed germination rate of our experiments in recent years proved this idea was not true (Zhang *et al*. 2005; Wu *et al*. 2007; Liu *et al*. 2011).

Furthermore, light condition may be another limiting factor for P. euphratica sexual regeneration (Zhang *et al*. 2005; Liu 2011). Therefore, we did a simple additional experiment about the effect of lightness on seed germination (Fig. 6). It is obvious that light significantly lowered the seed vitality. In nature, the extremely high illumination intensity there made the ground temperature rise to over 70°C with rare vegetation, definitely leading to the death of weak seeds and seedlings. Along the river bank, the conditions of vegetation were various and only the suitable shading condition provided by the abundant plants could pretend them from being burnt by sun.

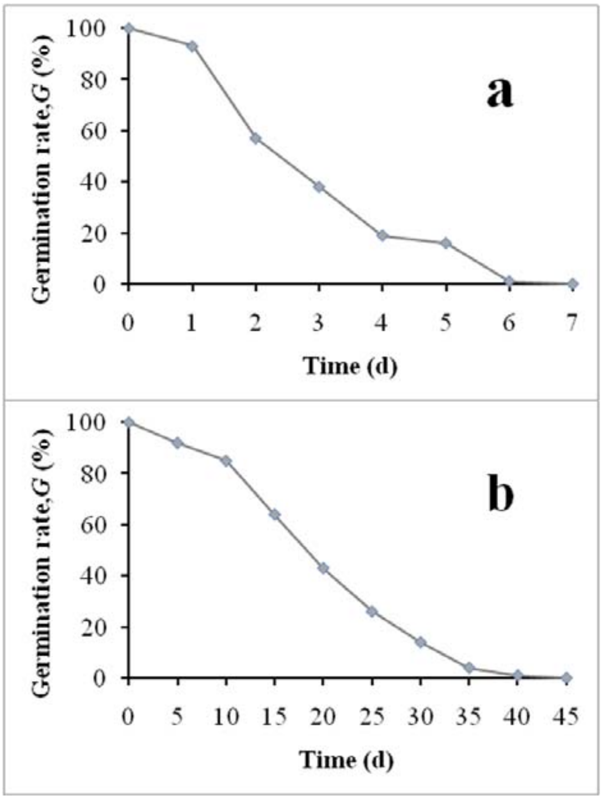


Fig. 6. Seeds vitality under both full light and shading conditions.
a: full light; b: shading.

In a word, the water supply amount and time were the keys for the successful sexual regeneration of P. ruphratica. It is badly in need of the reasonable water adjustment and the foundation of river bank vegetation to protect the P. euphratica forest in Ejina Oasis.

4.3 Suggestions for artificial seedling cultivation

Through our experiment connecting two essential environmental factors with seed and seedling periods, we provided some useful suggestions for artificial seedling cultivation of P. euphratica: 1) the harvested seeds should be germinated in the pure water which can guarantee the highest germination rate; 2) the germinated seeds should be kept in the water for 8-10 days to elongate their taproots quickly; 3) after that, seedlings should be transplanted in the river bank soil or the other soils with suitable moisture (20%) and nutrients, and at the same time, the light condition should be gradually added.

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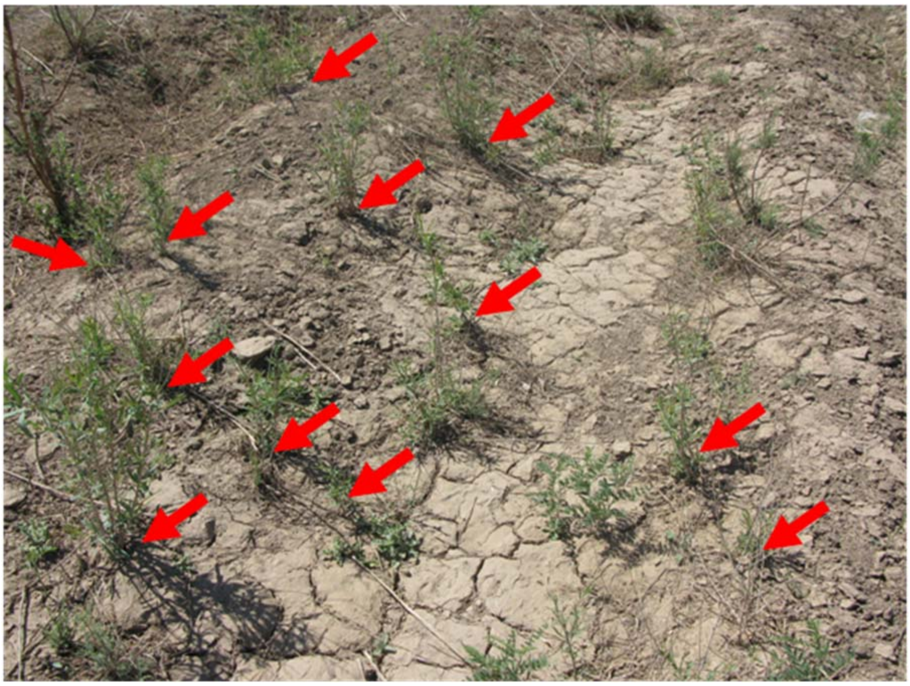

Photo 1. Natural seed-originated seedlings on a flooded river bank in Ejina Oasis (N41°54.804', E101°04.387') (Taken in June, 2010)

Photo 2. A flooded river bank suitable for *P. euphratica* seedlings in Ejina Oasis (N41°54.804', E101°04.387') (Taken in June, 2010)