

Original article:**COMPARATIVE GERMINATION OF *Tamarix ramosissima* SPRING AND SUMMER SEEDS**Cheng Yan¹, Yan Wei^{2*} and Meilin Yang^{1,3}¹ Xinjiang Ecology and Geography Research Institute, the Chinese Academy of Sciences, Urumqi, 830011, Xinjiang China² College of Pratacultural & Environmental Science, Xinjiang Agricultural University, Urumqi, 830052, Xinjiang China³ Graduate University of the Chinese Academy of Sciences, 100049, Beijing China* corresponding author: Yan Wei; e-mail: weiyan1966@163.com; Tel: 086-13899933211**ABSTRACT**

Tamarix ramosissima has bi-seasonal flowering and fruit-setting characteristics. This study compared the morphology and germination characteristics of seeds from *Tamarix ramosissima* plants during the spring flowering period and the summer flowering period. The results are as follows: there is no significant difference in morphology, such as size and thousand-seed weight, between seeds from different the spring and summer flowering periods. Freshly harvested spring and summer flowering period seeds can both rapidly germinate under the 5/15 °C, 5/25 °C, 15/25 °C or 25/35 °C thermoperiods. Average germination time of the spring flowering period seeds is shorter than that of the summer flowering period seeds, with a TG₅₀ of 2-18 hours (TG₅₀ = the time when germinate percentage reached 50 %). NaCl solutions at concentrations below 0.2 mol/L have no significant effect on the germination of *Tamarix ramosissima* seeds from either flowering periods. However, at above 0.4 mol/L, higher NaCl concentrations adversely affect the *Tamarix ramosissima* seeds' germination rates. Seeds from the summer flowering period have higher tolerance of NaCl solutions than seeds from the spring flowering period. Seeds that were incubated in NaCl solution for 8 days cannot recover after being transferred to distilled water (0 % recovery), indicating that NaCl treated seeds permanently lost their germination capability. After storing in room temperature for 2 months, *Tamarix ramosissima* seeds from both spring and summer flowering periods maintain above 80 % viability. After 2 months, the seed viabilities decrease as the storage time lengthens. The viabilities of seeds from both spring and summer flowering periods decrease to about 40-50 % after storage for 6 months. This study can provide scientific basis for rational utilization of the biological resources in arid desert ecosystems.

Keywords: bi-seasonal seed, germination, salinity, *Tamarix ramosissima***INTRODUCTION**

Bi-seasonal flowering refers to the phenomenon when plants have two distinct flowering periods during one growth cycle or have two flowering peaks during one extended-flowering period, and can produce mature seeds throughout the flowering period (Picó and Retana, 2003; Vaknin et al.,

1996). This flowering pattern is the result of plants' life-history strategies and coevolution in ecological communities (Ohara et al., 2001). Plants' phenological characteristics have significant implications to their reproductive successes (Rathcke and Lacey, 1985; Neill, 1997; Picó and Retana, 2000). Seed-germination behaviors are also critical to population sustainability, population dy-

namics and community structures (Bischoff et al., 2006). Seeds are the unique reproductive organs of seed plants. Seed germination characteristics are determined by the environmental conditions encountered both by the seed during its germination process and by its parent during fruit-setting and maturation (Picó and Retana, 2000). According to Gutterman (1991), in some plants, mature seeds from the same plant, but from different sunshine lengths and temperatures, may have different germination abilities. Because of this seed heterogeneity, only a portion of all seeds germinate at the same time even under optimal conditions. This phenomenon is critical to the survival of seeds, especially under the extreme conditions of the desert where the total amount and distribution of rainfall is unpredictable.

Tamarix ramosissima is a deciduous shrub or small arbor in the *Tamarixaceae* family, *Tamarix* genus. In China, it is mainly distributed in the southern and northern Xinjiang, valleys, the Piedmont Gobi desert and the Hexi Corridor. It is an ideal species for desert forestation. *Tamarix ramosissima* has two distinct flowering periods in the spring and the summer and can continuously produce germinateable seeds during both flowering periods (Wang and Yin, 1991). What are the morphology and germination characteristics of seeds from *Tamarix ramosissima* plants during different flowering periods? How do these characteristics fit *Tamarix ramosissima*'s habitat? This study compared the morphology and germination characteristics of seeds from *Tamarix ramosissima* plants during the spring flowering period and the summer flowering period. It has great value in understanding plant reproductive ecology, adaptive mechanisms and evolution. It also provides scientific basis for rational utilization of the biological resources in arid desert ecosystems.

MATERIAL AND METHODS

Materials and natural profile of the study area

Tamarix ramosissima has bi-seasonal flowering characteristics. The inflorescences of the spring flowering period form panicles that grow laterally on old branches from the previous year. The spring flowering period lasts from late April to early- to mid-May. The inflorescences of the summer flowering period are large panicles formed by varying number of racemes, growing on the tips of new branches of the current year. The summer flowering period lasts from mid-June to August. The seeds mature 8-12 days after flowering.

Tamarix ramosissima seeds used in this study were collected from the Chinese Academy of Sciences Turpan Desert Botanical Garden during late-May (spring flower seeds) and early-July (summer flower seeds) in 2006.

Chinese Academy of Sciences Turpan Desert Botanical Garden is located in the Turpan Basin (89°11'E, 40°51'N, altitude -95 ~ -76m). The annual average temperature is 13.9 °C; monthly average temperature of January is -9.5 °C; monthly average temperature of July is 32.7 °C; highest recorded temperature is 47.6 °C; lowest recorded temperature is -28.0 °C. Average annual rainfall is only 16.4 mm; annual evaporation is 2837.8 mm. It belongs to warm and arid desert climate (Wang and Yin, 1991).

RESEARCH METHODS

Observation of seed morphology

Plump *Tamarix ramosissima* seeds were selected under naked eyes. Seed weights were determined by 1000 seeds as a group using one ten-thousandth gram balance. Weighing was repeated four times and the average was calculated accordingly. The length and width of 30 seeds were measured using the Motic B5 microscope.

Germination of the seeds under different temperature

Plump seeds were selected from the newly harvested mature seeds, 50 seeds per group, for 3 groups. Seeds were placed in 90 mm petri dishes with two layers of filter papers as padding. The filter papers were kept moist until the end of germination.

Based on the climatic characteristics of Xinjiang (Wang and Yin, 1991), four thermoperiods – 5/15 °C, 5/25 °C, 15/25 °C and 25/35 °C – were selected. Germination experiments were conducted in the GXZ Intelligent Light Incubator under 3000 lx illumination (dark/light = 12/12h) and continuously observed for 8 days.

Germination of the seeds in NaCl solutions of different concentrations

NaCl solutions of 0.05, 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00 mol/L were used with distilled water as control. Germination of seeds were tested at 15/25 °C (dark/light = 12/12 h). Under this thermoperiod, germination rates of *Tamarix ramosissima* seeds in NaCl solutions of different concentrations were observed. After 8 days of incubation, ungerminated seeds were transferred to distilled water and incubated for another 8 days to determine the seeds' recovery germination rate and final germination rate.

Data analysis

During the germination process, the seeds were examined once every 2 hours during the first day, followed by once every 24 hours in the subsequent days. The germinated seedlings were removed immediately upon identification.

Germination results were expressed in both germination percentages and recovery germination percentages:

Germination percentages = number of germinated seeds / 50 × 100 %

Recovery germination percentages = [(A-B) / (C-B)] × 100 %, wherein A represents the total number of germinated seeds, B represents the number of seeds germinated in NaCl solutions, C represents the total number of seeds under treatment in the experimental group.

Germination speeds are calculated using the initial germination times and also the time when germinate percentage reached 50 % (TG₅₀).

The data was processed and analyzed using the SPSS 11.5 software. First, all experimental data was tested for normal distribution using the “one-sample Kolmogorov-Smirnov (K-S) test.” For the data points that were consistent with normal distribution, “Independent Samples T Test,” “Paired-Samples T Test” and “One-Way ANOVA” were conducted to determine the differences. For the data points that were inconsistent with normal distribution, the nonparametric, two independent samples “Kolmogorov-Smirnov Z Test” was conducted to examine the differences.

RESULTS

Seed morphology

The *Tamarix ramosissima* seeds are small, brown and rod-shaped. There are clusters of seed hairs on the top of the seeds. The length of the seed hair is about 2 to 4 times the length of the seed. The seeds are wind-dispersed. The morphologies of *Tamarix ramosissima* seeds from both flowering periods are listed in Table 1.

Table 1: The Seed morphology of *Tamarix ramosissima*

| | Spring Seed | Summer Seed |
|---------------------------|--------------|--------------|
| Thousand Seed Weight (mg) | 19.16 ± 0.39 | 18.26 ± 0.54 |
| Length (mm) | 0.97 ± 0.03 | 0.94 ± 0.01 |
| Width (mm) | 0.46 ± 0.02 | 0.40 ± 0.01 |

Values are Mean ± SEM, n = 4 (Thousand Seed Weight), n = 30 (Length and Width).

Effect of thermoperiods on germination of *Tamarix ramosissima* seeds

As shown in Figure 1, under 5/15 °C, 5/25 °C, 15/25 °C or 25/35 °C thermoperiods, final germination rates of *Tamarix ramosissima* seeds from both flowering periods reach above 80 %, with no significant difference (One-Way ANOVA, Independent Samples T Test, Paired-Samples T Test: $P > 0.05$) (Figure 1).

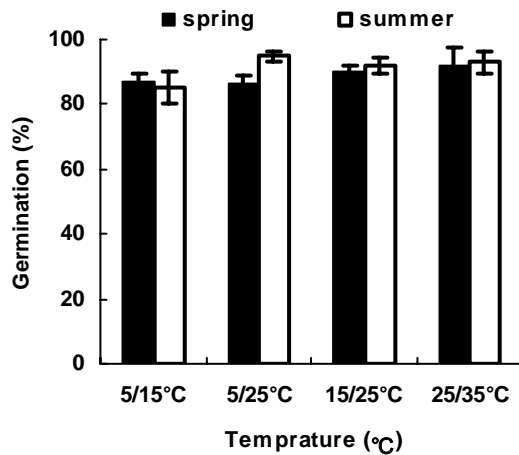


Figure 1: Final germination percentages (\pm s.e.) of seeds of *Tamarix ramosissima* at different constant thermoperiods after 8 days of incubation.

5/15, 5/25, 15/25 and 25/35 °C were four alternating temperature regimes with a corresponding 12 h light : 12 h dark photoperiod

However, the initial germination time and TG_{50} of *Tamarix ramosissima* seeds from both flowering periods under different thermoperiods have significant difference. The summer flower seeds have faster rate of germination. Spring flower seeds begin to germinate within 24 hours of getting in contact with water, and reaches TG_{50} within 48 hours. The higher the temperature, the faster the germination speed is. In contrast, the initial germination time and TG_{50} of summer flower seeds under 5/15 °C, 5/25 °C and 15/25 °C thermoperiods are all significantly shorter than those of spring flower seeds (Figure 2, Figure 3).

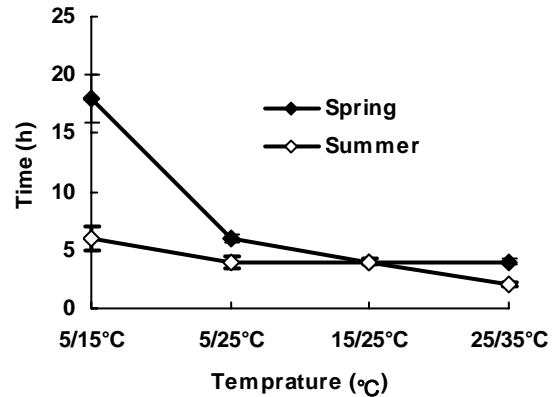


Figure 2: Initial germination time of *Tamarix ramosissima* seeds under different thermoperiods.

5/15, 5/25, 15/25 and 25/35 °C were four alternating temperature regimes with a corresponding 12 h light : 12 h dark photoperiod

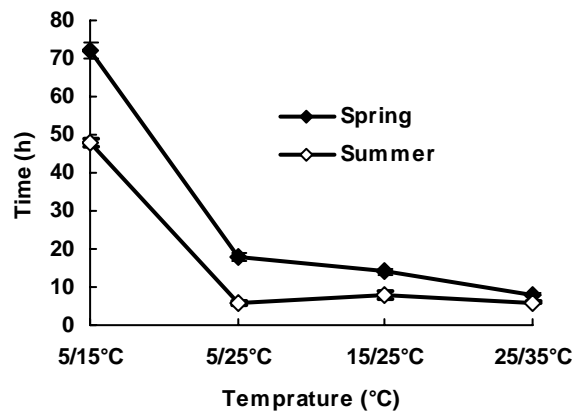


Figure 3: TG_{50} of *Tamarix ramosissima* seeds under different thermoperiods

TG_{50} = The time when germinate percentage reached 50 %

5/15, 5/25, 15/25 and 25/35 °C were four alternating temperature regimes with a corresponding 12 h light : 12 h dark photoperiod

Effect of salt on germination of *Tamarix ramosissima* seeds

After 8 days of incubation, germination rates of *Tamarix ramosissima* seeds from both flowering periods decrease with similar trends (Table 2). Both germination rates have no significant difference from control in 0.05, 0.1 and 0.2mol/L NaCl solutions. However, in higher concentrations of NaCl such as 0.4, 0.6 and 0.8 mol/L, the effect is significant: salt inhibits seed germination and decreases the seed germination rates.

Table 2: Effects of different concentration of NaCl solution on the germination of *Tamarix ramosissima* seed

| Salinity (mol/L) | Germination Rate | | Initial Time (h) | | TG ₅₀ (h) | |
|------------------|--------------------------|--------------------------|--------------------------|-------------------------|-------------------------|----------------------|
| | Spring Seed | Summer Seed | Spring Seed | Summer Seed | Spring Seed | Summer Seed |
| 0 | 84.00±3.06 ^a | 90.00±1.15 ^a | 7.00±0.00 ^a | 3.30±0.00 ^a | 15.00±0.00 ^a | 7±0.67 ^a |
| 0.05 | 80.33±4.67 ^b | 89.67±1.76 ^{ab} | 8.00±0.00 ^a | 2±0.67 ^a | 18.00±0.00 ^b | 6.00±0 ^a |
| 0.1 | 80.00±1.15 ^{ab} | 89.67±1.33 ^{ab} | 9.33±0.67 ^a | 4±0.67 ^a | 21.00±0.00 ^c | 8±3.33 ^a |
| 0.2 | 66.67±2.40 ^{ab} | 88.00±5.29 ^b | 14.33±0.67 ^b | 6±0.67 ^b | 60±0.67 ^d | 24±0.67 ^b |
| 0.4 | 55.67±2.91 ^c | 45.67±5.46 ^c | 48.00±0.00 ^c | 13.00±0.00 ^c | 110±8.00 ^e | — |
| 0.6 | 3.33±5.46 ^d | 12.33±1.76 ^d | 144.00±0.00 ^d | — | — | — |
| 0.8 | 0 ^e | 2.00±3.06 ^e | — | — | — | — |
| 1.0 | 0 ^e | 0 ^f | — | — | — | — |

Values are Mean ± SEM, n = 3. Values with different alphabetical superscripts (a, b, c, d, e, ab) along a column are significantly different at P<0.05.

TG₅₀ = The time when germinate percentage reached 50 %.

On the other hand, germination rates of the summer flower seeds are significantly higher than those of the spring flower seeds (Paired-Samples T Test: $t = -6.495$, $P=0$), indicating that summer flower seeds have higher salt tolerance than spring flower seeds.

After 8 days of incubation in salt solutions of various concentrations, ungerminated seeds were transferred to distilled water in order to determine the seeds' recovery germination after inhibition by salt. Results show that neither spring flower seeds nor summer flower seeds germinate after being transferred back to water, both recovery germination rates being 0.

DISCUSSION

Plant's flowering time and number of days during the flowering period may be affected by adverse weather conditions (Rose et al., 1998) or different biological factors (Biere and Honders, 1996). Therefore the plants with bi-seasonal flowering characteristics may have different seed morphology (TSG or size) and different germination characteristics in seeds from different time periods (Guterman, 1991). *Tamarix ramosissima* seeds from the spring and summer flowering periods have no significant difference in morphology (length and width) and germination rates under four different thermoperiods all reach above

80 %, with no significant difference. Germination speeds increase when temperature increases. The TG₅₀ is 144 hours, whereas at 25/35 °C, the TG₅₀ is only 2-4 hours. This ability to rapidly germinate under a wide temperature range enables *Tamarix ramosissima* to take advantage of any available flood or precipitation to quickly sprout seedlings during the May to September fruiting season. This ability to rapidly germinate under a wide temperature range has also been observed in seeds of other desert plants, such as *Blepharis spp.* (Guterman, 1972), *Salsola kali* (Wallace et al., 1968), *Salsola affinis* (Wei et al., 2007). This ability to rapidly germinate under a wide temperature range enables majority of seeds to germinate and generate seedlings within suitable seasons, therefore, is beneficial to the evolution and renewal of the population.

Tamarix ramosissima seeds from both flowering periods have the ability to germinate with some salt tolerance. Summer flower seeds have higher salt tolerance than spring flower seeds. In low concentration NaCl solutions, seeds from both flowering periods have higher germination rates. However, germination rates decrease as the salt concentration increases, till no germination is observed. Seeds cannot recover after being transferred back to water. The effect of salt on seed germination is gener-

ally understood as ion toxicity and osmotic effect (Ungar, 1987). Low germination or no germination after being transferred back into water indicate that the seeds were already killed by the toxicity of sodium ions. Similar phenomenon has been observed in *Medicago sativa* (Redmann, 1974), *Pisum sativum* (Manohar 1966), *Sporobolus airoides* (Hyder and Yasmun, 1972), *Haloxylon recurvum* (Khan and Ungar, 1996), and *Aeluropus lagopoides* (Gulzar and Khan, 2001). The differences in germination speed and salt tolerance between *Tamarix ramosissima* seeds from spring and summer flowering periods may be caused by environmental differences encountered by the seeds during maturation. Such differences have also been observed between *Cheiridopsis aurea* (Aiozaceae) summer and winter seeds (Guterman, 1991).

Desert plants generally have special combinations of survival mechanisms (Guterman, 1993). Different desert plants have a variety of different seed dispersal and seed germination mechanism combinations, whereas special seed dispersal and germination mechanisms adapted to the environment are key to desert plants' survival and proliferation (Wei et al., 2007). Seeds have greater tolerance to stress than adult plants. Hence plants often form soil seed banks (Chang et al., 2001). In particular, persistent soil seed banks can not only effectively extend the useful lives of seeds and preserve their germination capabilities, but also spread out the seed germination times, thereby reducing risks seeds face during germination and contributing to population renewal and recovery (Guterman, 1993). However, *Tamarix ramosissima* seeds have a shorter life span, can rapidly germinate under a wide range of temperatures, and have an "opportunistic" germination strategy (Venable, 1985), therefore, can not form a persistent soil seed bank.

On the other hand, the *Tamarix ramosissima* plant can produce seeds with high germination capabilities during both spring and summer reproductive seasons, and seeds produced by summer flowers in Au-

gust to September still have more than 80 % viability in the beginning of winter (November, 2 months after maturation). Studies have shown that the below freezing temperature is the optimal long-term dry storage temperature for seeds of many arbores and shrubs, maintaining good seed viability. In the following spring when air temperature rises and snow melts, viable summer flower seeds can have guaranteed germination and successful settlement in the early spring. *Tamarix ramosissima*'s continuous flowering and continuous fruiting reproductive strategies allow it to create a "live seed-bank", and its rapid germination tactics ensure seeds germination into seedlings under suitable conditions, so as to achieve reproductive success.

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