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SEED PROPAGATION STRATEGY INVOLVING RAPID GERMINATION WITH A HIGH FINAL PERCENTAGE IN TWO PERENNIAL CLONE PLANTS IN THE TAKLIMAKAN DESERT

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Abstract

Alhagi sparsifolia and *Karilinia caspica* are two important perennial clone plants of the Taklimakan Desert that also produce a large number of seeds every year. In their natural environment, the two species always reproduce asexually. It is necessary, from the perspective of both basic science and applied science (e.g. vegetation restoration), to explore why clone plants such as these produce seeds and under what conditions they become viable. Accordingly, in this study, the effects of light and temperature on germination and embryo stretch under five temperature treatments (15°C, 20°C, 25°C, 30°C and 35°C) and two light treatments (LIGHT/DARK and DARK) were investigated. Significant differences were detected in terms of the final germination percentage, germination rate, and the lengths of the plumule, radicle, and seedling, leading us to further investigate the germination strategies of the two plant species under the different light and temperature conditions. The results showed that both light and temperature had a significant effect on germination and embryo stretch in the two plants, yet they were not limiting factors. In shielding the adverse effects of external factors through use of an incubator, the two species' seeds showed a seed propagation strategy involving rapid germination with a high final germination percentage.

Key words: Germination, Embryo stretch, Light, Temperature, Taklimakan Desert.

Introduction

Seeds are a key component of the life history of higher plants. Because the embryo contained within a seed is structurally and physiologically equipped, the next generation of plants can develop independently (Bewley, 1997). Thus, seeds play an important role in plant colonization, providing opportunities to settle in new environments and avoid adverse conditions (Bu *et al.*, 2007). Seed germination and stretching of the embryo are critical phases in the early life of higher plants (Cheng *et al.*, 2015). They will affect the germination percentage, survival rate, and the distribution and abundance of the species (Washitani & Nishiyama, 1999; Olff *et al.*, 1994; Valverde *et al.*, 2004). However, from seed germination to stretching of the embryo is the weakest link in the life cycle of higher plants in desert areas (Wang *et al.*, 2008; Shen *et al.*, 2015). Therefore, carrying out research that broadens our knowledge of seed germination in plants living in desert environment can assist in improving artificial vegetation restoration.

The Taklimakan Desert is the second largest mobile desert in the world; the mean annual precipitation amount is approximately 33 mm, and potential annual evaporation is approximately 2600 mm (Zeng *et al.*, 2016). Environmental conditions are extremely poor, with very little vegetation able to survive. Indeed, the region is often referred to as the "sea of death" (Wang *et al.*, 2014). Nevertheless, two very important perennial plants do exist in the Taklimakan Desert: *Alhagi sparsifolia* and *Karilinia caspica*. Under natural conditions, these species propagate by producing clones of them asexually. They are often associated with each other and their roots penetrate deep below the surface to the groundwater level (Gui *et al.*, 2013). Perhaps not surprisingly, *A. sparsifolia* and *K. caspica* have developed xeromorphic characteristics as a result of long-term adaptation to drought, high temperatures, salinity, high light intensity, strong winds, and sand movement (EL-Khatib, 2000; Hassanein & Mazen, 2001). Consequently, owing to their ability to survive this harsh desert environment, these two plants play a key role in vegetation restoration, serve as windbreaks, and promote sand fixation. In addition, *A. sparsifolia* is an excellent livestock feed (Zeng *et al.*, 2002), and an important fodder for animals of local farmers because of its high protein content (Gui *et al.*, 2013). *K. caspica*, meanwhile, can contribute to the conversion of saline–alkali soil into farmland (Jia & An, 2004). Regrettably, however, these two plants are clone plants, which are very difficult to reproduce by seed in their natural environment. Even with frequent irrigation, only a very low number of seedlings can be found in the *A. sparsifolia* community, and none at all in the *K. caspica* community (Guo *et al.*, 2008). Therefore, studying germination strategies in these two plants is imperative for the advancement of seed-based vegetation restoration techniques.

Temperature and light are two of the most important environmental factors for successful germination (Ghaderi *et al.*, 2008; Koger *et al.*, 2004). However, our understanding of the effects of these two factors on seed germination and embryo stretching in *A. sparsifolia* and *K. caspica* is limited. The present study attempts to address this knowledge gap.

Materials and Methods

Seed sources and processing: Seeds were collected from *A. sparsifolia* and *K. caspica* plants in their natural environment at the southern edge of the Taklimakan Desert. The climate of this region is extremely dry (Thomas *et al.*, 2006), with annual precipitation of approximately 35.1 mm and annual evaporation of more than 2600 mm (Zeng *et al.*, 2006). The maximum temperature in summer is 42°C. Completely mature and undamaged seeds were chosen for the experiment.

Since *A. sparsifolia* seeds have a hard coat, they were soaked in 98% concentrated sulfuric acid for 3 minutes to soften the seed coat. Seeds were rinsed repeatedly with sufficient water to remove the sulfuric acid after soaking, and were then placed on a piece of white paper to dry. After drying, we checked for a yellow or gray mark on the white paper to ensure no residual sulfuric acid remained. In order to avoid mildew growth during the germination of *K. caspica* seeds, the seeds were soaked with 1% $HgCl_2$ for 7 minutes, and then repeatedly rinsed (more than seven times) with distilled water after disinfection. Finally, the seeds were dried. All seeds (both species) were placed in distilled water for 12 hours before germination.

Light exposure: The experimental design was a completely randomized 2×5 factorial arrangement (light versus temperature). Experiments were carried out in a constant-temperature light incubator (YGZ-300F, Shanghai, China). Seeds were exposed to alternating light (12 hours of light and 12 hours of dark) in the LIGHT/DARK treatment and continuous darkness in the DARK treatment. Light was provided by twelve light bulbs (14W, 220V, 50Hz). The light intensity reached 15000 Lx in the LIGHT/DARK treatment. Light regimes were selected to reflect seeds buried in the soil, or exposed at the soil surface, in their natural environment.

Temperature: Constant temperature effects were examined using the incubator, and the error was calculated to be less than 0.1°C. To ensure the environmental conditions met the requirements, the incubator was left running for 1 hour prior to each experiment. The temperature ranged from 15°C to 35°C in 5°C intervals, i.e., there were five temperatures tested: 15°C, 20°C, 25°C, 30°C and 35°C. These temperature treatments were selected to represent several temperatures that are common during the natural and artificial regeneration period of these two perennial desert plants.

Germination and stretching of the embryo test: The treated seeds were evenly distributed in culture dishes with two layers of filter paper soaked with distilled water. There were 50 seeds in each culture dish, and six dishes per treatment of one plant. Three of the six dishes were used to determine germination, and the remaining three for testing the stretching of the embryo. The dishes were placed in the incubator, which had been pre-running for an hour.

We added moderate amounts of water to each dish and recorded the number of germinations every 12 hours. Seeds were recorded as germinated when the length of the protruding radicle was ≥1 mm, as defined by Imanishi (2014). When there were no new germinations for three consecutive days, it was taken to be the end of the germination period. At that point, we calculated and gathered statistics regarding final germination (FG) (El-Keblawy *et al.*, 2014), the germination rate (GR) (Edmond & Drapala, 1958), and the daily germination rate (DGR) (Zucareli *et al.*, 2015). FG (%) was calculated as:

$$FG = (G_T / 50) \times 100,$$

where G_T is the total number of germinated seeds. GR was determined using the average number of days spent germinating (Zucareli *et al.*, 2015):

$$GR = ((G_1 \times D_1) + (G_2 \times D_2) + \ldots + (G_n \times D_n)) \div (G_1 + G_2 + \ldots + G_n),$$

where G_1, G_2, …, G_n is the number of germinated seeds computed in the first, second, …, last count; and D_1, D_2, …, D_n is the number of days after the experimental layout. DGR (%) was calculated as

$$DGR = G_n / 50 \times 100,$$

where G_n is the number of germinated seeds in n days.

When all seeds had finished germinating, we selected the five longest seedlings from each dish to measure the length of the plumule, radicle and seedling. We selected the average value of each index to represent the corresponding index in the dish, with three dishes as three replications in each treatment.

Statistical analysis: To examine the effects of light and temperature on germination and stretching of the embryo, we analyzed five indicators (FG, GR, plumule length, radicle length, and seedling length) by constructing a two-way analysis of variance (two-way ANOVA), with the five indicators as the response variables and two factors (light, temperature) and their interaction (light × temperature) as the explanatory variables. The data were subjected to ANOVA for significance, and the least-squares difference (LSD) test was used to compare means at 5% probability. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Final germination: As shown in Fig. 1A, the FG of *A. sparsifolia* seeds in LIGHT/DARK was significantly lower than that in DARK ($p<0.05$) except at 30°C. Under the conditions of LIGHT/DARK, seeds germinated at 20°C had a lower FG compared to seeds germinated at other temperatures ($p<0.05$), with an average value of 82.67%, and the highest FG was observed at 30°C, with an average value of 97.33% ($p<0.05$). Under DARK conditions, the lowest FG also appeared at 20°C ($p<0.05$), with only 84% of seeds germinating. No significant differences in FG were observed among the 15°C, 25°C, 30°C and 35°C treatments ($p>0.05$).

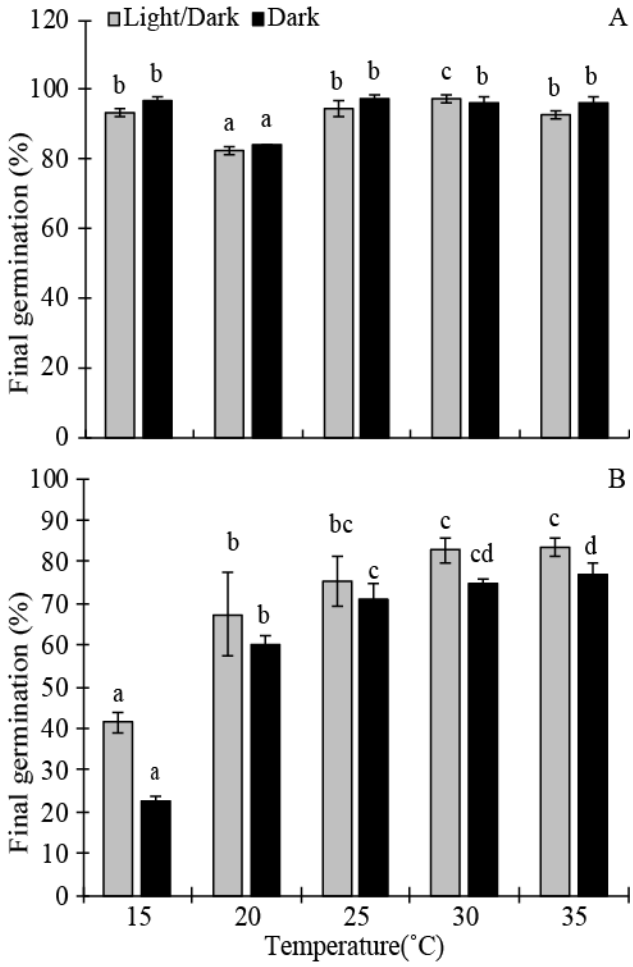

Fig. 1. Final germination of *Alhagi sparsifolia* (A) and *Karilinia caspica* (B) seeds under different light and temperature treatments, noted as mean ± SD (n = 3). Means with the same letters are not significantly different among temperatures at the p = 0.05 level, based on LSD mean separation.

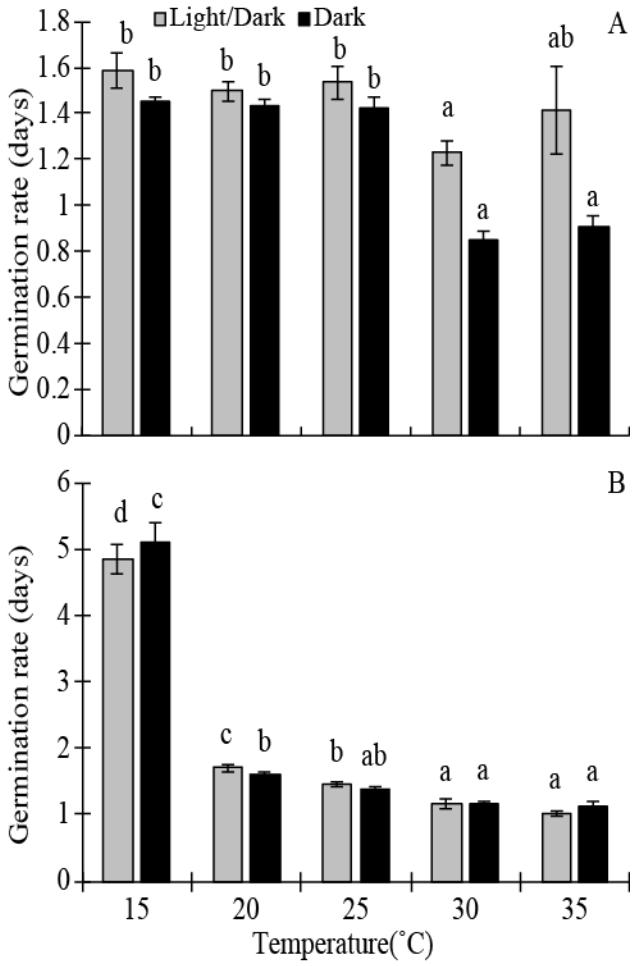

Fig. 2. Germination rate of *Alhagi sparsifolia* (A) and *Karilinia caspica* (B) seeds under different light and temperature treatments, noted as mean ± SD (n = 3). Means with the same letters are not significantly different among temperatures at the p = 0.05 level, based on LSD mean separation.

The results of the two-way ANOVA for the effects of light and temperature on FG (Table 1) showed that the FG of both *A. sparsifolia* and *K. caspica* seeds was significantly different ($p < 0.01$) under different light and temperature treatments, but not significantly different ($p>0.05$) in their interactions, except for the GR of *A. sparsifolia*.

In *K. caspica*, the FG was improved by light exposure ($p<0.05$) (Fig. 1B), being higher in LIGHT/DARK than in DARK ($p < 0.05$). Furthermore, under both LIGHT/DARK and DARK conditions seed germination increased gradually with an increase in temperature ($p<0.05$). FG in DARK was 22.7% at 15°C, while it increased to 237.8% when temperature reached 35°C. Similarly, in LIGHT/DARK, the FG at 15°C (41.3%) was also lower than the FG achieved under the other (higher) temperature treatments ($p<0.05$). When the temperature exceeded 30°C, no significant differences in FG were observed compared to the 35°C treatment ($p>0.05$).

The process of germination: Light, temperature and their interaction had an extremely significant impact ($p<0.01$) on the GR of *A. sparsifolia*, as shown in Table 1. The average numbers of days spent germinating in DARK were less than those in LIGHT/DARK at the five different temperatures (Fig. 2A) ($p<0.05$). GR showed a gradual declining trend with increasing temperature, in both LIGHT/DARK and DARK. There were no significant differences ($p>0.05$) among the 15°C, 20°C and 25°C treatments. The number of days under the 15°C, 20°C and 25°C treatments was significantly ($p<0.05$) greater than that at 30°C and 35°C. No significant differences ($p>0.05$) were observed between the 30°C and 35°C treatments, either in LIGHT/DARK or in DARK. There were also no significant differences between Light/Dark and DARK under the 15°C, 20°C and 25°C treatments; however when temperature increased to 30 and 35°C, a significant difference in the GR was observed.

Figure 3A shows the DGR of *A. sparsifolia* in LIGHT/DARK. The time need for first germination was 0.5 days for all temperature treatments except that of 15°C, which needed 1 day. Furthermore, the increase in temperature pushed the peaks of DGR forward: 1.5 days at 15°C; 1 day at 20°C; 1 day at 25°C; 1 day at 30°C; and 0.5 days at 35°C. The DGR curves for DARK (Fig. 3C) were similar to those for LIGHT/DARK at the same temperature; plus, the time needed for the first germination at 35°C was 0.5 days. The DARK treatment increased the peak value of the DGR under all temperatures and shortened the time to germination at 20°C, 25°C, 30°C and 35°C.

Table 1. F-values of two-way ANOVA for effects of light and temperature on final germination (FG) and germination rate (GR).

Treatment	*Alhagi sparsifolia*		*Karilinia caspica*.	
	FG (%)	GR (days)	FG (%)	GR (days)
Light	12.25**	70.78**	31.46**	0.61ns
Temperature	85.53**	46.77**	119.81**	1.02E3**
Light × Temperature	2.72ns	9.31**	2.32ns	1.97ns

Note: ** Significant difference at the 0.01 level; * Significant difference at the 0.05 level; ns, No significant difference at the 0.05 level

Table 2. F-values of two-way ANOVA for effects of light and temperature on seedling growth.

Treatment	*Alhagi sparsifolia*			*Karilinia caspica*		
	Length of plumule (mm)	Length of radicle (mm)	Length of seedling (mm)	Length of plumule (mm)	Length of radicle (mm)	Length of seedling (mm)
Light	38.42**	214.04**	128.20**	269.13**	491.92**	84.70**
Temperature	145.11**	126.28**	177.91**	318.05**	38.05**	220.06**
Light × Temperature	4.15*	12.66**	7.99*	41.29**	18.77**	12.21**

Note: ** Significant difference at the 0.01 level; * Significant difference at the 0.05 level; ns No significant difference at the 0.05 level

Temperature had an extremely significant impact (p<0.01) on the GR of *K. caspica*, and there were no significant differences (p>0.05) in light and interaction between light and temperature (Table 1). Figure 2B shows that the average number of days spent germinating in LIGHT/DARK and DARK decreased with increasing temperature. The GR, in LIGHT/DARK was 4.8% at 15°C, and then began to decline sharply (by 64.8%) at 20°C (p<0.05), and further still (78.8%) when the temperature reached 30°C. Similarly, in DARK, the GR at 15°C (5.1%) was also higher than the GR under the other (higher) temperature treatments (p<0.05). When the temperature exceeded 30°C, no significant differences in GR were observed compared to the 35°C treatment (p>0.05) both in LIGHT/DARK and DARK.

As shown in Fig. 3, with increasing temperature, the time of first germination and the DGR peak of the two seeds advanced in both LIGHT/DARK and DARK. In *A. sparsifolia*, the peak DGR appeared in the first 1.5 days at 15°C, both in LIGHT/DARK and DARK (Fig. 3A, Fig. 3C). However, in *K. caspica*, it appeared within the first 1 day at 35°C, again both in LIGHT/DARK and DARK (Fig. 3B, Fig. 3D).

Stretching of the embryo: The results in terms of the effects of light and temperature on stretching of the embryo in *A. sparsifolia* and *K. caspica* are shown in Table 2. The indices for this aspect include the length of the plumule (LP), radicle (LR), and seedling (LS). The differences in the LP, LR and LS of *A. sparsifolia* and *K. caspica* were highly significant (p<0.01) under the different light and temperature treatments. There were significant differences (p<0.05) in the effect of the interaction between light and temperature on the LP and LS of *A. sparsifolia*, and highly significant differences (p<0.01) in the effect on the LR of *A. sparsifolia* and the LP, LR and LS of *K. caspica*.

Figures 4A, 4C and 4E provide details on the stretching of the embryo in *A. sparsifolia*. The values of LP, LR and LS of *A. sparsifolia* in DARK were significantly higher than in LIGHT/DARK (p<0.05). As shown in Fig. 4A, LP increased with the increase in temperature under the same light treatments. Minimum LP occurred at 15°C (p<0.05), with the values in LIGHT/DARK and DARK being 2.06 mm and 8.19 mm, respectively. Meanwhile, in LIGHT/DARK, the LP at 35°C was significantly higher (p<0.05) than at other temperatures; and in DARK, there were no significant differences (p>0.05) in LP at 25°C, 30°C and 35°C, but it was significantly higher (p<0.05) than that in the 15°C and 20°C treatments. Figure 4C shows that, in both LIGHT/DARK and DARK, LR increased with increasing temperature at first, reached its peak value at 30°C, and then began to decline at 35°C. The LR at 30°C was significantly higher (p<0.05) than at the other temperatures, with values of 65.51 mm and 89.25 mm in the LIGHT/DARK and DARK treatments, respectively. As shown in Fig. 4E, the LS in DARK was significantly higher than in LIGHT/DARK (p<0.05). Both in the LIGHT/DARK and DARK treatments, the LS increased with the increase in temperature, and reached its peak value at 30°C. When the temperature reached 35°C, the LS reduced significantly in DARK (p<0.05), but there were no significant differences in LIGHT/DARK (p>0.05).

Figures 4B, 4D and 4F provide details on the stretching of the embryo in *K. caspica*. The values of LP and LS in LIGHT/DARK were significantly lower (p<0.05) than in DARK, while the LR in LIGHT/DARK was significantly higher (p<0.05) compared with that in DARK. As Fig. 4B shows, seeds did not grow plumules at 15°C in LIGHT/DARK, and no significant difference (p>0.05) in LP were measured among the 25°C, 30°C and 35°C treatments. The highest (13.63 mm) and lowest (4 mm) LP values were observed at 35°C and 15°C, respectively, in DARK. As shown in Fig. 4D, the LR increased significantly (p<0.05) with increasing temperature in LIGHT/DARK. When the temperature was maintained at 25°C, there were no longer obvious differences (p>0.05) in LIGHT/DARK; however, with the increasing of temperature the LR began to reduce, but was still higher than the15°C treatment (p<0.05) in DARK. As temperatures increased, the LS presented an increasing trend (Fig. 4F). Significantly higher (p<0.05) LS values were observed from seeds that germinated at 35°C in DARK and at 25°C, 30°C and 35°C in LIGHT/DARK.

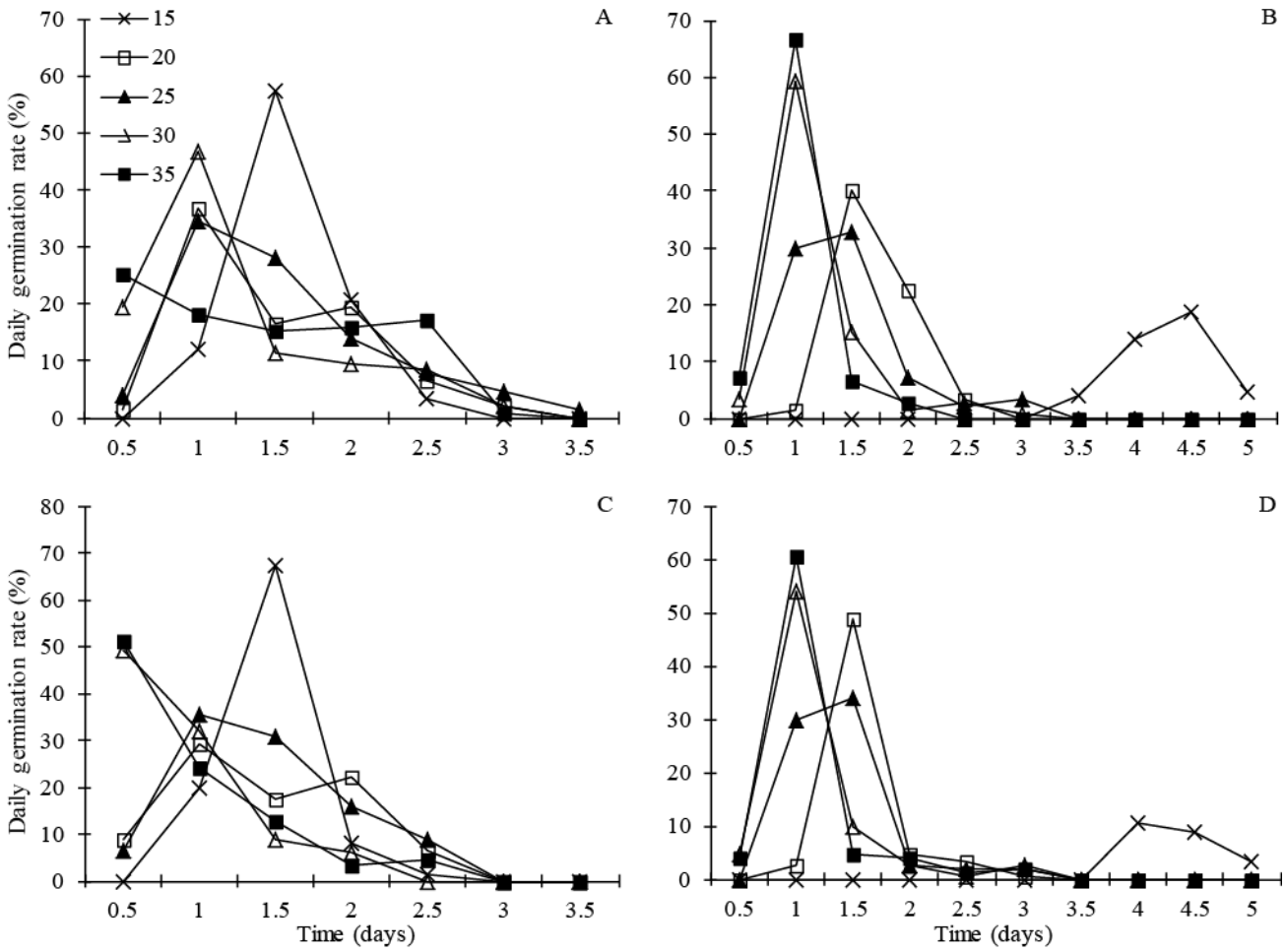

Fig. 3. Daily germination rate of *Alhagi sparsifolia* and *Karilinia caspica* seeds subjected to different light treatments: *Alhagi sparsifolia* seeds in LIGHT/DARK (A); *Karilinia caspica* seeds in LIGHT/DARK (B); *Alhagi sparsifolia* seeds in DARK (C); *Karilinia caspica* seeds in DARK (D) (values are the average percentages of daily germination).

Discussion

Effects of light on seed germination and embryo extension: The two plants examined in this study showed two different germination patterns under two light treatments, as described for other species (Burrows, 1995; Nicotra *et al.*, 1999; Huang *et al.*, 2003; Kettenring *et al.*, 2006). Light treatment affected the process of germination, final germination, and stretching of the embryo in both *A. sparsifolia* and *K. caspica*.

Seeds are controlled by phytochromes (Prober *et al.*, 1985; Lariguet *et al.*, 2013), and the phytosensitivity of seeds guides the suitability of a particular location for germination. If the location is not appropriate, the phytosensitivity prompts seeds to not germinate, or germinate in small amounts (Khan, 2004). Different plants respond differently to light factors (Gul & Weber, 1999); for instance, some seeds need light to break from dormancy, while others do not (Sederias & Colman, 2007). The phytosensitivity of desert plants is relatively more diverse than in other ecosystems (Gutterman, 1993). In our study, light slowed the progression of seed germination in *A. sparsifolia*. Moreover, the germination rate, which characterizes seed vigor (Zucareli *et al.*, 2015), was higher in the LIGHT/DARK treatment for *A. sparsifolia* seeds, demonstrating that this species possesses lower seed vigor under these conditions, as compared to the conditions represented by the DARK experiment (Fig. 2A). Ultimately, this led to the final germination percentage in LIGHT/DARK being lower than in DARK. Therefore, seed germination in *A. sparsifolia* is inhibited by its photosensitivity. In contrast, the greater exposure by half a day in LIGHT/DARK compared to that in DARK accelerated seed germination, improved seed vigor increased the daily germination rate (Fig. 3B, Fig. 3D) and resulted in an increase in the percentage of final germination (Fig. 1B) in *K. caspica*. Therefore, seed germination in *K. caspica* is promoted by its photosensitivity.

Compared with seed germination, the response of the embryo to light was more rapid. The photosensitivity of the hypocotyl improves its extension (Kato-Noguchii & Hasegawa, 1992), and light as a signal function acts on the endosperm to affect the embryo's extension (Powell *et al.*, 1984). As with germination, this aspect can be divided into two effects—inhibition and promotion (Kettenring *et al.*, 2006). Based on our research, the extension of *A. sparsifolia* embryos was inhibited by half a day's exposure - whether the plumule, radicle or seedling (Fig. 4A, Fig. 4C, Fig. 4E). Although light promoted radicle extension in *K. caspica*, growth of the plumule and seedling was inhibited by light. Therefore, embryo extension in the two plants' seeds is inhibited by light.

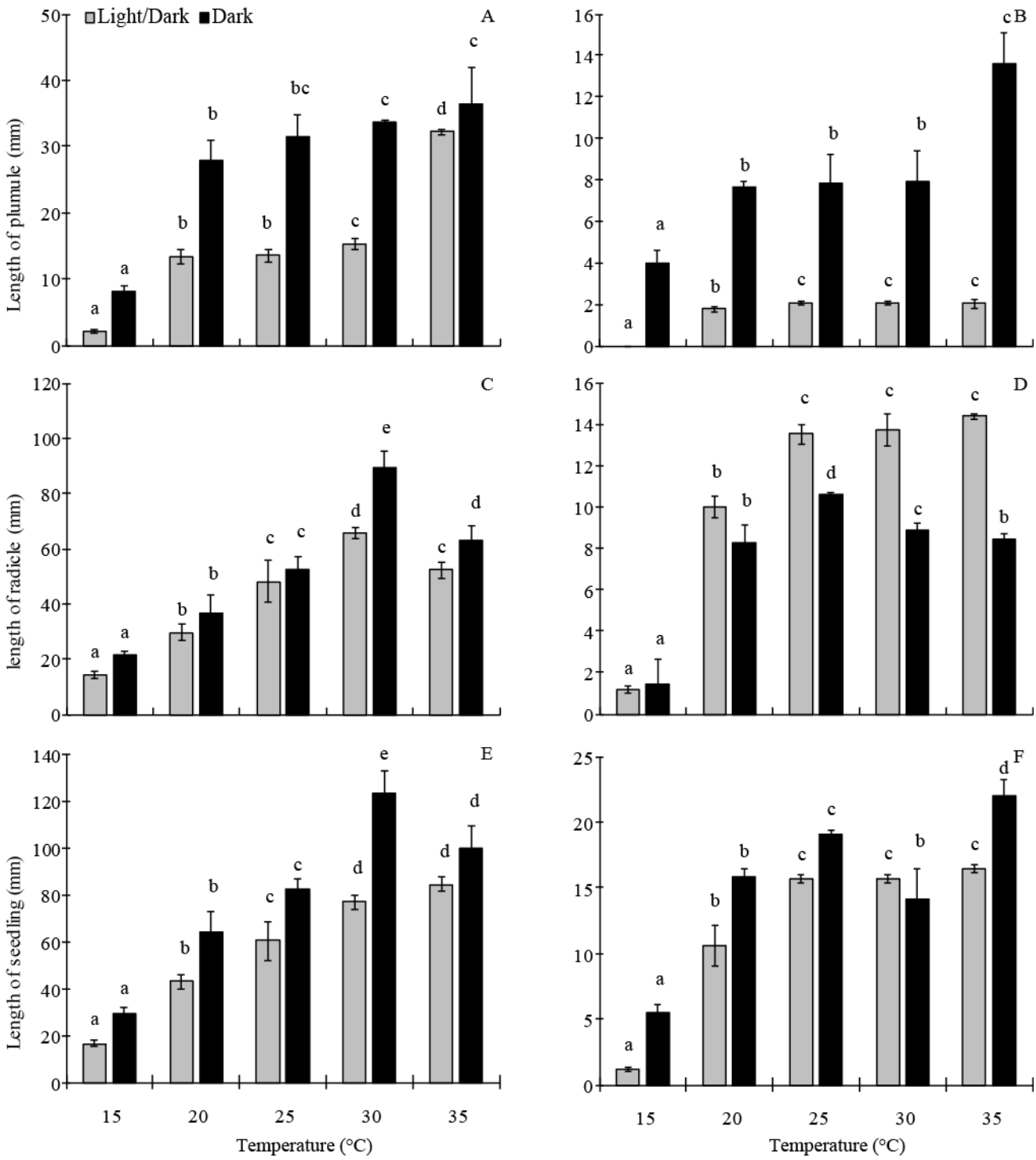

Fig. 4. Stretching of the embryo in *Alhagi sparsifolia* (A, C, E) and *Karilinia caspica* (B, D, F) seeds under different light and temperature treatments, noted as mean ± SD (n = 3). Means with the same letters are not significantly different among temperatures at the p = 0.05 level, based on LSD mean separation.

Effects of temperature on seed germination and embryo extension: Based on the results of this study, temperature is not only a significant factor during the process of germination (i.e., once seeds begin to germinate), but also in the onset of germination (i.e., whether or not seeds are able to begin germinating). Low temperature (15°C) clearly delayed the first germination and decreased the first germination percentage both in *K. caspica* and *A. sparsifolia*; the higher the ambient temperature, the earlier germination occurred (Fig. 3). The reason is that seeds require sufficient accumulative temperature to break from dormancy (Kettenring & Galatowitsch, 2007), with the level of accumulated temperature varying among different kinds of plants (Budelsky & Galatowitsch, 1999; Daws *et al.*, 2007). Moreover, after breaking from dormancy, different seeds require different temperatures for the whole germination process (Guillemin *et al.*, 2012; Rahimi, 2013), and this was reflected in the present study. For instance, the time of first germination at 15°C was delayed by 3 days

compared with that under other temperature conditions, and was only 0.5 days late in *A. sparsifolia*. All seeds of *A. sparsifolia* at 15°C to 35°C had a higher final germination; plus, they germinated more rapidly at 30°C and 35°C. Similarly, the temperature treatments of 30°C and 35°C were also suitable for *K. caspica*.

This study shows that the extension of the embryo is also influenced by temperature. Like other plants (Voyiatzis, 1995; Gutterman, 2000; Boeken *et al.*, 2004), higher temperatures promote the extension of the embryo in both *A. sparsifolia* and *K. caspica*. The main reason is that the active ingredients in seed embryos. Higher temperatures increase the vitality of the active ingredients in the embryo, such that the embryo can better obtain phosphorus and other nutrients to promote its extension (Islam *et al.*, 2007; Seyyedi *et al.*, 2015). At 35°C, the embryo of *K. caspica* showed its furthest extension. However, the ability of the embryo to spread did not always increase with temperature. In fact, the growth of the embryo in *A.sparsifolia* even declined. In summary, 30°C and 35°C are the most suitable temperatures for *A. sparsifolia* and *K. caspica* seeds, respectively.

Breeding strategies: The two kinds of plants' seeds were produced under different treatments. The final germination percentage of *A. sparsifolia* reached more than 80%, while that of *K. caspica* was more than 22%, with an average value of 65%. Nevertheless, the two plants reproduce asexually, not sexually, in their adaptation to the arid environment of the desert (Liu *et al.*, 2013). Many plants adopt a hybrid propagation model, with both sexual and asexual reproduction demonstrated (Richards, 1986). These two clone plant species produce a large number of seeds for sexual reproduction, which, despite being ready to germinate constantly, are prevented from doing so by the lack of suitable environmental conditions (Baskin & Baskin, 1985). The experiments carried out in the present study were performed in an incubator, which shielded the seeds from adverse factors such as aridity, salt, and sand. Plus, the temperature and light conditions were controlled. This was the reason for the high germination rate observed.

Conclusion

Both light and temperature are factors of influence in seed germination and the stretching of embryos in the two desert plant species examined in this study; and yet, they are not limiting factors. The two species' seeds have adopted a rapid and high percentage germination strategy in a relatively suitable environment. The findings suggest that seeds of *A. sparsifolia* and *K. caspica* should be bred in greenhouses in desert areas, where temperatures should be controlled at 30°C and 35°C, respectively. The whole process should be controlled in a dark environment.

Acknowledgements

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