

The effect of light, temperature and bracteoles on germination of polymorphic seeds of *Atriplex centralasiatica* Iljin under saline conditions

W. LI^{1,2}, P. AN^{1*}, X. LIU², M.A. KHAN³, W. TSUJI⁵ AND K. TANAKA⁴

¹ Arid Land Research Center, Tottori University, Hamasaka, Tottori 680-0001, Japan;

(E-mail: wqli@ms.sjziam.ac.cn; an.ping@alrc.tottori-u.ac.jp; tsun@alrc.tottori-u.ac.jp)

² Center for Agricultural Resources Research, Institute of Genetic and Developmental Biology, Chinese Academy of Science, Shijiazhuang, 050021 P. R. of China; (E-mail: xjliu@ms.sjziam.ac.cn)

³ Department of Botany, University of Karachi, Karachi-75270, Pakistan; (E-mail: ajmalk@super.net.pk)

⁴ Faculty of Agriculture, Tottori University, Koyama, Tottori 680-8553, Japan
(E-mail: jotanaka@muses.tottori-u.ac.jp)

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Summary

Atriplex centralasiatica, a salt-secreting herb in the family Chenopodiaceae, is one of the most economically important halophytes in China. Seeds were collected from Huanghua City and were separated to get humped-black, humped-brown, flat-black and flat-brown seeds on the basis of the type of bracteoles and seed coat color. The four kinds of seeds were screened for germination with NaCl solutions of different concentration. Results showed that the effects of fruit types were not significant on polymorphic seed germination. Multi-factorial analysis of variance revealed significant differences in the effects of seed color, temperature, salinity, light and their interactions on seed germination. Maximum germination occurred in distilled water, and an increase in NaCl concentration progressively inhibited germination in all the four types of seeds. A temperature regime of 15/25°C resulted in the maximum germination. Brown seeds had a higher germination percentage than black seeds in mild temperature regime and high salinity; while black seeds had a higher germination percentage than brown seeds in higher temperature and lower salinity. Germination of black seeds was sensitive to light in NaCl solution but not of brown seeds. Attached bracteoles inhibited the germination of both black and brown seeds. The black seeds were more sensitive to presence of bracteoles than brown ones. In conclusion, the polymorphic seeds showed bet-hedging ecological strategies.

Introduction

Soil salinity of agricultural lands is increasing and the availability of fresh water is becoming limited due to overexploitation and wastage (Khan and Weber, 2006). Several approaches to incorporate salt and/or drought tolerance in crops have showed only limited success. Therefore, utilization of salt-tolerant plants as potential cash-crops is being suggested (Lieth *et al.*, 1999; Hamdy *et al.*, 1999; Zhao *et al.*, 2002, Khan and Weber, 2006). *Atriplex centralasiatica* Iljin, a herbaceous salt-secreting halophyte in the family

* Author for correspondence

Chenopodiaceae, is widely distributed in China (Zhao, 2002). This species is a potential source of oilseed, food, high quality fodder and a tool for reclamation of saline soils (Zhao *et al.*, 2002; Bai *et al.*, 2003; Yi and Liu, 2003, 2004; Wang and Hou, 2005). *Atriplex centralasiatica* shows high tolerance to salinity and warm temperatures during growth (Qiu *et al.*, 2003; Qiu and Lu, 2003). However, there is little information about its salt tolerance during seed germination. *Atriplex centralasiatica* produces two morphologically different types of bracteoles in dispersal unit, humped (convex) or flat (He and Li, 1995; Wang and Hou, 2005). Both types of dispersal units have brown and black fruits. Therefore, there are four morphologically different fruit types in total. In the investigation, the four kinds of fruits were screened for seed germination. Hence, seeds mean fruits in the paper. Seed dimorphism (or polymorphism) is a common phenomenon in a number of halophytic taxa like *Arthrocnemum*, *Chenopodium*, *Cakile*, *Salicornia*, *Salsola*, *Spergularia*, *Suaeda* and *Trianthema* including *Atriplex* (Ungar, 1977; Galinato and van der Valk, 1986; Khan and Ungar 1984; Ungar, 1988; Morgan and Myers, 1989; Khan and Gul, 1998; Imbert, 2002). This seed polymorphism can enable halophytes to adapt to varying salt-marsh or dry salt desert environments. It also enhances chances for seedling establishment and survival in a saline environment (Philippillai and Ungar, 1984; Venable, 1985; Imbert, 2002).

Seed germination is a critical stage for the establishment of a species. Salt (usually dominated by NaCl) inhibits seed germination in saline soil in spring and high salinity is injurious to most glycophytes. However, seeds of halophytes can show complete germination once the salinity stress is removed, which indicates the inhibition was an osmotic effect of salinity (Hardegree and Emmerich, 1990). Interaction between temperature and salinity also affects the seed germination of halophytes (Gul and Khan, 2002) and halophytes germinate at times when there is an optimal combination of day length, temperature regime, and salinity. Germination of some halophytes seeds is completely inhibited in dark environment, while others are not sensitive to light (Khan and Gul, 2002). Studies on seed germination of *Atriplex* spp. [e.g. *A. triangularis* (Khan and Ungar 1986; Ellison, 1987); *A. nummularia* (Uchiyama, 1981); *A. dimorphostegia* (Koller, 1957); *A. prostrata* (Ungar and Khan, 2001); *A. patula* (Katembe *et al.*, 1998); *A. griffithii* (Ungar and Khan, 2001); *A. sagittata*, *A. hortensis*, *A. tatarica*, *A. rosea* (Mandák, 2003; Khan *et al.*, 2004); *A. confertifolia*, (Meyer *et al.*, 1998); *A. cordobensis*, (Aiazzi and Argüello, 1992)], showed a variable level of salt tolerance ranging from 200 mM NaCl to 1000 mM NaCl in seed germination. Bracteoles affect *Atriplex* seed germination in many ways. They could cause change of the availability of light or red:far-red ratio, modify salt stress and water stress, cause allelopathy, do mechanical inhibition and have negative effects of growth regulating chemicals (Ungar and Khan, 2001). The main inhibition mechanism is different in different *Atriplex* species (Ungar and Khan, 2001). A better understanding of the processes involved in alleviation of *A. centralasiatica* seed dormancy may help in its future management as an economic crop.

The presence of different kinds of bracteoles and seed/fruit morphs appears to be related to the ecological strategies of *Atriplex* species to maximize survival under variable environmental stresses. There is little information available on germination of polymorphic seeds of *A. centralasiatica*. The present investigation was designed to study (1) the variation in weight and ratio characters of the polymorphic dispersal units and seeds;

(2) the effect of salt on germination of the polymorphic seeds; (3) the water absorbing process of polymorphic seeds; (4) the effect of light, temperature, bracteoles and their interactions on the germination of *A. centralasiatica* seeds under salt stress.

Materials and methods

Dispersal units of *A. centralasiatica* were collected in mid October, 2004 from coastal saline soils of Huanghua City, Hebei Province of north China. These dispersal units were air dried for a few days and stored in paper bags at room temperature in the laboratory. Three groups of 5 g mixed dispersal unit were separated into humped and flat ones based on the shape of bracteoles and the number of each kind of dispersal units was counted. Four hundred flat and humped dispersal units were randomly collected and weighed. The biomass distribution is shown in figure 1. When the bracteoles were removed, both dispersal units contained fruits with different coat colors (brown or black), leading to four fruit morphs. In the investigation, the four kinds of fruits were screened for seed germination. Hence, seeds refer to fruits in the paper. Then four kinds of seeds were separated from inflorescence. The four kinds of seeds were: black ones from flat dispersal units (flat-black), brown ones from flat seeds (flat-brown), black ones from humped seeds (humped-black) and brown ones from humped seeds (humped-brown). Numbers and weight of the four kinds of seeds were recorded.

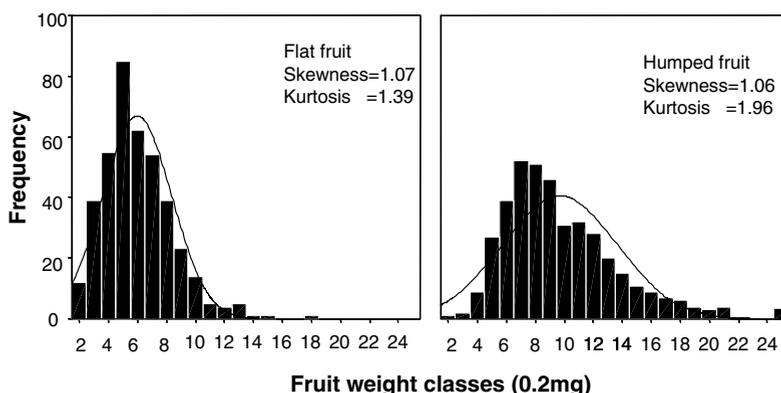


Figure 1. Frequency distribution of weight of particular Humped and flat fruits of *A. centralasiatica* (400 flat and humped fruits were used for analysis). Characteristics of the frequency distribution (skewness and kurtosis) are showed.

Seeds and dispersal units were treated using 0.5% NaOCl (Wako, Japan) for 3 minutes and rinsed with distilled water before using in germination studies. Germination was carried out in 50×9mm tight-fitting plastic Petri plates with two layers of qualitative filter paper (Toyo Roshi Kaisha, Ltd. Japan) and 1ml of test solution. Four replicates of 35 seeds each were used for each treatment. Seeds were scored as germinated at radicle protrusion. The germination test was carried out in 0, 60, 120, 240, 360 and 480 mM NaCl solution at constant temperature of 23°C in Petri dishes lined with filter paper, in light (fluorescent lamp, intensity approximately 100 mmol m⁻² s⁻¹). For measuring water

uptake, seeds were weighed every 2 h after immersing in distilled water for 48 h or until the first seed germinated. A kimwipe tissue paper (Kimwiper S-200, Kimberly-Clark Co., supplied by Kulesia, Tokyo) was used to remove seed surface water before weighing.

To determine the effect of NaCl and light on germination, black and brown seeds from humped and flat dispersal units were germinated in 0, 60, 120, 240, 360, 480 and 600 mM NaCl solution at constant 23°C temperature in Petri dishes on filter paper, in light (fluorescent lamp, intensity approximately 100 mmol m⁻² s⁻¹) or darkness (Petri dishes were wrapped in aluminum foil and kept in a black box). After 11 days the germination percentage was noted.

To determine the interaction of temperature and salinity on germination, in a 10h/14 h night and day cycle, 10/20, 15/25, 20/30 and 25/35°C temperature regimes were used. The four kinds of seeds were germinated in 0, 240, and 480 mM NaCl solution at the above-mentioned temperature regimes. The germination percentage was checked every 24 h and seedlings were removed.

To determine the effect of bracteoles, three replicates of 50 seeds (mixture of black and brown ones) each with bracteoles (bracteoles attached to seeds), seeds+bracteoles (bracteoles removed from seeds but placed in Petri dish with seeds) and bare seeds without bracteoles (bracteoles were removed from dispersal unit, no bract) were germinated in 9 cm Petri dish with 2 ml water for 16 d in incubators with 12 h photoperiod (fluorescent lamp, intensity approximately 100 mmol m⁻²s⁻¹) and 12 h thermoperiod (light: dark) in a temperature regime of 15/25°C. When all seeds had germinated the un-germinated fruits (seed with bracteoles) were checked to find the number of un-filled fruits. Germination percentage of fruits was calculated. Fruiting bracts (5 g) of humped and flat fruits were soaked separately in distilled water (50ml) for 24 hours at 20°C. Extract was filtered through qualitative filter paper (Toyo Roshi Kaisha, Ltd. Japan) and stored in the refrigerator for germination test. *Atriplex* seeds (flat seeds and humped seeds) were used for checking the effect of leachate on germination (seeds+bract leachate).

Data from germination experiments were transformed before analysis to achieve normality (homogeneity of variance). Multi-way analysis of variance (ANOVA) was used to test for the effect of different factors and their interactions among final germination percentages. A Bonferroni test was carried out to determine whether significant (at 1 and 5% levels of probability) differences occurred between individual treatments. SPSS version 10.0 software package was used in the process.

Results

Variation in weight of particular dispersal units and seeds types

As shown in figure 1, humped dispersal units (9.7±0.5mg) were significantly heavier than flat dispersal units (6.0±0.2mg) (P<0.001). Humped dispersal units exhibited greater size variation (coefficient of variation, CV=49.5%) than flat ones (CV=40.1%). Brown seeds (flat-brown 1.9±0.1mg, humped-brown 2.3±0.3mg) were significantly heavier than black ones (flat-black 1.5±0.1mg, humped-brown 1.6±0.0mg, P<0.05) from both flat and humped dispersal units. Brown and black seeds from humped dispersal units were significantly heavier than that from flats ones (P<0.05) respectively. Humped dispersal

units (50%) contained more black seeds than flat ones (16%). Bracteoles of flat/humped-black dispersal units were found to be harder and more compact than flat/humped-brown ones.

Variation in germination of four kinds of seeds under NaCl stress

Three-way ANOVA test showed a significant effects of NaCl, seed type and their interactions for germination ($P < 0.05$), while there is no significant interaction between dispersal unit types and salinity for seed germination ($P > 0.05$). Both black and brown seeds showed maximum germination (nearly 100%) in distilled water; black seeds germinated later than the brown ones, for example, brown seeds reach 50% germination in 2 days later while black seeds in 3 days later in distilled water (figure 2). An increase in NaCl concentration progressively inhibited germination in all four kinds of seeds. Few black seeds germinated at 480 mM NaCl. There were significant differences between the final germination percentages of black and brown seeds in all salinity treatments but not

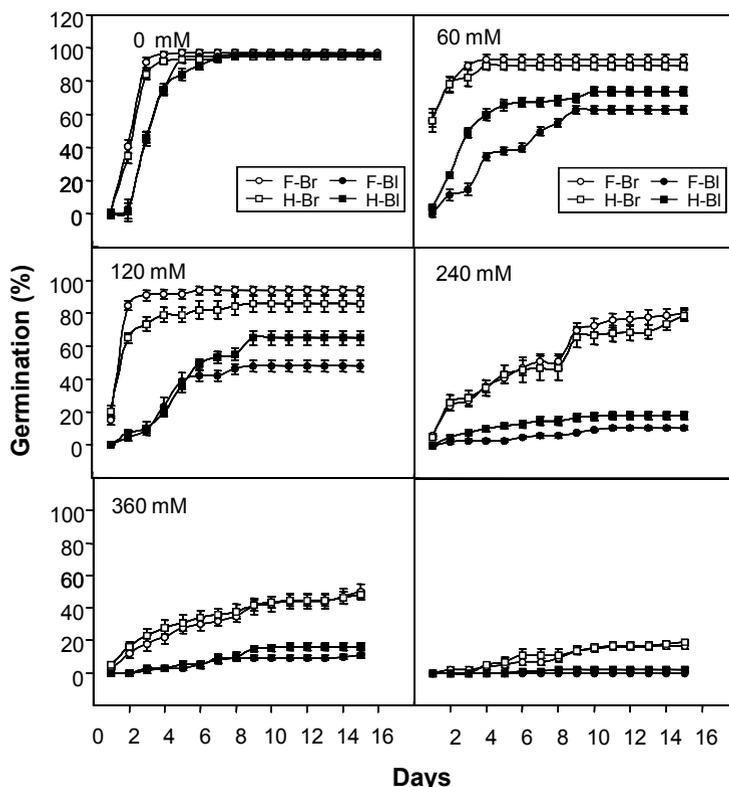


Figure 2. Percentage of germination of Flat-brown (F-Br), Flat-black (F-BI), Humped-brown (H-Br) and Humped-black (H-BI) seeds of *A. centralasiatica* in NaCl solution. The four kinds of seeds were sown in 0, 60, 120, 240, 360 and 480 mM NaCl solution at constant temperature of 23°C in Petri dishes lined with filter paper, in light (fluorescent lamp, intensity approximately 100 mmol m⁻² s⁻¹). To calculate the average rate of germination more than 100 seeds were used for each treatment. The experiments were repeated 3 times and S.E was calculated.

between flat-brown and humped-brown seeds. At lower levels of salinity (60 and 120 mM NaCl solution), humped-black seeds showed significantly higher germination percentage than flat-black seeds; while at higher salinity (240 and 480 mM NaCl solution), there was no significant difference ($P < 0.05$) between them. Generally, brown seeds had a higher germination percentage than black seeds in all salinity treatments. There was no significant interaction between dispersal unit types and salinity for seed germination, indicating that there was little difference between the seed with same color but from different dispersal units.

Water absorption process

The brown seeds absorbed water much faster than the black seeds (figure 3) under all salinity levels. Water absorption in brown seeds decreased with an increase in salinity but salinity had little effect on water absorption by the black seeds (figure 3). Brown seeds germinated in 14 hours, while black seeds took 18 hours after being treated with 60 mM NaCl solution. Brown seeds started germination when relative water content approached 60% in all treatments; while black seed started germination when relative water content approached 30%.

Effect of light

Four-way ANOVA test showed a significant effects of NaCl, seed type, light and their interactions for germination while there is no significant effect of dispersal unit type and interactions between bracteole types, seed type, light and salinity for seed germination (table 1). For brown seeds, light had little effect on the germination in both non-saline and saline conditions (table 1). However, under saline condition, light appeared to promote germination (table 1) for the black seeds. At 240 mM NaCl solution, about 10% black seeds germinated in dark in comparison to 30% germination in light.

Interaction effect between temperature and salinity

A four-way ANOVA of germination showed significant ($P < 0.01$) effects of salinity, temperature, seed color and their interactions. However, there is no significant effect of dispersal unit type and interactions between dispersal unit types, seed type, temperature and salinity for seed germination. Seed germination decreased with an increase in salinity and was maximum at 15/25°C temperature regime (figure 4), while both the higher and the lower temperature gave lower germination percentage. Distilled water showed little effect on germination percentage (figure 4). Salinity (240mM or more) inhibited seed germination, and few black seeds germinated at 480mM NaCl solution. At high temperature regime (25/35 C), brown seeds showed lower germination percentages than black seeds in distilled water (figure 4); while brown seeds showed higher germination percentages than black seeds in all temperature regimes when NaCl solution was more than 240 mM.

Effect of bracteoles

Two-way ANOVA showed significant effect of dispersal unit type, different bracteoles treatments and their interaction for seed germination. One-way ANOVA indicated that

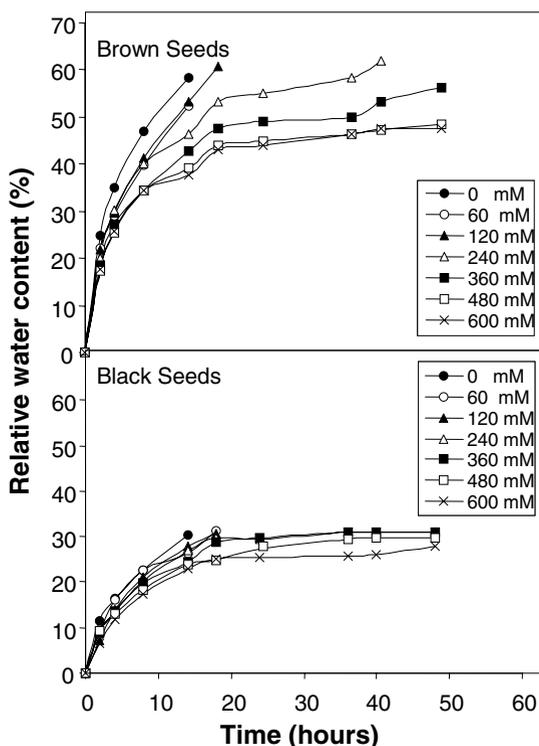


Figure 3. Water uptake of brown and black seeds (from humped fruits) of *A. centralasiatica* in 0, 60, 120, 240, 360, 480 and 600mM NaCl solution at constant temperature of 23°C in Petri dishes lined with filter paper, in light (fluorescent lamp, intensity approximately 100 mmol m⁻² s⁻¹). The brown and black seeds were weighed with regular intervals for 48 hours. Tissue paper was used to remove seed surface water before weighing. The experiments were repeated 3 times and S.E was calculated. Relative water content (%) was based on the weight of initial dry seeds, as shown in the following equation: $RWC = \frac{W_w - D_w}{D_w} \times 100\%$; RWC: relative water content; Ww: Wet weight of seeds; Dw: initial Dry weight of seeds.

the presence of bracteoles and bracteole extraction significantly affected final germination percentage of seeds from humped dispersal units ($P < 0.05$); but showed little effects on seeds from flat ones ($P > 0.05$). However, the presence of bracteoles and bracteole solution affected germination of seeds from both flat and humped dispersal units (figure 5). The inhibitory effect of bracteoles on germination was stronger for humped seeds than the flat ones (figure 5). During germination, flat seeds germinated earlier and quicker than the humped ones (figure 5). All the un-germinated seeds were black ones.

Discussion

Dimorphic dispersal units and polymorphic seeds

Previous investigations showed that *A. centralasiatica* produced polymorphic/dimorphic

Table 1. Final germination percentage of four kinds of *A. centralasiatica* seeds in 0, 60, 120, 240, 360, 480 mM NaCl at 23°C in light and dark.

Seed type	Dispersal unit type	Light	NaCl concentration								
			0 mM	60 mM	120 mM	240 mM	360 mM	480 mM			
Black	Humped	Dark	91.6±2.0 ^a	58.9±1.7 ^a	47.7±1.8 ^a	9.3±1.8 ^a	6.2±1.2 ^a	0.0±0.0 ^a			
		Light	96.1±1.7 ^b	74.1±1.2 ^b	64.1±2.1 ^b	30.4±1.4 ^b	15.7±0.9 ^b	2.0±0.6 ^b			
	Flat	Dark	92.3±1.2 ^a	55.0±2.9 ^a	45.0±0.6 ^a	11.7±1.2 ^a	5.9±0.9 ^a	0.0±0.0 ^a			
		Light	92.7±1.5 ^a	74.0±1.5 ^b	61.0±3.2 ^b	31.0±2.3 ^b	18.3±0.9 ^b	2.3±0.3 ^b			
Brown	Humped	Dark	95.5±2.4 ^a	89.4±2.3 ^c	84.9±1.7 ^c	76.9±2.1 ^c	49.9±1.3 ^c	21.7±0.9 ^c			
		Light	94.4±0.7 ^a	88.7±1.5 ^c	85.6±1.4 ^c	78.7±0.9 ^c	47.7±2.0 ^c	19.7±0.6 ^c			
	Flat	Dark	94.3±1.2 ^a	91.3±0.9 ^c	81.7±2.0 ^c	76.0±3.0 ^c	49.0±2.1 ^c	19.0±2.1 ^c			
		Light	93.3±1.2 ^a	89.0±2.1 ^c	83.3±2.4 ^c	79.0±1.0 ^c	45.3±0.7 ^c	17.3±1.5 ^c			
ANOVA											
Source of Variation	D×ST	D×S	ST×S	D×ST×S	D×L	ST×L	D×ST×L	S×L	D×S×L	ST×S×L	D×ST×S×L
Germination	ns	ns	***	ns	ns	***	ns	***	ns	***	ns

Within columns, means±se followed by the same small letter are not significantly different (P<0.05), by Bonferroni test. Four-way ANOVA tests were used to test the effect of dispersal unit type (D), seed type (ST), light (L), NaCl (S) and their interactions on the transferred (arcsine) final germination percentage. ***, **, * Significant at the 0.001 and 0.01 probability levels respectively, ns, not significant.

dispersal units (flat and humped or convex fruits) (He and Li, 1995), while we found that *A. centralasiatica* produced polymorphic seeds from dimorphic dispersal units. Other investigations here shown that *Atriplex* spp. produce polymorphic/dimorphic dispersal units or polymorphic/dimorphic seeds (Frankton and Bassett, 1968; Mandák and Pyšek, 2001a,b; Ellison, 1987). Our investigation showed that *A. centralasiatica* not only produces dimorphic dispersal units but also produces dimorphic seeds from each dispersal unit type (leading to four types of seeds).

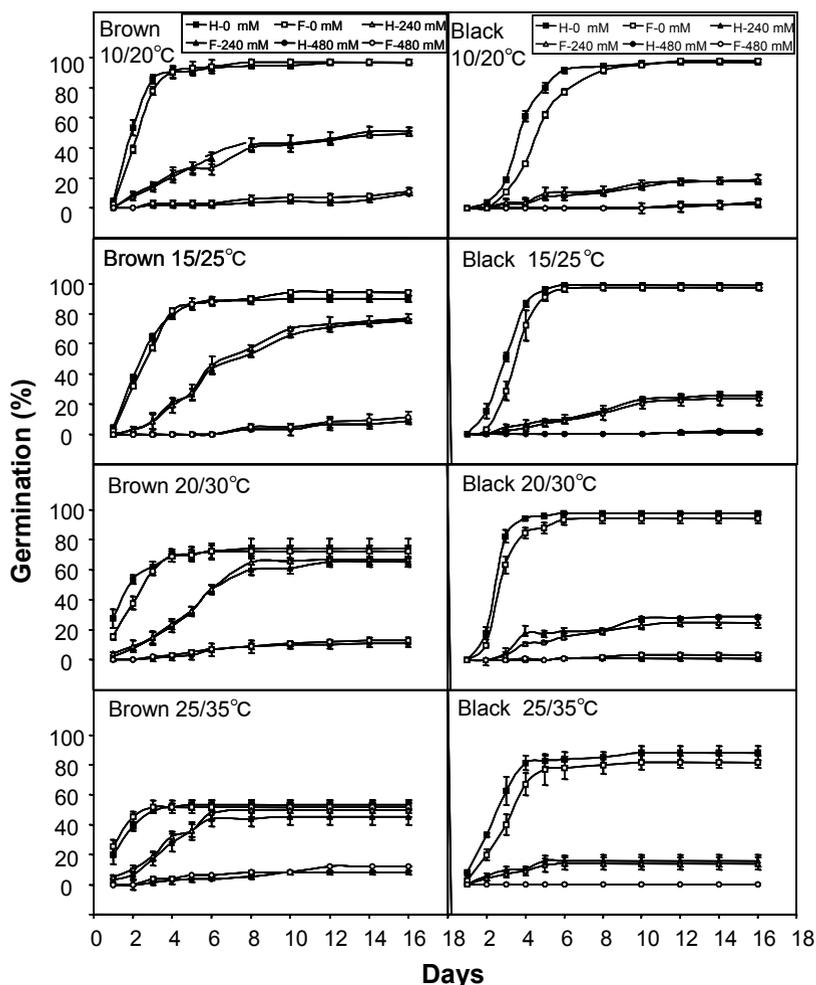


Figure 4. Percentage of germination of brown and black seeds from humped (H) and flat (H) dispersal units of *A. centralasiatica* in 0, 240, and 480 mM NaCl solution at 10/20°C, 15/25°C, 20/30°C and 25/35°C temperature regime in a 10h/14 h night and day cycle. To calculate the average rate of germination more than 100 seeds were used for each treatment. The experiments were repeated 3 times and S.E was calculated.

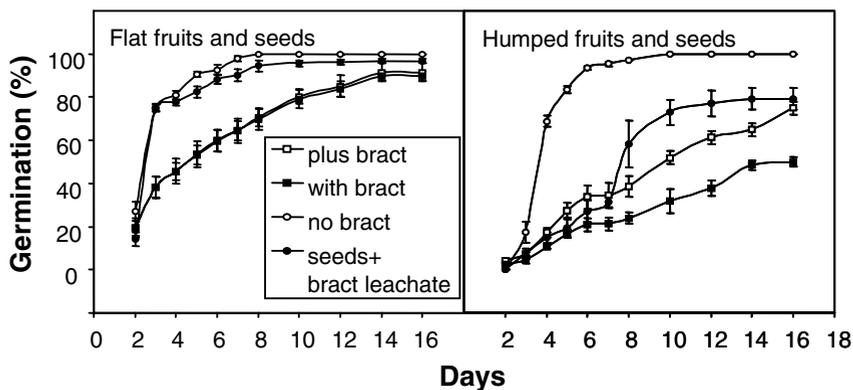


Figure 5. Percentage of germination of flat and humped seeds of *A. centralasiatica* in the presence or absence of bracteoles with continuous light at 23°C. With bract, bracteoles attached to seeds; plus bract, bracteoles removed from seeds but placed in Petri dish with seeds; no bract, bracteoles removed from seeds; seeds+bract leachate, bare seeds were placed to bracteoles leachate with bracteoles:water = 1:10 ratio.

Ecological adaptations of polymorphic seeds.

Seed polymorphism and/or fruit heterocarpy is very common in *Atriplex* spp. and is related to their dispersal ability, time of germination, competitive abilities, and seed bank dynamics (Mandák, 1997; Mandák and Pyšek, 2001a,b; Imbert, 2002). The flat dispersal units are dispersed by wind while humped dispersal units are not (personal observation). It implies that the production of flat and humped dispersal units for *A. centralasiatica* indicates differences in dispersal mechanism. The flat dispersal units contained high proportion of brown seeds (figure 2). They tend to germinate soon after dispersal if conditions are favorable resulting in seedling establishment over a short time span by a high-risk strategy (Venable, 1985; Venable *et al.*, 1987). In contrast, the humped dispersal units are non-dispersing, germinating slowly and ensure long-term reproduction and survival in the field by protracted germination (figure 2), a low-risk strategy (Venable, 1985). The occurrence of two dispersal strategies represents a kind of bet-hedging strategy (Venable, 1985; Mandák, 1997; Imbert, 2002) for *A. centralasiatica* adapted to coastal saline soils.

Humped-black seeds showed higher germination rate than flat-black seeds in low salinity (figure 2), and this may be related to seed size (figure 1). Seed size also affected seed germination in salt or drought stress in such *Atriplex* spp. as *A. nummularia* Lindl, *A. prostrata* and *A. patula* (Uchiyama, 1981; Katembe *et al.*, 1998). The color of testa may be correlated with its permeability to water uptake or gas diffusion. The water imbibitions study showed that the light colored testa was more permeable than dark colored testa to water (figure 3). The results are similar to those reported for *A. semibaccata*, *A. spongiosa*, *A. inflata*, *A. sagittata* and *Suaeda salsa* (Beadle, 1952; Mandák and Pyšek, 2001a,b; Li *et al.*, 2005). Recent studies showed that seed size has strong effects on *A. sagittata* entire life cycle and it has an important impact on population regeneration in successive years (Mandák and Pyšek, 2005).

Effect of light

Black seeds were more sensitive to light than brown ones (table 1). Similar results have been observed in *A. triangularis* (Khan and Ungar, 1986) and *Suaeda salsa* (Li *et al.*, 2005). For *A. dimorphostegia*, both flat and humped dispersal units showed high germination in darkness than in light (light-inhibited) (Koller, 1957), while germination of seeds of *A. sagittata* (Mandák and Pyšek, 2001a) and *A. prostrata* was light-stimulated (Young *et al.*, 1980). Other *Atriplex* species, such as *A. halimus*, *A. semibaccata* and *A. lentiformis* (Koller, 1957; Young *et al.*, 1980) were found to be light-insensitive. Cresswell and Grime (1981) suggested that the green bracteoles in *A. hastata* or other fruiting structure in other species could alter red:far-red ratio perceived by seeds and may be significant in inducing a light requirement for germination. In *A. centralasiatica*, both bracteoles and seed (or fruit) coats (testa, especially black ones) might be affecting red:far-red ratio and thus seed germination.

Interaction between temperature and salinity

Germination of coastal halophytes in north China is affected by variation in temperature under saline condition. Significant interactions between temperature and salinity have been reported by several workers for halophyte seed germination (Khan and Ungar, 1999; Khan and Gulzar, 2003). High temperature and salinity inhibited halophyte seed germination (Mikhieil *et al.*, 1992; Aiazzi *et al.*, 2002; Gul and Khan, 2002; Song *et al.*, 2006). Our results showed that the optimal temperature regime for germination of both black and brown seeds was 15/25°C. Low salinity showed little inhibition of seed germination in low temperature or optimal temperature for both black and brown seeds, while it showed significant inhibition at high temperature for black seeds but not for brown seeds. It might be concluded that brown seeds were more sensitive to temperature than the black ones; while black seeds were more sensitive to salinity than brown ones. Therefore, the combination of high temperature and salinity prevents dimorphic seed germination, and different seeds germinate only when the soil salt content declines which may be an important strategy to survive in their habitat (Khan and Rizvi, 1994).

Effect of bracteoles

Removal of dispersal units bracteoles significantly improved seed germination of *A. dimorphostegia* (Koller, 1957), *A. cordobensis* (Aiazzi and Argüello, 1992) *A. halimus*, *Salsola vermiculata* (Osman and Ghassali, 1997), *A. griffithii* (Ungar and Khan, 2001) and *A. nummularia* (Abu-Zanat and Samarah, 2005). However, in *A. polycarpa* (Sankary and Barbour, 1972) and *A. sagittata* (Mandák and Pyšek, 2001a) and *A. prostrata* (Ungar and Khan, 2001) seed germination was not affected by their bracteoles. Seed germination of *A. codonocarpa* was also affected by its leaf leachates and by seed dimorphism (Jefferson and Pennacchio, 2003). Our experiment showed that *A. centralasiatica* brown seeds germinated readily, while black seed germination was inhibited by bracteole leachate. Possible reasons for the inhibition of seed germination of *Atriplex* by bracteoles could be induction of light requirement, mechanical inhibition, osmotic stress or specific ion effect of soluble salts, negative effects of growth regulating substances (*e.g.*, ABA), and presence of allelopathic compounds (saponin) (Beadle, 1952; Koller, 1957; Cresswell

and Grime, 1981; Aiuzzi and Argüello, 1992; Ungar and Khan, 2001). Germination of black seeds of *A. centralasiatica* was inhibited by bracteoles in the presence of salinity. Because the black seeds are sensitive to light and salinity (table 1 and figure 2) and bracteoles leachates contains more NaCl, the inhibition might be because of increased salinity. The leachates also affected winter wheat and *Arabidopsis* seed germination and seedling growth by dose response (data not showing). When we removed bracteoles from utricles, we found that black seeds bracteoles were harder and more compact than the brown ones for both humped and flat fruits. It suggested that mechanical force might have also affected black seed germination. Similar results were reported by Aiuzzi and Argüello (1992) for *A. cordobensis* where bracteoles formed a mechanical barrier to water uptake. Effect of bracteoles on seed germination was affected not only by leachate concentration but also by seed types.

In the natural environment, the concentration of inhibiting chemicals and property of bracteoles would often be determined by a number of important factors, such as the density of bracteoles (ratio of bracteole and seed), rate of the material decomposition, rainfall, soil type, micro-organisms, and the concentration of the chemicals (Jefferson and Pennacchio, 2003). Seed germination was regulated by the environmental factors through bracteoles. Bracteoles also played a significant role in the dispersal of seeds and they serve to maintain a bet-hedging strategy in some *Atriplex* spp. (Ungar and Khan, 2001). The role of humped and flat bracteoles in *A. centralasiatica* may affect dispersal ability.

Conclusions

Atriplex centralasiatica not only produces dimorphic dispersal unit but also dimorphic seed from each dispersal unit type. It is seed type and not the dispersal unit type which affected *A. centralasiatica* seed germination characters. Results of this study also suggested that seed germination in *A. centralasiatica* was related to both seed and bracteoles type in natural environment. Environmental factors, such as light, temperature and salinity affected the seed germination. The germination occurred only when temperature and edaphic conditions were favorable. Polymorphic seed germination characters are most likely an important adaptive strategy in *A. centralasiatica* to survive in saline environment. It is a kind of bet-hedging strategy. Flat dispersal units contain more brown seeds and their germination is not sensitive to light, salinity and presence of bracteoles. This type of seeds should be more successful than seeds from humped dispersal units during seedling establishment. Humped dispersal units contain more black seeds and their germination is sensitive to light, salinity, bracteoles and can maintain germination in later period.

References

- Abu-Zanat, M.M.W. and Samarah, N. (2005). Physical and chemical treatments for enhancing seed germination of Oldman saltbush (*Atriplex nummularia*). *African Journal of Range and Forage Science*, **22**, 141-145.
- Aiuzzi, M.T., Carpane, J.A., Di Rienzo, J. and Argüello, J.A. (2002). Effects of salinity and temperature on germination and early seedling growth of *Atriplex cordobensis* Gandoger et Stucker (Chenopodiaceae). *Seed Science and Technology*, **30**, 329-338.

- Aiazzi, M.T. and Argüello JA. (1992). Dormancy and germination studies on dispersal unites of *Atriplex cordobensis* (Gandoger and Stucker) (Chenopodiaceae). *Seed Science and Technology*, **20**, 401-407.
- Bai, Y., Liu, X. and Li, W. (2003). Primary analysis of four salt tolerant plants growing in Hai-He Plain, China. In *Cash crop Halophytes: Recent Studies*, (ed. H. Lieth), pp. 135-138, Kluwer, Great Britain.
- Beadle, N.C.W. (1952). Studies in halophytes I. the germination of the seed and establishment of the seedlings of five species of *Atriplex* in Australia. *Ecology*, **33**, 49-62.
- Cresswell, E.G. and Grime, J.P. (1981). Induction of light requirement during seed development and its ecological consequences. *Nature*, **291**, 583-585.
- Ellison, A.M. (1987). Effect of seed dimorphism on the density-dependent dynamics of experimental populations of *Atriplex triangularis* (Chenopodiaceae). *American Journal of Botany*, **74**, 1280-1288.
- Frankton, C. and Bassett, I.J. (1968). The genus *Atriplex* (Chenopodiaceae) in Canada. I. Three introduced species: *A. heterosperma*, *A. oblongifolia*, and *A. hortensis*. *Canadian Journal of Botany*, **46**, 1309-1313.
- Galatino, M.I. and van der Valk, A.G. (1986). Seed germination traits of annuals and emergent recruited during drawdowns in the Delta marsh, Manitoba, Canada. *Aquatic Botany*, **26**, 89-102.
- Gul, B. and Khan, M.A. (2002). Seed germination of halophytes exposed to high salinity and temperature in the seed bank. In *Halophytes Utilization and Regional Sustainable Development of Agriculture*, (eds. X. Liu and M. Liu), pp. 69-76, Meteorological Press of China, Beijing.
- Hamdy, A.H., Lieth, H., Todorovic, M. and Moschenko, M. (1999). *Halophyte uses in different climates. II. Halophyte crop development: pilot studies*. Backhuys Publishers, Leiden.
- Hardegree, S.P. and Emmerich, W.E. (1990). Partitioning of water potential and specific salt effects on seed germination of four grasses. *Annals of Botany*, **66**, 587-595.
- He, X.Q. and Li, F.Z. (1995). [In Chinese with English abstract] Seed morphology of *Atriplex* L. from China and its taxonomic significance. *Bulletin of Botany Research*, **15**, 65-71.
- Imbert, E. (2002). Ecological consequences and ontogeny of seed heteromorphism. *Perspectives in Plant Ecology, Evolution and Systematics*, **5**, 13-36.
- Jefferson, L.V. and Pennacchio, M. (2003). Allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination. *Journal of Arid Environments*, **55**, 275-285.
- Katembe, W.J., Ungar, I.A., and Mitchell, J.P. (1998). Effect of salinity on germination and seedling growth of two *Atriplex* species (Chenopodiaceae). *Annals of Botany*, **82**, 167-175.
- Khan, M.A. and Gul, B. (1998). High salt tolerance in germinating dimorphic seeds of *Arthrocnemum indicum*. *International Journal of Plant Science*, **159**, 826-832.
- Khan, M.A. and Gul, B. (2002). Some ecophysiological aspects of seed germination in halophytes. In *Halophytes Utilization and Regional Sustainable Development of Agriculture*, (eds. X. Liu and M. Liu), pp. 59-68, Meteorological Press of China, Beijing.
- Khan, M.A., Gul, B. and Weber, D.J. (2004). Temperature and high salinity effects in germinating dimorphic seeds of *Atriplex rosea*. *Western North American Naturalist*, **64**, 193-201.
- Khan, M.A. and Gulzar, S. (2003). Light, salinity, and temperature effects on the seed germination of perennial grasses. *American Journal of Botany*, **90**, 131-134.
- Khan, M.A. and Rizvi, Y. (1994). The effect of salinity, temperature and growth regulators on the germination and early seedling growth of *Atriplex griffithii* Moq. var. *stocksii* Boiss. *Canadian Journal of Botany*, **71**, 475-479.
- Khan, M.A. and Ungar, I.A. (1984). The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. *American Journal of Botany*, **71**, 481-489.
- Khan, M.A. and Ungar, I.A. (1986). Life history and population dynamics of *Atriplex triangularis*. *Vegetatio*, **66**, 17-25.
- Khan, M.A. and Ungar, I.A. (1999). Effect of salinity on the seed germination of *Triglochin maritime* under various temperature regimes. *Great Basin Naturalist*, **59**, 144-150.
- Khan, M.A. and Weber, D.J. (2006). *Ecophysiology of High Salinity Tolerant Plants*. Springer, Netherlands.
- Koller, D. (1957). Germination-regulation mechanisms in some desert seeds, IV. *Atriplex dimorphostegia* Kar. et Kir. *Ecology*, **38**, 1-13.
- Li, W., Liu, X., Khan, M.A. and Yamaguchi, S. (2005). The effect of plant growth regulators, nitric oxide, nitrate, nitrite and light on the germination of dimorphic seeds of *Suaeda salsa* under saline conditions. *Journal of Plant Research*, **118**, 207-214.
- Lieth, H., Moschenko, M., Lohmann, M., Koyro, H.W. and Hamdy, A. (1999). *Halophyte uses in different climates. I. Ecological and eco-physiological studies*, Backhuys Publishers, Leiden.

- Mandák, B. (1997). Seed heteromorphism and the life cycle of plants: a literature review. *Preslia*, **69**, 129-159.
- Mandák, B. (2003). Germination requirements of invasive and non-invasive *Atriplex* species: a comparative study. *Flora*, **198**, 45-54.
- Mandák, B. and Pyšek, P. (2001a). The effect of light quality, nitrate concentration and presence of bracteoles on germination of different fruit types in the heterocarpous *Atriplex sagittata*. *Journal of Ecology*, **89**, 149-158.
- Mandák, B. and Pyšek, P. (2001b). Fruit dispersal and seed banks in *Atriplex sagittata*: the role of heterocarpy. *Journal of Ecology*, **89**, 159-165.
- Mandák, B. and Pyšek, P. (2005). How does seed heteromorphism influence the life history stages of *Atriplex sagittata* (Chenopodiaceae)? *Flora*, **200**, 516-626.
- Meyer, S.E., Carlson, S.L. and Garvin, S.C. (1998). Seed germination regulation and field seed bank carryover in shadscale (*Atriplex confertifolia*: Chenopodiaceae). *Journal of Arid Environments*, **38**, 255-267.
- Mikhiel, G.S., Meyer, S.E. and Pendleton, R.L. (1992). Variation in germination response to temperature and salinity in shrubby *Atriplex* Species. *Journal of Arid Environments*, **22**, 30-49.
- Morgan, W.C. and Myers, B.A. (1989). Germination of salt-tolerant grass *Diplachne fusca*. I. Dormancy and temperature responses. *Australian Journal of Botany*, **37**, 225-237.
- Osman, A.E. and Ghassali, F. (1997). Effects of storage conditions and presence of fruiting bractes on the germination of *Atriplex halimus* and *Salsola vermiculata*. *Experimental Agriculture*, **33**, 149-155.
- Philippillai, J. and Ungar, I.A. (1984). The effect of seed dimorphism on the germination and survival of *Salicornia europaea* L. populations. *American Journal of Botany*, **71**, 542-549.
- Qiu, N. and Lu, C. (2003). Enhanced tolerance of photosynthesis against high temperature damage in salt-adapted halophyte *Atriplex centralasiatica* plants. *Plant Cell and Environment*, **26**, 1137-1145.
- Qiu, N., Lu, Q. and Lu, C. (2003). Photosynthesis, photosystem II efficiency and the xanthophylls cycle in the salt-adapted halophyte *Atriplex centralasiatica*. *New Phytologist*, **159**, 479-486.
- Sankary, M.N. and Barbour M.G. (1972). Autocology of *Atriplex polycarpa* form California. *Ecology*, **53**, 1155-1162.
- Song, J., Feng, G. and Zhang, F. (2006). Salinity and temperature effects on germination for three salt-resistant euhalophytes, *Halostachys caspica*, *Kalidium foliatum* and *Halocnemum strobilaceum*. *Plant and Soil*, **279**, 201-207.
- Uchiyama, Y. (1981). [In Japanese with English abstract]. Studies on the germination of saltbushes I. The relationship between temperature and germination of *Atriplex nummularia* Lindl. *Japanese Journal of Tropic Agriculture*, **25**, 62-67.
- Ungar, I.A. (1977). Salinity, temperature, and growth regulator effects on seed germination of *Salicornia europaea* L. *Aquatic Botany*, **3**, 329-335.
- Ungar, I.A. (1988). Effects of the parental environment on the temperature requirements and salinity tolerance of *Spergularia marina* seeds. *Botanical Gazette*, **149**, 432-436.
- Ungar, I.A. and Khan, M.A. (2001). Effect of bracteoles on seed germination and dispersal of two species of *Atriplex*. *Annals of Botany*, **87**, 233-239.
- Venable, D.L. (1985). The evolutionary ecology of seed heteromorphism. *American Naturalist*, **126**, 577-595.
- Venable, D.L., Burque, A.M., Corra, G., Morales, E. and Espinosa, F. (1987). The ecology of seed heteromorphism in *Heterosperma pinnatum* in Center Mexico. *Ecology*, **68**, 65-76.
- Wang, Y. and Hou, X. (2005). [In Chinese with English abstract]. The Exploitation and research on halophyte *Atriplex centralasiatica*. *Chinese Wild Plant Resources*, **24**, 36-37.
- Yi, D. and Liu, F. (2003). [In Chinese with English abstract]. Study on extraction technology of leaf protein from halophyte *Atriplex centralasiatica*. *Advances in Marine Science*, **21**, 83-88.
- Yi, D. and Liu, F. (2004). [In Chinese with English abstract]. The study on the extraction process of insoluble dietary fiber from *Atriplex centralasiatica* leaf residue. *Journal of Chinese Institute of Food Science and Technology*, **4**, 1-5.
- Young, J.A., Kay, B.L., George, H. and Evans, R.A. (1980). Germination of three species of *Atriplex*. *Agronomy Journal*, **72**, 705-709.
- Zhao, K., Fan, H. and Ungar, I.A. (2002). Survey of halophyte species in China. *Plant Science*, **163**, 491-498.