PART OF A HIGHLIGHT SECTION ON SEEDS

Variation of seed heteromorphism in *Chenopodium album* and the effect of salinity stress on the descendants

Shixiang Yao, Haiyan Lan* and Fuchun Zhang

Xinjiang Key Laboratory of Biological Resources and Genetic Engineering, College of Life Science and Technology, Xinjiang University, Urumqi 830046, China
**For correspondence. E-mail lanhaiyan@xju.edu.cn*

• *Background and Aims Chenopodium album* is well-known as a serious weed and is a salt-tolerant species inhabiting semi-arid and light-saline environments in Xinjiang, China. It produces large amounts of heteromorphic (black and brown) seeds. The primary aims of the present study were to compare the germination characteristics of heteromorphic seeds, the diversity of plant growth and seed proliferation pattern of the resulting plants, and the correlation between NaCl stress and variation of seed heteromorphism.

• *Methods* The phenotypic characters of heteromorphic seeds, e.g. seed morphology, seed mass and total seed protein were determined. The effects of dry storage at room temperature on dormancy behaviour, the germination response of seeds to salinity stress, and the effect of salinity on growth and seed proliferation with plants derived from different seed types were investigated.

• *Key Results* Black and brown seeds differed in seed morphology, mass, total seed protein, dormancy behaviour and salinity tolerance. Brown seeds were large, non-dormant and more salt tolerant, and could germinate rapidly to a high percentage in a wider range of environments; black seeds were salt-sensitive, and a large proportion of seeds were dormant. These characteristics varied between two populations. There was little difference in growth characteristics and seed output of plants produced from the two seed morphs except when plants were subjected to high salinity stress. Plants that suffered higher salinity stress produced more brown (salt-tolerant) seeds.

• *Conclusions* The two seed morphs of *C. album* exhibited distinct diversity in germination characteristics. There was a significant difference in plant development and seed proliferation pattern from the two types of seeds only when the parent plants were treated with high salinity. In addition, seed heteromorphism of *C. album* varied between the two populations, and such variation may be attributed, at least in part, to the salinity.

Key words: *Chenopodium album*, development of descendants, salinity tolerance, seed germination, variation of seed heteromorphism.

INTRODUCTION

Seed germination is the crucial stage in life cycles of many plant species (Khan and Ungar, 1996) and for the annuals growing in unpredictable environments, the germination strategy may be the most significant factor determining seedling survival and the maintenance of their populations from one year to the next (Keiffer and Ungar, 1997). In the course of evolution, the ability of some plant species to produce different types of seeds in a single plant, i.e. seed heteromorphism, has been mostly observed in those species distributed in semi-arid, saline and other unfavorable environments (Sorensen, 1978; Imbert, 2002). Heteromorphic seeds often differ in colour, shape and mass, and are frequently accompanied by differences in dispersal, germination characteristics, dormancy behaviour, ability to persist in a seed bank and seedling growth (Baker and O'Dowd, 1982; Mandák and Pyšek, 2001; for an overview, see Imbert, 2002; Brändel, 2004).

The most obvious morphological differences between heteromorphic seeds are often in their seed coat structure and seed size. Thickness of the seed coat plays an important role in germination, as it may restrict water uptake and/or gas diffusion, and prevent radicle protrusion (Mohamed-Yasseen *et al.*, 1994). Large seeds often have high reserve mass which is stored in the embryo and/or endosperm (Imbert, 2002), consequently, seedlings derived from large seeds are usually larger and may have higher seed output (Ellison, 1987; Imbert *et al.*, 1996; Mandák and Pyšek, 2005). The variation in dormancy level between heteromorphic seeds also exerts influence by extending the germination period of these species and the formation a seed bank for the long-term recruitment of seedlings (Ungar, 1987). Furthermore, a growing body of evidence has shown that different types of heteromorphic seeds in many species respond to environmental stress differently. Large or brown seeds are usually more salt tolerant than the small or black ones (Khan *et al.*, 2004), for example in *Atriplex centralasiatica* (Li *et al.*, 2008), *Halopyrum mucronatum* (Khan and Ungar, 2001), *Suaeda aralocaspica* (Wang *et al.*, 2008) and *S. splendens* (Redondo-Gómez *et al.*, 2008). Taken together, seed heteromorphism can be considered a bet-hedging strategy to ensure seedling establishment and population succession of those plant species in the seasonally fluctuating and disturbed areas (Venable, 1985).

Although much emphasis has been put on the differences between heteromorphic seeds, and the consequences of such

differences for seed germination, plant growth and seed pro-liferation, a few questions are still incompletely answered. Firstly, few previous studies of seed heteromorphism have con-firmed variation in seed heteromorphism (e.g. hetero-seeds ratio, differences in germination of hetero-seeds, etc.) among populations, although some researchers found that the hetero-seeds ratio might change in response to changing environ-ments (Brändel, 2007; Song *et al.*, 2008). Secondly, many plant species distributed in semi-arid areas, where soil salini-zation is becoming a more serious problem, have been reported to exhibit seed heteromorphism. Even so, few studies have focused on the response of seed germination and the develop-ment of plants from these seeds to salinity (Wei *et al.*, 2007; Wang *et al.*, 2008). Thirdly, little information is available about the effect of seed heteromorphism on plant growth and seed proliferation of the offspring derived from different types of seeds, especially under different environmental stres-ses (Mandák and Pyšek, 2005).

Chenopodium album (Chenopodiaceae) produces two dis-tinct lots of seeds (morphs), some (approx. 3·0 %) with a brown seed coat are non-dormant and germinate rapidly after the initial harvest, the majority with a black seed coat, are dormant (Williams and Harper, 1965). Therefore, most pre-vious research on *C. album* has been aimed at investigation of the germination strategy of the black seeds in relation to a better control of this harmful weed (Williams and Harper, 1965; Henson, 1970; Karssen, 1976; Roberts and Benjamin, 1979; Saini *et al.*, 1985; Ahmed, 1987).

Chenopodium album is distributed widely over the semi-arid areas in Xinjiang province, China (Commissione Redactorum Florae Xinjiangensis, 1994). Among the many environmental factors in this area, the combination of high temperature and strong sunlight during the summer season leads to high water evaporation, which, together with little precipitation, causes a gradually increasing salinity in the soil surface (Khan and Ungar, 1996; Song *et al.*, 2005; Munns and Tester, 2008). This becomes an important limiting factor for plant growth and survival (Rozema and Flowers, 2008). It was found that *C. album* located in semi-arid areas of Xinjiang produces heteromorphic seeds, >50 % of which were brown (Yao *et al.*, unpubl. res.), an observation that was significantly different from previous studies (Williams and Harper, 1965; Karssen *et al.*, 1989). Moreover, plants of *C. album* could grow normally under 300 mmol L^{-1} NaCl stress (Yao *et al.*, 2010), and may be considered as a salt-resistant species (Hamidov *et al.*, 2007). However, the responses of germination and the growth of descendants from heteromorphic seeds of *C. album* to salinity were poorly documented, although these may provide evidence for the introduction of this promising annual herb to amend saline soils (Yao *et al.*, 2010).

In the present study, seeds of *C. album* were collected from several populations located in different micro-habitats, in a highly unpredictable semi-arid environment of Xinjiang pro-vince. The germination of heteromorphic seeds of *C. album* from different populations were investigated, and it was deter-mined whether significant differences exist in the growth and seed proliferation pattern between plants from different seed types under different conditions (with or without salt treat-ment). To this end, the following aspects were studied: (*a*)

The phenotypic differences between heteromorphic seeds, including seed coat morphology and colour, seed mass/seed reserve mass, total seed protein; (*b*) the effect of dry storage at room temperature on dormancy behaviour of heteromorphic seeds; (*c*) the response of heteromorphic seeds to salinity during germination; (*d*) the effect of salt stress on growth and hetero-seed ratio of the descendants.

MATERIALS AND METHODS

Seed collection

The mature seeds of *Chenopodium album* were collected in November 2007, from naturally dry inflorescences of plants grown in a semi-arid environment (43 °45′N, 87 °37′E; 910 m a.s.l.), near the southern edge of the Junggar Basin in Xinjiang. Meteorological data based on the previous 3 years (2005–2007) indicated that the mean temperature of the warmest month (July) was 24·6 °C and the coldest month (January) was −13·1 °C. Annual precipitation (melting snow plus the rainfall) was 310·6 mm; while the mean evaporation from May to September amounted to 945·7 mm. Seeds from the same microhabitat were pooled to form one population. Population 1 was located on relatively flat ground; population 2 was grown on a slope. These two populations were selected for study following initial work on four populations. The two chosen showed contrasting germination behaviour for the black and brown seeds within the population.

Seeds were naturally air-dried and stored at 20–25 °C, 18–25 % relative humidity (RH) in brown paper bags for various experiments.

Determination of the brown : total seeds ratio

Three replicates with 1000 randomly chosen seeds from two populations (poorly developed or shrivelled seeds were excluded) were divided into black or brown seeds depending on the seed coat colour and measured the ratio of brown seeds in the total.

Seed morphology observation

A stereomicroscope SMZ800 (Nikon, Japan) was employed to examine the dry seed and surface and longitudinal sections. For seed coat observation, dry seeds were mounted directly on stubs using double-sided adhesive tape, and sputter-coated with gold, then imaged using a LEO1430VP scanning electron microscope (LEO Corp., Germany). Thickness of the seed coat was measured using the NIS-Elements imaging software (Nikon, Japan). Digital photographs were manipulated with Adobe Photoshop (Adobe Photosystems) to prepare figures.

Phenotypic characters of heteromorphic seeds

Determination of seed mass. Three replicates of 1000 black or brown seeds from each of the two populations were sorted for each seed type; the seed coats were separated from the embryo and endosperm, and the mass of each part weighed.

Total seed protein content analysis. To gain insight into the total protein variability that may correlate with seed

heteromorphism in *C. album*, the total seed protein was measured. Black and brown seeds (100 mg of each) were homogenized in liquid nitrogen, and then transferred to a fresh 1·5 mL micro-tube with 500 μL extraction buffer (12·5 mmol L^{-1} Tris-Cl, pH 6·8). The homogenate was centrifuged at 10 000 *g* for 15 min at 4 °C. The resulting supernatant was stored at 4 °C until use. The protein content of the extract was determined by the method of Bradford (1976).

Seed germination experiments

Three replicates of 40 seeds from each seed type were tested in each treatment. For germination, seeds were incubated on two layers of filter paper in a 9-cm Petri dish, to which 5 mL of distilled water or aqueous solutions of NaCl were added. The filter paper was renewed every 2 d to keep the NaCl concentration unchanged. All Petri dishes were placed in an illuminated incubator (LRH-400-GII; Guangdong Medicine Apparatus Manufactory, China), and subjected to a 16-h daily photoperiod (light flux: approx. 116 μmol m^{-2} s^{-1}) at 25 °C. A seed was considered to be germinated when the emerging radicle was at least 0·5 mm. Non-germinated seeds were checked under a stereo microscope to see if the embryos were white and firm, thus indicating they were alive; if not, they were considered dead and excluded from further calculation (Baskin and Baskin, 2001). Germination was recorded every 12 h during a 2-week period to calculate germination rate (expressed in mean time to germination, MTG for short). MTG was calculated by using the equation: $MTG = \Sigma_i(n_i \times d_i)/N$, where *n* is the number of seeds germinated at day *i*; *d* is the incubation period in days and *N* is the total number of seeds germinated in the test (Redondo-Gómez *et al.*, 2008). According to this equation, a lower value indicates more rapid germination. The final germination was counted on the 15th day.

Storage at room temperature for 12 months. Storage at room temperature was used to test the effect of laboratory conditions on the dormancy behaviour of seeds. Heteromorphic seeds from each population were stored at 20–25 °C, 18–25 % RH, and retrieved monthly for seed germination tests, beginning at the end of November 2007 (approx. 1 month after harvest) and ending at the end of November 2008.

Salinity treatments. To investigate the effect of salinity on germination of heteromorphic seeds, different concentrations of NaCl solution (0, 50, 100, 200, 300 and 400 mmol L^{-1}, respectively) were applied in the germination test. In a seed recovery experiment, seeds that failed to germinate after 60 d in the NaCl treatment were rinsed three times with distilled water, and then set to germinate with distilled water.

Testa treatment. This experiment aimed to investigate the germination response of black seeds to salinity when dormancy was alleviated. Seed coats covering the radicle of black seeds (population 2) were removed carefully, and then the seeds were transferred to Petri dishes containing 0, 50, 100, 200 or 300 mmol L^{-1} NaCl solutions. Germination conditions were the same as described in the experiment 'Salinity treatments'.

Determination of plant growth and seed proliferation of the descendants derived from heteromorphic seeds under different salinity treatments

In October, 2008, black and brown seeds of population 2 were sown in round plastic pots [filled with perlite : vermiculite (1 : 3)], then cultivated in a greenhouse at a temperature regime of 25–32 °C, 28–52 % RH and natural sunlight (light flux during the day: 364–1743 μmol m^{-2} s^{-1}). Hoagland solution was supplied once per month, tap water was added at other times when necessary. When seedlings were 4 months old (before anthesis) they were treated with Hoagland solutions containing different concentrations of NaCl as follows: 0 as control, 50 mmol L^{-1} as lower and 300 mmol L^{-1} as higher salinity. Hoagland solutions containing NaCl were supplied once per month. At the full flowering stage, plant height and number of branches (primary) were recorded. Then the mature black and brown seeds were separately collected from each plant per day for nearly 75 d (the period when plants with the 300 mmol L^{-1} treatment completed their life cycles), and the total number of seeds and the hetero-seeds ratio per plant were calculated.

Statistical analysis

All data were expressed as mean ± standard error. Percentages were arcsine transformed before statistical analysis to ensure homogeneity of variance. Unpaired *t*-test was used to compare two samples (or treatments). For more than two samples (or treatments), one- or two-way ANOVA was employed to compare treatment effects using the GraphPad Prism Version 4·02 for Windows (GraphPad Software, San Diego, CA, USA). When significant main effects existed, differences were tested by a multiple comparison Tukey test at the 0·05, 0·01, 0·001 and 0·0001 significance levels.

RESULTS

Variation of the seed heteromorph ratio and morphology between two populations

Seeds of *C. album* from two populations grown in different micro-habitats were collected in the autumn. The two seed lots matured almost simultaneously with no special dispersal structure; a proportion of the seed would be dispersed by wind while some would drop on the ground surrounding the mother plant. Both of the two populations had a higher proportion of brown seeds (>50 %) than black seeds. Moreover, the percentage of brown seeds was significantly higher in population 1 than in population 2 (65 % and 52 %, respectively; unpaired *t*-test, *P* < 0·01).

Both the black and brown seeds from the two populations had an oblate spheroid shape, and had black and brown testae, respectively (Fig. 1A–D). In longitudinal section, both seed morphs presented as a circular embryo surrounding the central endosperm (Fig. 1E–H). Further inspection of the germination process indicated that, in all seeds from both populations, the radicle had to break through the seed coat mechanically for protrusion and germination (Fig. 1I–L). Scanning electron microscopy revealed that the testa of the black seeds from both populations was more than twice as
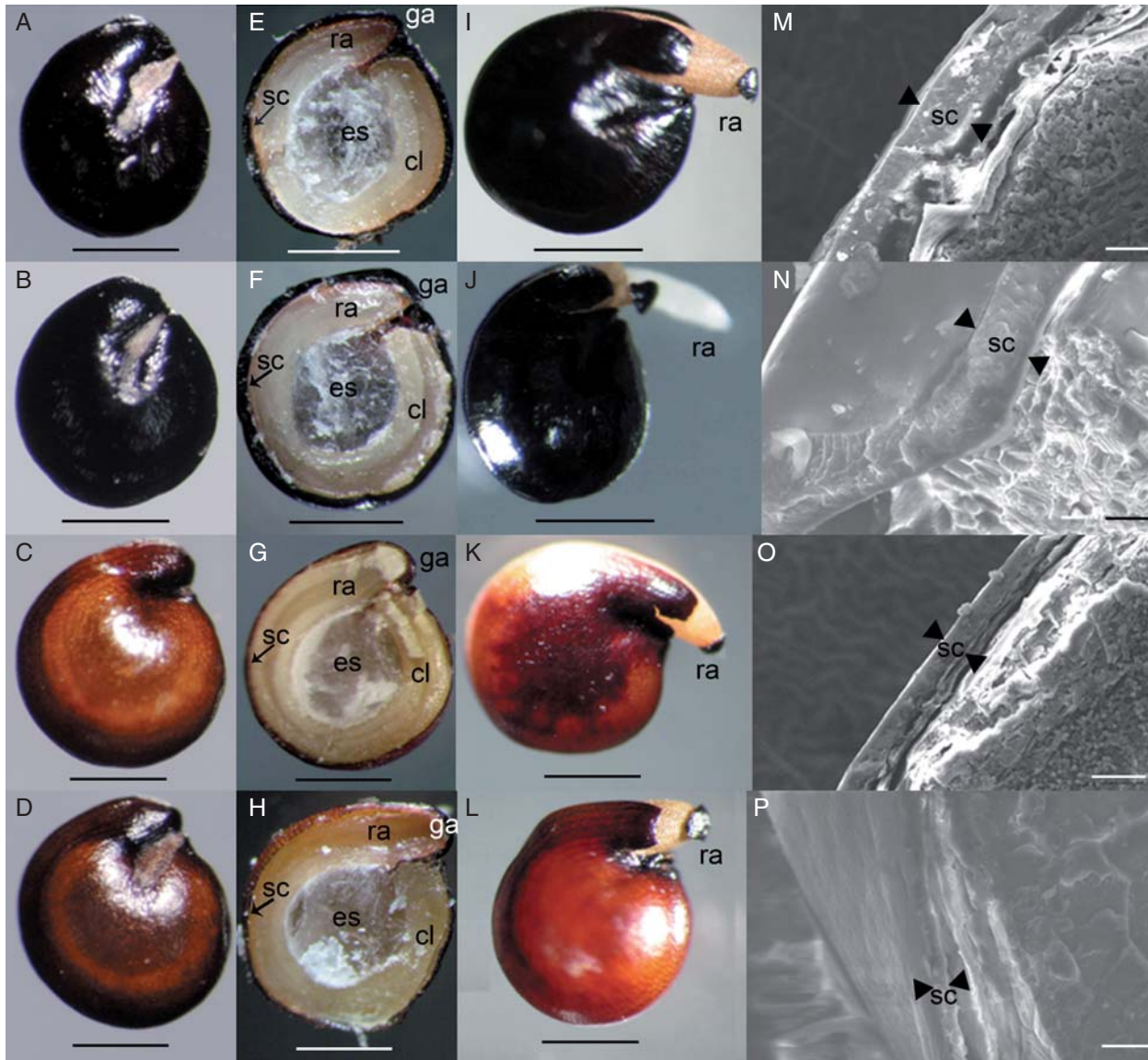
FIG. 1. Morphology of heteromorphic seeds of *Chenopodium album*: (A, E, I, M) the black seed from population 1; (B, F, J, N) the black seed from population 2; (C, G, K, O) the brown seed from population 1; (D, H, L, P) the brown seed from population 2; (A–D) dry mature seeds; (E–H) longitudinal section of seeds; (I–L) position of radicle protrusion; (M–P) scanning electron micrographs of seed coat, showing the thickness of the testa. Scale bars: (A–L) 0·5 mm; (M–P) 20 μm. Abbreviations: es, endosperm; cl, cotyledon; ra, radicle; ga, germinal aperture; sc, seed coat.

thick as that of the brown seeds (Fig. 1M–P). Furthermore, there was no significant difference between populations in the thickness of the seed coat of either black or brown seeds (unpaired *t*-test, $P > 0.05$). However the seed coat of the black seed from population 2 was much harder compared with that of population 1.

Differences in some characteristics of heteromorphic seeds and germination behaviour between two populations

Seed mass distribution. Within the two populations, a significantly lower proportion of total seed mass was allocated to the seed reserve mass (embryo plus endosperm) in the black seed compared with the brown seed (Fig. 2A; unpaired *t*-test, population 1, $P < 0.001$; population 2, $P < 0.0001$). In

addition, the seed reserve mass in the black seed from population 1 was higher than that from population 2 (unpaired *t*-test, $P < 0.01$), although there was no significant difference in the brown seed between two populations (unpaired *t*-test, $P > 0.05$).

Total seed protein content. The total seed protein content was much lower in the black seeds than in the brown seeds (Fig. 2B; unpaired *t*-test, population 1, $P < 0.001$; population 2, $P < 0.001$). There was a small but non-significant difference in the protein content of the black seeds in the two populations (unpaired *t*-test, $P > 0.05$), whereas the protein content of the brown seeds in population 1 was much lower than that in population 2 (unpaired *t*-test, $P < 0.001$).
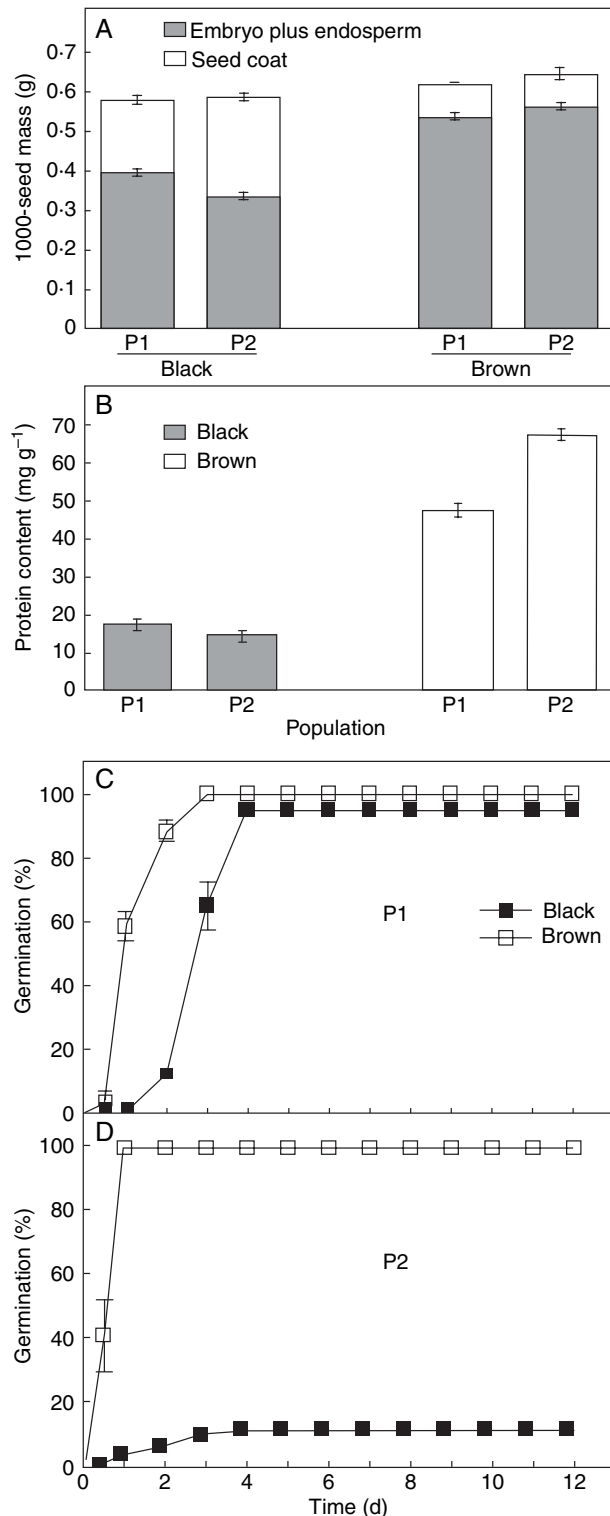
FIG. 2. Mass distribution between seed coat and embryo plus endosperm (A), total seed protein content (B) and germination in distilled water (C, D) of black and brown seeds in *Chenopodium album*. P1, Population 1; P2, population 2. Values are means ± s.e. from three replicates.

Germination in distilled water. The germination behaviour of the heteromorphic seeds in distilled water differed dramatically between the two seed morphs and populations

(Fig. 2C, D). The brown seeds germinated more quickly, and to a higher final percentage, than the black seeds within both populations. The brown seeds of population 1 germinated more slowly than those from population 2 (unpaired *t*-test, $P < 0.0001$), although both reached a final germination percentage of nearly 100 %. In contrast, the black seeds of population 1 had a much higher final germination (almost 100 %; Fig. 2C) than population 2 (10·8 %; Fig. 2D, unpaired *t*-test, $P < 0.0001$).

Effect of storage at room temperature (RT) for 12 months on seed germination

The effect of dry storage at RT on the seed dormancy characteristics varied between morphs and populations (Fig. 3). Dry storage at RT had little effect on the germination percentage of the brown seeds from both populations (two-way ANOVA: time, $P = 0.154$; population, $P = 0.192$; time × population, $P = 0.066$). The freshly mature brown seeds in both populations were non-dormant, and germinated approx. 100 % for the whole year (Fig. 3A and C). In contrast, there was considerable variation in the dormancy behaviour of the black seeds between the two populations. At the beginning of the experiment, only 20 % of the black seeds in population 2 germinated (Fig. 3C), while nearly 100 % germination in population 1 was observed during most of the year (Fig. 3A; data available only from April to November). The dormancy of the black seeds in population 2 was reduced by after-ripening during winter and early spring (November to April of the next year), as germination increased to the maximum percentage of approx. 50 % after storage for 6 months (April; Fig. 3C). However, the germination of the black seeds subsequently fell to <20 % in May, and remained low from May to November (Fig. 3C).

The rate of germination also varied with dry storage at RT (Fig. 3B, D), and a significant 'population' × 'time' interaction in two-way ANOVA analysis confirmed such an effect (Fig. 3B, D; black seeds, $P < 0.05$; brown seeds, $P < 0.01$). The germination rate of the black seeds in population 2 increased from November to June of the next year (decreased MTG; Fig. 3B). In population 1, there was a small decrease in MTG, i.e. germination rate increased, from April to June, and then decreased from July to November. A similar trend was found in brown seeds.

Effect of salinity stress on seed germination

There was no effect of salinity on the seeds of either population from 0 to 200 mmol L^{-1} NaCl (Fig. 4A and B). At higher salinities, a small proportion (20 %) of brown seeds from both populations germinated at 300 mmol L^{-1} NaCl (Fig. 4C, D), but none at 400 mmol L^{-1} NaCl, while no black seeds germinated at either 300 or 400 mmol L^{-1}.

After 60 d of exposure to various NaCl concentrations, seeds that failed to germinate under 300 and 400 mmol L^{-1} NaCl were set to germinate with distilled water (Fig. 4C and D). The re-germination of the brown seeds in both populations was similar, reaching a final percentage of >80 %. Further tests showed that non-germinated brown seeds had decayed. In contrast, the response of black seeds from the two
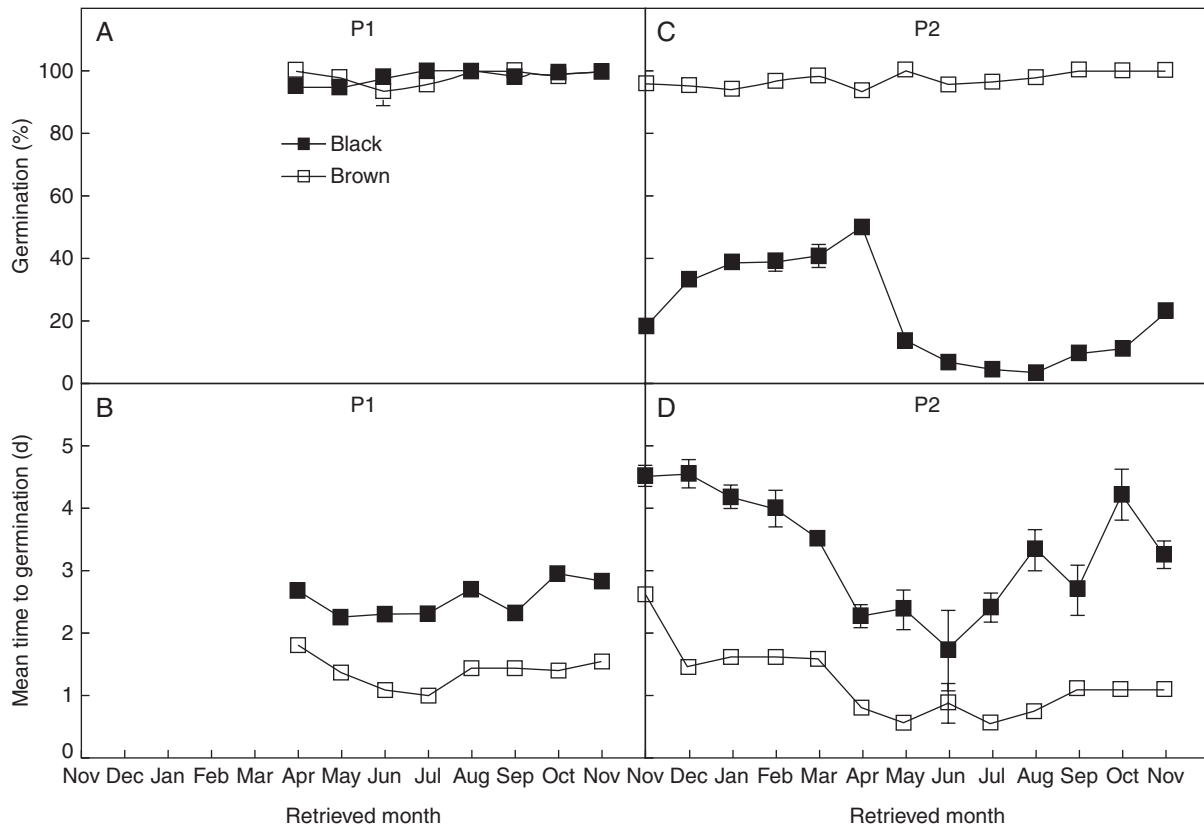
FIG. 3. Final germination percentage (A, C) and mean time to germination (MTG; B, D) of black and brown seeds of *Chenopodium album* in two populations following 1–13 months of storage at room temperature (20–25 °C) in the dark. P1, Population 1; P2, population 2. Values are means $\pm$ s.e. from three replicates.
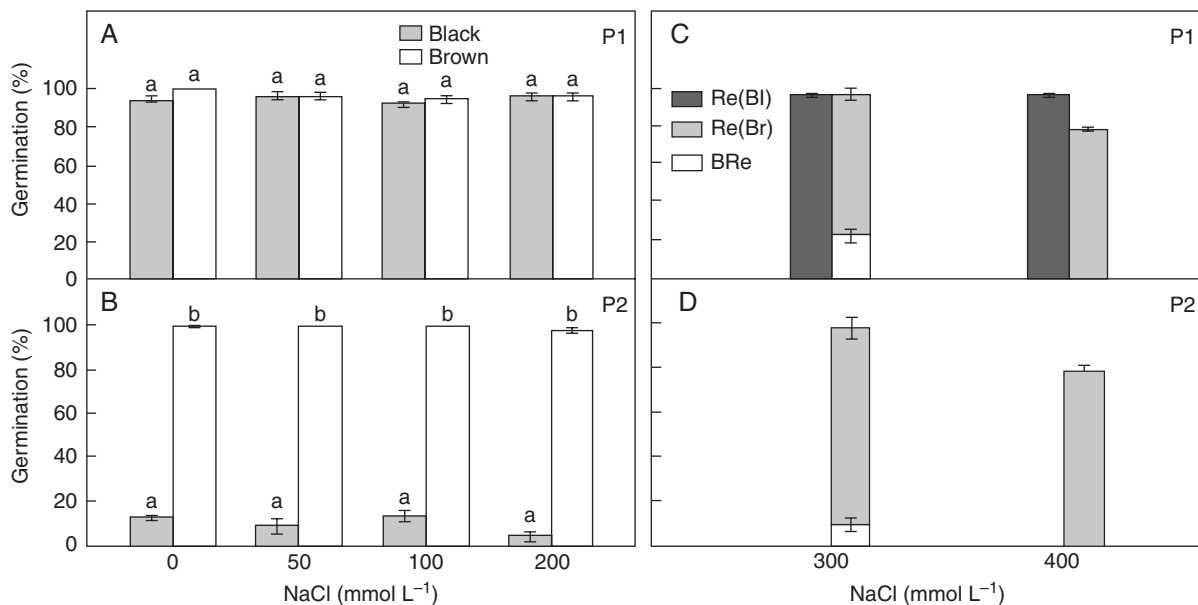

FIG. 4. Effect of NaCl on final germination percentage of heteromorphic seeds of *Chenopodium album* in population 1 (A, C) and population 2 (B, D). After seeds were treated with 300 and 400 mmol L^{-1} NaCl for 60 d, non-germinated seeds were transferred to distilled water for recovery and germination for another 12 d. Different letters indicate significant differences at the same NaCl concentration ($P < 0.05$). Re(Bl), after recovery of black seeds; Re(Br), after recovery of brown seeds; BRe, before recovery. Values are means $\pm$ s.e. from three replicates.

populations to re-germination was significantly different. Following both the 300 and 400 mmol L^{-1} NaCl pre-treatments, the black seeds of population 1 were able to

recover germination to 100 % (Fig. 4C), while black seeds from population 2 failed to recover. However, further tests indicated that non-germinated black seeds remained alive.

To investigate further the response of dimorphic seeds to salt stress, the seed coat covering the radicle of the black seeds was removed before germination in saline conditions. The germination of black seeds having had part of the seed coat removed, improved under salinity stress in comparison to non-treated black seeds (Fig. 5; two-way ANOVA, $P <$ 0·0001). However, inhibition of germination by salinity was still greater in the black seeds with dormancy relieved than it was in brown seeds, as indicated by a significant 'seed type' × 'salt concentration' interaction ($P <$ 0·0001).

Effect of NaCl stress on plant growth and seed proliferation of the descendants derived from heteromorphic seeds

Plant growth. The effect of salinity on the growth of the plants derived from black and brown seeds was different (Fig. 6). The plant height from brown seeds was greater than that from black seeds under various NaCl concentrations, but only 300 mmol L^{-1} NaCl resulted in a significant difference (Fig. 6A; plant type in two-way ANOVA, $P <$ 0·05). A similar trend was
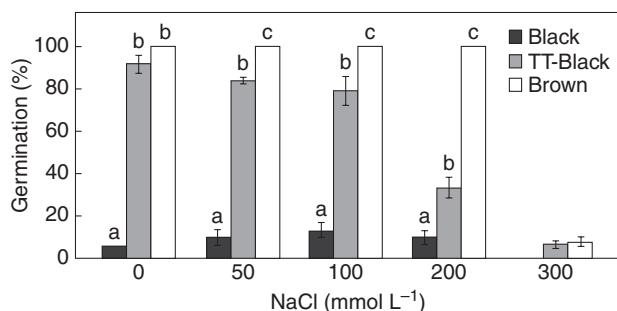
FIG. 5. Effect of salinity on germination of non-treated black seeds (Black), testa-treated black seeds (TT-Black) and non-treated brown seeds (Brown) of *Chenopodium album*. All of these seeds were from population 2. For a given NaCl concentration, values sharing a common letter are not significantly different at $P <$ 0·05. Testa treatment means seed coat covering radicle was removed. Values are means ± s.e. from three replicates.
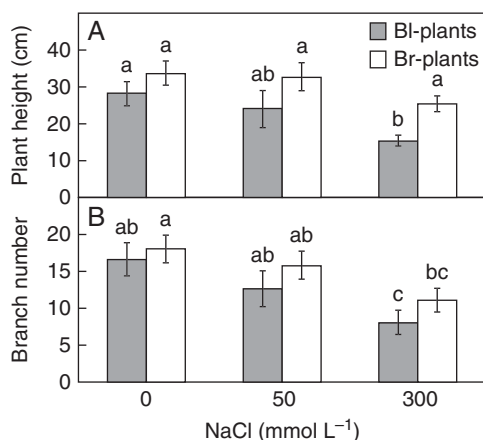
FIG. 6. Effect of salt stress on (A) plant height and (B) branch number of *Chenopodium album* plants derived from heteromorphic seeds. The growth characters were recorded after plants were treated with salinity for 3 months. Values are means ± s.e. from ten plants per treatment. Different letters above the bars indicate significant differences ($P <$ 0·05). Bl-plants, Plants grown from black seeds; Br-plants, plants grown from brown seeds.

also observed with the branch number, although no significant difference was shown (Fig. 6B; plant type in two-way ANOVA, $P >$ 0·05).

Seed proliferation. The seeds set on plants from the dimorphic seeds matured and began shedding 5 months after being sown. Seeds were collected upon the first seed maturity. The number of the two types of seeds did not differ significantly on plants from the two morphs at the beginning of maturation at any of the NaCl concentrations applied during plant growth (Fig. 7). After the first 30 d, the pattern of production of the dimorphic seeds apparently changed. In the 0 or 50 mmol L^{-1} NaCl treatment, plants from the two morphs produced black seeds more quickly than brown seeds, although only plants from brown seeds showed a significant difference (Fig. 7A, B, D and E; time × seed type interaction in two-way ANOVA, Br-plants: 0 mmol L^{-1}, $P <$ 0·0001; 50 mmol L^{-1}, $P <$ 0·0001). However, when under 300 mmol L^{-1} NaCl stress, plants from two morphs produced black seeds much more slowly than brown seeds (Fig. 7C and F; time × seed type interaction in two-way ANOVA, Bl-plants: $P <$ 0·01; Br-plants: $P <$ 0·0001).

The output of total seeds was higher for plants from brown seeds than from black seeds grown under various NaCl concentrations. The highest total number of seeds ($>$500) was observed for the plants from brown seeds at 50 mmol L^{-1} NaCl, the lowest ($<$200) for the plants from black seeds at 300 mmol L^{-1} NaCl. A significant difference in total seeds between two types of plants was observed at 300 mmol L^{-1} (Fig. 7; unpaired *t*-test, $P <$ 0·05). Furthermore, plants from the two morphs produced $>$70 % of brown seeds under 300 mmol L^{-1} NaCl treatment, compared with $<$50 % of brown seeds for 0 or 50 mmol L^{-1} NaCl (Fig. 8; NaCl concentration in two-way ANOVA: $P <$ 0·0001). Plants from black seeds produced more brown seeds than plants from brown seeds, but a significant difference was only observed at 50 mmol L^{-1} NaCl (unpaired *t*-test, $P <$ 0·05), suggesting that the ratio of brown seeds in total was promoted significantly by high salinity within both plant types from dimorphic seeds.

DISCUSSION

Many plant species inhabiting unpredictable and stressful environments have been reported to exhibit seed heteromorphism (dimorphism), which enables germination to occur at the right time in a favourable place to ensure population establishment and succession (Venable, 1985; Imbert, 2002). *Chenopodium album* is a serious weed species with seed heteromorphism, which is widely distributed all over the world (Williams and Harper, 1965; Ahmed, 1987), including the semi-arid areas in Xinjiang province, China (Commissione Redactorum Florae Xinjiangensis, 1994; Yao *et al.*, 2010), and has been observed as a salt-tolerant species (Hamidov *et al.*, 2007). The present study has shown that *C. album* grown in semi-arid areas produced two distinct types of seeds, black and brown, which differ in morphology, germination behaviour, dormancy and salinity tolerance, and these characteristics varied between populations. Furthermore, higher salinity could lead to variation in seed heteromorphism within plants of either morph. Salinity stress is suggested as one of the environmental factors that may induce the variation
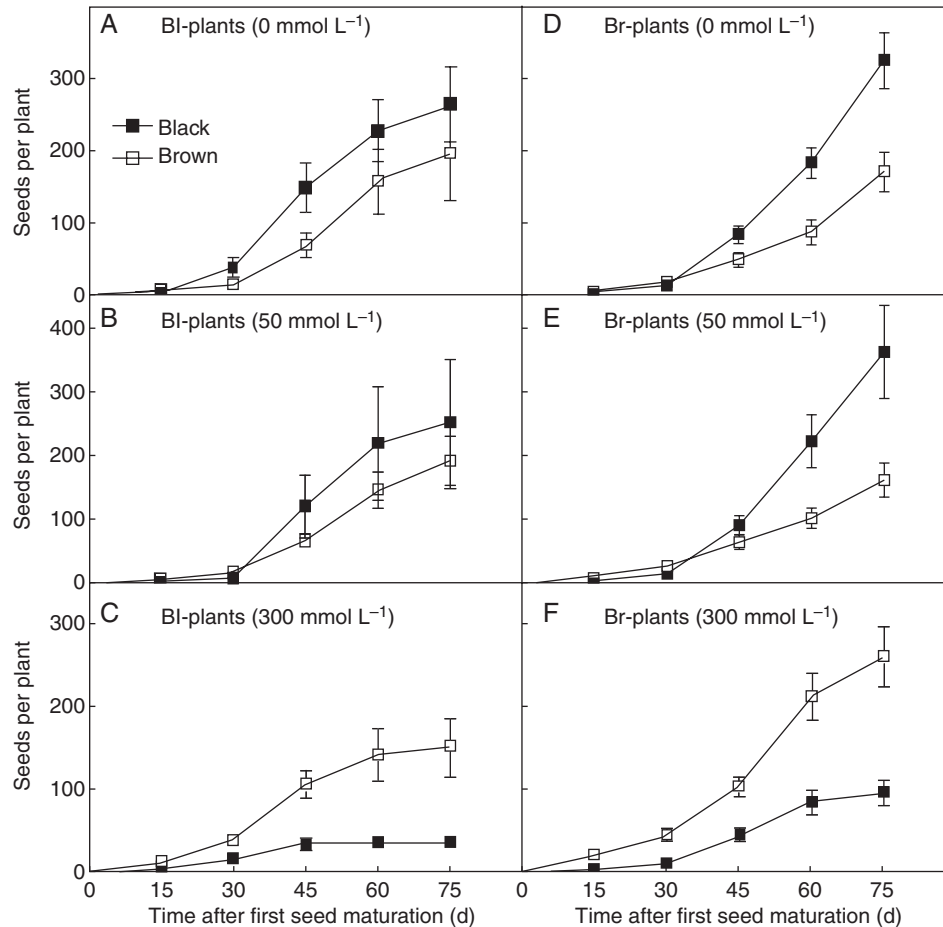
FIG. 7. Effect of salinity on dimorphic seeds setting of each plant derived from black and brown seeds of *Chenopodium album*, respectively. Bl-plants, Plants grown from black seeds; Br-plants, plants grown from brown seeds. Values are means ± s.e. from ten plants per treatment.
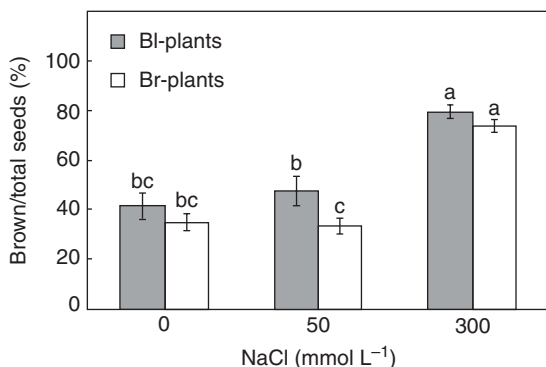

FIG. 8. Effect of salinity on brown : total seeds ratio of *Chenopodium album*. Values are means ± s.e. from ten plants per treatment. Different letters above the bars indicate significant differences ($P < 0.05$). Bl-plants, Plants grown from black seeds; Br-plants, plants grown from brown seeds.

in seed heteromorphism of *C. album* in semi-arid and light-saline areas seen in the present study.

Different populations within heteromorphic species may face diverse environmental conditions in heterogeneous areas (Imbert, 2002), therefore it is unlikely that seed heteromorphism (reflected as either hetero-seeds ratio or differences in

germination of hetero-seeds) would remain stable among populations (Venable *et al.*, 1987; Van Molken *et al.*, 2005). Indeed there is evidence indicating the possibility of variation in seed heteromorphism among populations, partially as the result of changing environments (Dakshini and Aggarwal, 1974; Brown and Mitchell, 1984). In addition, the proportion of larger seeds might increase in response to more stressed conditions in some species, e.g. *Atriplex triangularis* (Ungar, 1987) and *Suaeda salsa* (Song *et al.*, 2008). The above observations support the hypothesis that differences in environmental conditions (e.g. soil moisture, consequently salinity, etc.) might be responsible for the variation of the proportion of heteromorphic seeds in *C. album*. In the present study, variation in seed heteromorphism (e.g. hetero-seeds ratio, varying of the difference in germination of hetero-seeds) of *C. album* between two populations revealed that the proportion of brown seeds was higher in population 1 (about 65 %) than in population 2 (about 52 %), and also significantly higher than those populations in a previous report (approx. 3 %) (Williams and Harper, 1965). The two populations had grown up in different micro-habitats, flat ground for population 1 and relatively sloping ground for population 2. The two micro-habitats were not far apart and differences in illumination and temperature were unlikely to exist in the two

environments. However, the soil moisture might differ, possibly leading to differences in the salinity in the upper layer of the soil in the summer, since evaporation under strong sunlight and high temperatures in semi-arid areas cause a dramatic increase in salinity of the soil surface by capillary movement (Khan and Ungar, 1996; Khan *et al.*, 2001; Song *et al.*, 2005). Brown seeds seem to be more advantageous in harsh and unpredictable conditions than black seeds (Imbert, 2002; Khan *et al.*, 2004; Song *et al.*, 2008), thus production of a high proportion of the brown morph may have an adaptive value in semi-arid environments. Apart from the hetero-seeds ratio, in the present study, variation of the heteromorphism was also exhibited in seed coat thickness and hardness, seed reserve mass, germination and dormancy behaviour, etc., with the black seeds in two populations.

Much attention has been paid to the mechanisms of germination in heteromorphic seeds, and the phenotypic differences have been considered as the main reasons for the diversity of germination behaviour (Imbert, 2002). In the present study, two populations were examined to obtain more information on the germination differences between two seed types of *C. album*. Brown seeds in both populations had thinner, softer testae than the black seeds and germinated to approx. 100 %. Differences in the germination of the black seeds in the two populations were not significantly related to differences in the colour and thickness of seed coat. However, the seed coat of the black from population 2 was much harder than that from population 1, and the improved germination following partial removal, suggested that the hardness, as well as the thickness, of the seed coat might prevent the seed from germinating, either as a result of mechanical resistance to radicle protrusion, or due to impermeability to water (Mohamed-Yasseen *et al.*, 1994; Debeaujon *et al.*, 2000). The higher seed reserve mass (i.e. larger seed size) and higher protein content of brown seeds suggested that more energy might be available for radicle to break the seed coat in the brown seeds, and contribute to the difference in the germination for the black seeds in the two populations. This was consistent with the higher germination of larger seeds in some heteromorphic species (Venable *et al.*, 1987; Katembe *et al.*, 1998; Van Molken *et al.*, 2005). Taken together, the thickness and hardness of the seed coat, combining with seed reserve mass and protein content may contribute to the variation in the germination of heteromorphic seeds in *C. album*.

In the present study, the brown and black seeds from population 1 were non-dormant and maintained a high germination percentage over the whole year; while a large proportion of the black seeds from population 2 were dormant, and exhibited an annual fluctuating pattern of germination with the highest germination percentage (>50 %) in April. A similar fluctuating germination pattern was observed for peripheral achenes of *Leontodon saxatilis* (Brändel, 2007), seeds of desert species *Mesembryanthemum nodiflorum* (Gutterman, 1980/1981). This might suggest that the germination of black seeds of *C. album* fluctuated as a response to the changes of environmental conditions, which might confer a selective advantage in adaptation to environments (Venable *et al.*, 1987; Carter and Ungar, 2003). In addition, the changes in the dormancy of black seeds over 1 year may extend the timing of germination and help to maintain the population (Wang *et al.*, 2008).

Seed germination under salinity in the present study suggested that the brown seeds of *C. album* were more salt tolerant than the black seeds. Similar cases have been reported in several halophytic chenopods having heteromorphic seeds, with brown and black seed types exhibiting different salt tolerance, as in *Atriplex centralasiatica* (Li *et al.*, 2008), *Suaeda aralocaspica* (Wang *et al.*, 2008), *S. salsa* (Li *et al.*, 2005) and *S. splendens* (Redondo-Gómez *et al.*, 2008). As far as we know, so far the reason why heteromorphic seeds display different salt tolerances is mostly unknown. We speculate that the more salt-tolerant nature of brown seeds may relate to their larger size and higher protein content.

The present study provided little evidence of differences in the growth characteristics of the plants from heteromorphic seeds. However, when the two types of plants were subjected to salinity stress, significant differences were exhibited for plant height and total seed output at 300 mmol L^{-1} NaCl treatment. The results suggested that higher salinity might enlarge the developmental differences between the two types of plants. Similarly, plants from brown and black seeds of *S. splendens* responded to salinity stress differently at a lower concentration (Redondo-Gómez *et al.*, 2008). However, further research is needed to elucidate the mechanism affecting their performance under salt stress.

Seed heteromorphism is thought to be a selective advantage for plant species inhabiting harsh and heterogeneous areas and to enable such species to allocate limited resources to different seed types in response to variable environments (Harper, 1977). However, insufficient information is available regarding the changes of morphs ratio per inflorescence (plant) and variation in environmental conditions. In the halophyte *S. salsa*, a population from saline inland areas produced fewer (70 %) brown seeds than a population from a higher salinity environment (81 %) (Song *et al.*, 2008). Similarly in *A. triangularis*, the ratio of large seeds versus small seeds was 1 : 60 in a population from less saline areas, increasing to 1 : 6 in populations from habitats of much higher salinity (Ungar, 1987). In the present study, high salinity treatment (300 mmol L^{-1} NaCl) increased the ratio of brown seeds to >70 %, significantly higher than that following treatment with 50 or 0 mmol L^{-1} NaCl (<50 % brown seeds), suggesting that salt stress may induce the plant to produce more brown seeds. There are however, many other environmental factors, such as high temperature, strong sunlight, shortage of water, that constitute the environmental stresses in semi-arid areas of Xinjiang (Song *et al.*, 2005) and significant changes in any of them might influence the morphs ratio, such as in the populations 1 and 2 in the present study. Salinity may therefore be only one of the factors influencing the proportion of black and brown seeds. However, producing more, larger, brown seeds with high germination percentage and greater salt tolerance, would undoubtedly be an advantageous strategy for plants with heteromorphism to respond and adapt to the stressful environments during the long term of evolution.

In conclusion, the present study demonstrated that the heteromorphic seeds of *C. album* differed in phenotypic characters, germination behaviour and salinity tolerance; variation of seed heteromorphism was also observed between populations, and such variation might partially be attributed to the salinity in environments. Based on this pattern, we
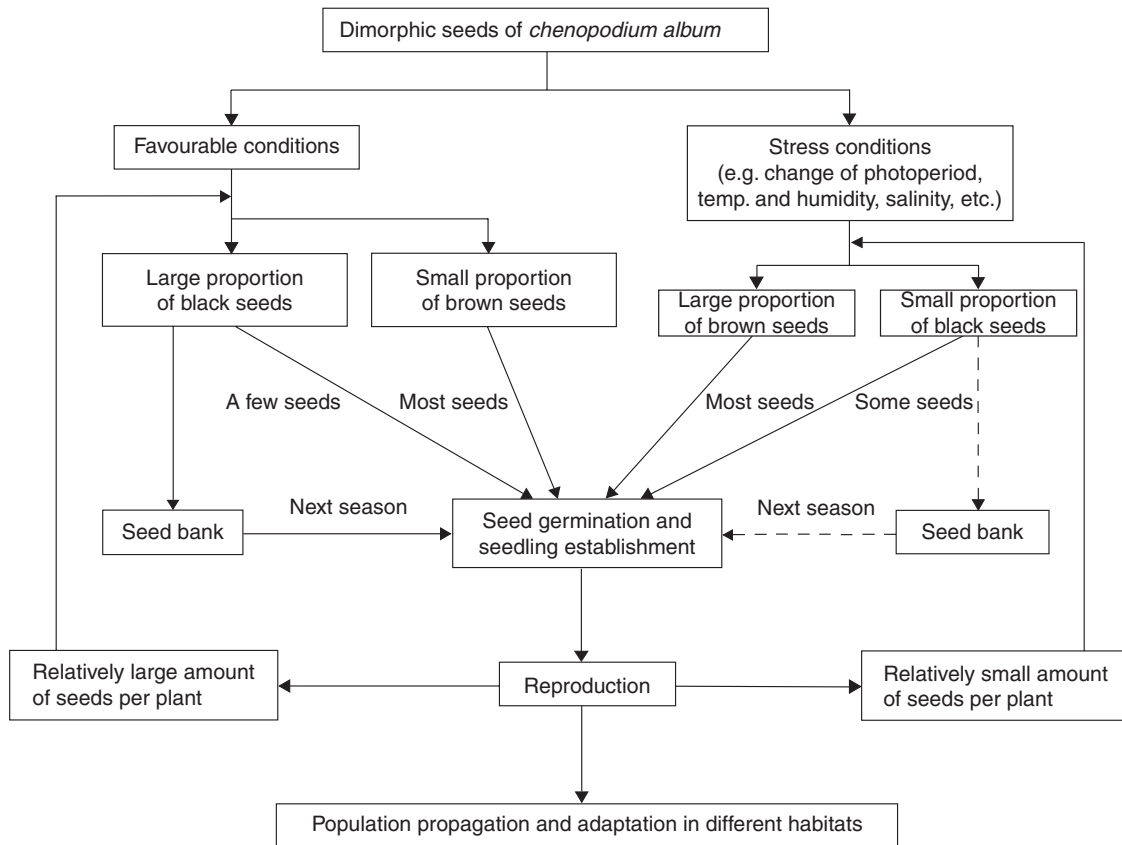
FIG. 9. Proposed model of dynamics of reproduction for dimorphic seeds of *Chenopodium album* under various environmental conditions.

propose a model for the cycle of heteromorphic seeds of *C. album* in the semi-arid areas as shown in Fig. 9. In favourable conditions, *C. album* produces a large amount of seeds and a greater proportion of black seeds. Some black seeds are expected to enter the seed bank and maintain a presence in environments to ensure population succession from one year to the next. In saline and other stressful environments, a smaller number of seeds and a greater proportion of brown seeds would be produced, the immediate germination of which may ensure the establishment and expansion of a population, to occupy limited resources which are transient in semi-arid areas (Ross and Harper, 1972; Song *et al.*, 2005). However, seedlings that develop from brown seeds run a higher risk of mortality because of unpredictable environmental stress occurring in early growing seasons (Li *et al.*, 2005). Therefore, the germination of the brown seeds in *C. album* may follow a 'high-risk' strategy, while the black seeds seem to take a 'low-risk' strategy, as described by Venable (1985). The combination of two opposing strategies in *C. album* could undoubtedly contribute to its seedling survival and population succession in semi-arid and harsh areas in Xinjiang.

ACKNOWLEDGEMENTS

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