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Research Note

Seed viability, germination and dormancy of *Nitraria roborowskii* (Nitrariaceae)

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Abstract

Nitraria roborowskii seeds were collected from plants in the desert of north-west China. Seed viability, germination response to temperature and dormancy characteristics were investigated. The tested seeds showed approximately 73% viability. The best values for germination percentage (GP), germination index and mean germination time were observed at 30°C. The sub-optimum temperatures for germination were 35°C, 20/30°C and 25/35°C. Temperatures below 25°C were unfavourable for seed germination. *N. roborowskii* seeds showed widely dispersed germination patterns. The highest GP occurred after 13 days of incubation at 30°C. The following parameters were recorded: thousand-seed weight, 44.96 g; drupe length, 7.08 mm; drupe width, 3.57 mm; putamen thickness, 0.95 mm; and pit number, 11.27 pits per drupe.

Experimental and discussion

Nitraria (Nitrariaceae) is an important component of the flora in the cold desert of north-west China. This genus has high tolerance to drought and salt, is well-adapted to sandy soil and thrives in dunes (Zeng *et al.*, 2014). Shoots and leaves of these plants have high forage value for herbivores, such as camels and sheep. Fruits (figure 1A) from *Nitraria* have high edible and pharmaceutical value (Zeng *et al.*, 2009). Local people drink the fruit juice and eat the mature fruit. *Nitraria roborowskii* Kom. is found in Russia, Mongolia, China and Kazakhstan (Pan *et al.*, 1999). *N. roborowskii* in China is mainly grown in the north-west provinces (Liu and Zhou, 2008).

Nitraria roborowskii is considered a good candidate for cultivation because it has the largest fruit and widest leaf in the *Nitraria* genus. Studies have reported two intraspecific differentiations in *N. roborowskii*, namely, male-fertile and male-sterile (Dou *et al.*, 2011). More sterile individuals were observed in populations that were seriously disturbed by human activities. The total resource of the *Nitraria* genus is recently shrinking because of environmental degradation and excess utilisation (Zhang *et al.*, 2013). Thus, this genus

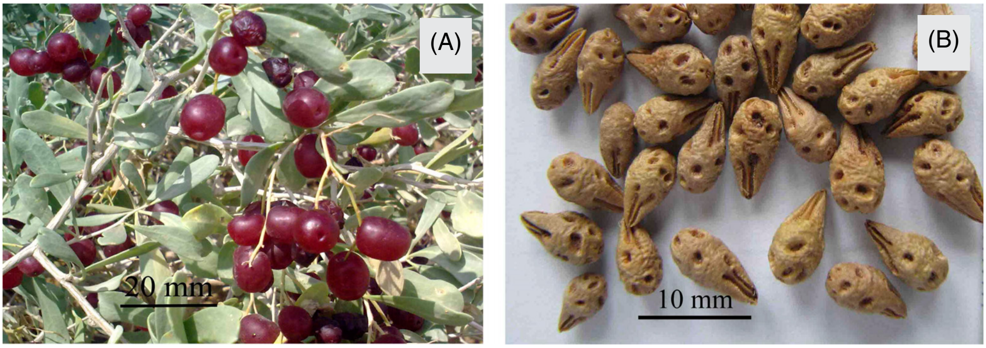

Figure 1. Ripening fruits (**A**) and drupes (**B**) of *Nitraria roborowskii*.

has been the focus of considerable attention. Studies on *N. roborowskii* have reported its reproductive allocation at the flowering stage (Li *et al.*, 2012), osmotic adjustment to salt stress (Wu *et al.*, 2012), salt tolerance (Zuo *et al.*, 2013), morphological and structural characteristics, and ecological adaptabilities (Zhang *et al.*, 2013), physiological and biochemical properties, and ultra-structure of the male sterile line (Dou *et al.*, 2013), spatial distribution patterns (Chu *et al.*, 2014) and phylogeographical patterns (Su *et al.*, 2016). However, its seed ecology is rarely investigated (Zeng and Wang, 2009).

Drupes of *N. roborowskii* have hard, bony endocarps (putamens) (figure 1B) and are difficult to germinate (Zhang *et al.*, 2013). Information on the seed viability, germination and dormancy of *N. roborowskii* is scarce. Thus, the seed germination test for this species frequently fails. This study investigated the seed viability, germination responses to temperature and dormancy characteristic of *N. roborowskii*. In particular, the optimum temperature for germination was determined to facilitate germination testing and raising of seedlings. Seed drupe traits and thousand-seed weight (TSW) were also investigated.

Seeds tested in this study were collected from plants in Mimqin County (125 mm average annual precipitation), Gansu Province north-west China, on 26 September 2009, when the fruits were dark red. The seed collection site was located at 103° 11' 42.4" E and 38° 52' 41.3" N, at 1,258 m a.s.l. The harvested fruits were sun-dried, mixed thoroughly and then stored in a closed seed chamber (38-45% relative humidity, 3-6°C) for 15 months before the commencement of experiments. Seed samples used in all experiments were drawn randomly from the stored dry fruits.

Seed mass is an extremely important trait in establishing a plant species. Colonisation theory predicts that large-seeded species are more likely to exhibit increased seedling recruitment in response to seed addition than small-seeded species. Thus, identifying the seed mass of a species significantly increases the predictability of its response to seed supplementation (Moles and Westoby, 2002, 2006). In addition, drupe mass and traits of endocarp of *Nitraria* species has high reference value in taxonomy and seed ecology (Pan *et al.*, 1999).

The TSW (drupes without sarcocarp) and drupe traits were measured using fully air-dried 30-day drupes. Four replications of 100 drupes were weighed and TSW determined to be 44.96 ± 0.87 g. Thirty drupes were measured: drupe length was 7.08 ± 0.13 mm;

drupe width, 3.57 ± 0.06 mm; putamen thickness, 0.95 ± 0.02 mm; and pit number, 11.27 ± 0.58 pits. In our previous work on its congeneric species, TSWs of *N. tangutorum* and *N. sibirica* were 27.03 and 12.70 g, respectively. The corresponding drupe lengths were 6.00 and 4.43 mm, and the corresponding drupe widths were 3.11 and 2.46 mm (Zeng *et al.*, 2010). *N. roborowskii* has evidently larger seed mass and size than the two other species. Thus, *N. roborowskii* should have better response to seed propagation than *N. tangutorum* Bobr. and *N. sibirica* Pall.

The putamen thickness and pit number (pits serve as water gap) of *N. roborowskii* were 0.95 mm and 11.27 pits, respectively. A previous study showed that the corresponding putamen thickness and pit number for *N. tangutorum* were 0.95 mm and 10.43 pits per drupe, respectively, whereas those for *N. sibirica* were 0.46 mm and 13.50 pits per drupe (Zeng *et al.*, 2010). The seeds of *N. roborowskii* and *N. tangutorum* evidently share some similar endocarp traits. However, these seeds significantly differ from *N. sibirica*, which might partly explain why the seeds of *N. roborowskii* and *N. tangutorum* showed slow and widely dispersed germination patterns (see below) compared with *N. sibirica*. Thicker bony endocarp provides better protection to the embryo than a thin bony endocarp.

The viability test was performed according to the tetrazolium testing method developed for *N. tangutorum* (Zeng *et al.*, 2009). Four independent replicates of 100 seeds were drawn at random. The percentages of viable, non-viable and empty drupes were 73, 14 and 13%, respectively. The viability of seeds of *N. tangutorum* and *N. sibirica* were 85 and 92%, respectively (Zeng *et al.*, 2009). However, *N. roborowskii* should not be presumed to produce a lower proportion of viable seeds than its congeners based on such a limited data set.

Dry drupes used in the experiment were soaked in water at room temperature for 12 hours. The sarcocarp was washed away, and the drupes were re-dried at room temperature for one week before commencement of the test. The germination experiment was performed under five constant temperatures (15, 20, 25, 30 and 35°C) and three alternating temperatures (15/25°C, 20/30°C and 25/35°C). Four replicates of 50 seeds were germinated between two layers of paper moistened with 6 mL distilled water in 90 mm-diameter Petri dishes. The seeds were incubated in the dark but were exposed to natural room light for a few minutes each day when checked for germination and when water was added. Radical emergence (≥ 3 mm) was the criterion for germination. Germinated seeds were removed from the test at any of the intermediate counts. Seeds were incubated for 29 days. The fresh and dead seeds were evaluated at the end of the germination test according to ISTA (1999). Then, the viability of the fresh seeds was tested by the tetrazolium testing method.

The results of the germination test are the averages of four 50-seed replicates and expressed as germination percentage (GP) on days after seeds were placed on substrates, germination index (GI) and mean germination time (MGT). GP is expressed as $(A/C) \times 100$, where A is the number of seeds that germinated and C is the number of seeds incubated. GI is calculated as $\sum (Gt / Dt)$, where Gt is the number of seeds germinated at t days and Dt denotes the number of days from the beginning of the test. MGT = $(\sum n_i \cdot t_i) / N$, where n_i is the number of seeds germinated in t_i days from the beginning of the test and N is the total number of germinated seeds at the end of the test (Naylor, 1981).

Non-germinated viable seeds at the end of the germination test were deemed as dormant seeds. Thus, dormant seed percentage (DSP) is expressed as $[D/(G+D)] \times 100$, where D is the number of viable seeds that did not germinate and G is the total number of germinated seeds.

The experimental data (without transformation) were analysed using SSPS 13.0 for Windows (SPSS Inc., Chicago, IL, USA) through one-way ANOVA. The means were then compared at 5% probability level using the Duncan post hoc test when the data met the assumptions of the analysis.

The highest GPs in the germination test were observed at 30 and 35°C, 20/30°C and 25/35°C after 29 days of incubation, with 59 to 67% germination and 9 to 13% fresh seeds that did not germinate. Moderately high germination was observed at 25°C and 15/25°C. Lower germination rates were observed at 15 and 20°C, with only 3 to 33% germination. The highest proportions of fresh seeds (FS) and dead seeds (DS) were observed at 15°C (figure 2A). Generally, *N. roborowskii* seeds showed widely dispersed germination patterns. The highest GP occurred after 13 days of incubation at 30°C (data not shown).

The highest GI was observed at 30°C. Moderately high GIs were observed at 25 and 35°C, 15/25°C, 20/30°C and 25/35°C. Lower GIs were observed at 15 and 20°C. The shortest MGT was observed six days after the beginning of the test at 30°C. Moderate MGTs were observed at 11 to 13 days at 25 and 35°C, 20/30°C and 25/35°C. The longest MGTs were observed at 15 and 26 days at 20 and 15°C, respectively (figure 2B).

Among the four constant temperatures, the highest DSP occurred at 15°C, with 96% dormant viable seeds. The lowest DSP was observed at 30 and 35°C, with 12 to 16% dormant seeds. DSP values (approximately 13 to 27%) were low among the three alternating temperatures, but a high value was observed at 15/25°C (figure 2C).

Temperature is the most important environmental variable responsible for the synchronisation of germination with conditions suitable for establishing seedlings (Probert, 2000). The germinated seedling thrives or suffers depending on the favourability of environmental conditions. From present study, the optimum and sub-optimum temperatures for the germination of *N. roborowskii* seeds were 30 and 35°C, 20/30°C and 25/35°C. These regimes evidently synchronise seed germination of *N. roborowskii* with the rainy season of its habitat. This season is characterised with high day temperature and low night temperature. Germination during this season will increase the survival probability of the juveniles of tested species.

The dormancy of seeds refers to the absence of the capacity of viable seeds to germinate in a specified period of time under any combination of normal physical environmental factors (Baskin and Baskin, 2004). The DSP of tested seeds were calculated to be 12-96% under darkness within the range of 15 to 35°C constant temperatures after 29 days of incubation and 13-27% for the alternating temperature regimes 15/25°C to 25/35°C. These results showed that some of the tested seeds have the dormancy characteristic. A previous study reported that seeds of *N. roborowskii* were difficult to germinate, implying that these seeds have the dormancy characteristic (Zhang *et al.*, 2013).

A comprehensive literature survey by Baskin and Baskin (2014) indicates that within *Nitraria*, some species have both non-dormant and physiologically dormant seeds (e.g. *N. tangutorum*) and others have physiologically dormant (PD) seeds (e.g. *N. billardierei*

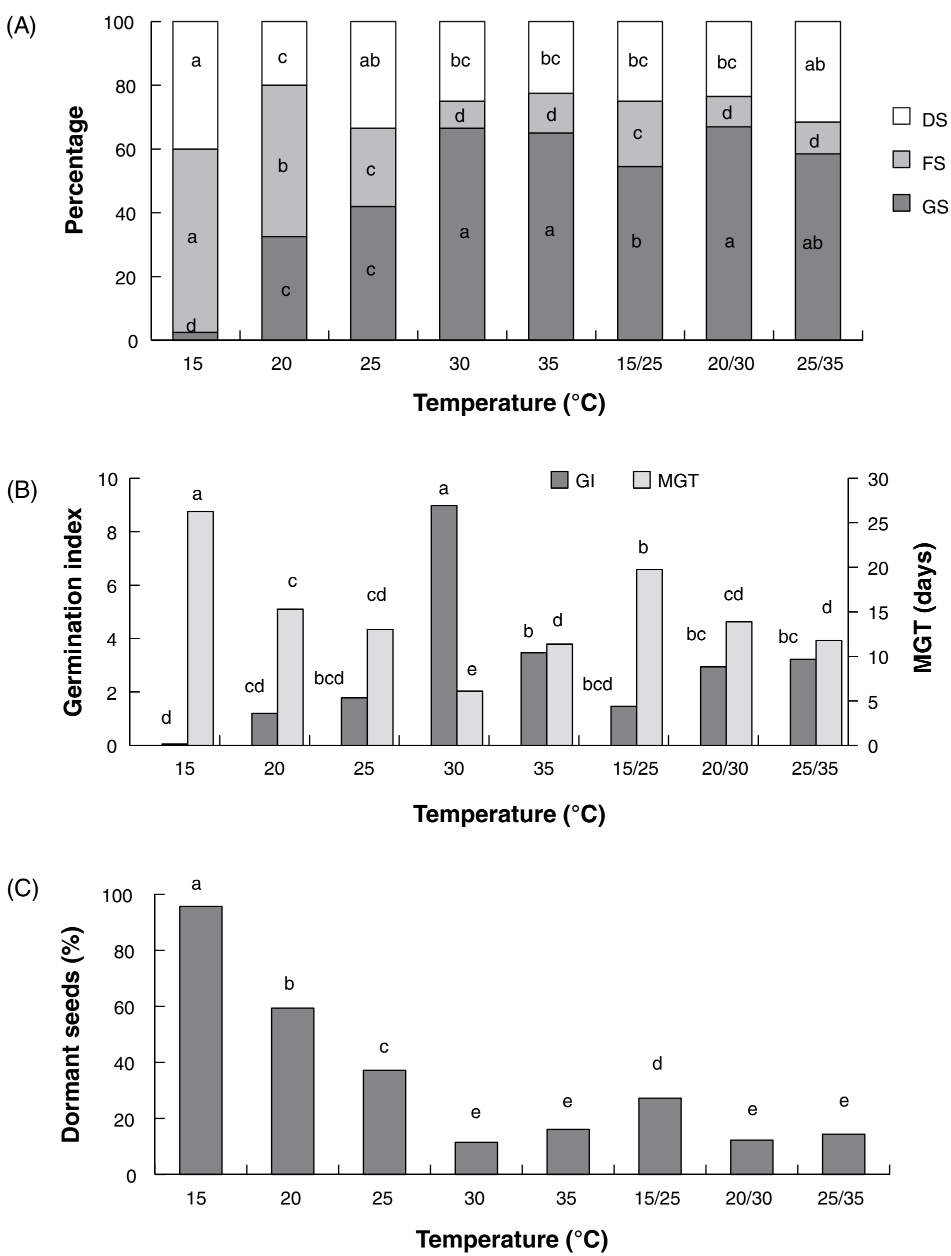

Figure 2. Comparisons of the proportions of (**A**) germinated seed (GS), fresh seed (FS), dead seed (DS) percentages; (**B**) germination index (GI) and mean germination time (MGT); and (**C**) dormant seeds at different temperatures, after 29 days incubation for *Nitraria roborowskii*. Values with different lower case letters for the same parameter are significantly different (Duncan, $P < 0.05$, $N = 4$).

DC., *N. praevisa* Bobr., *N. sibirica*, *N. sphaerocarpa* Maxim.). In the case of present study, seeds were stored for 15 months at low temperature in dry conditions that might have played some role to break PD, if any. Anyways, it is premature to conclude PD in *N. roborowskii* without testing germination and dormancy patterns for fresh mature seeds.

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