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### Short communication

## Germination characteristics of early successional annual species after severe drought in the Mongolian steppe



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#### ABSTRACT

Post-drought recovery of vegetation is known to depend largely on seedling establishment of ruderal annuals. We observed the transition of dominant annuals over several years during the recovery period after a drought in the Mongolian steppe, and we hypothesized that the transition was a result of the difference in germination characteristics among those species. Germination of five major annuals in the Mongolian steppe, Bassia dasvphylla, Chenopodium acuminatum, Chenopodium album, Chenopodium aristatum, and Salsola collina, was examined at seven temperatures ranging from 5 to 35 °C under three light conditions (red, far-red light, and dark). The germination percentage was very high for S. collina irrespective of temperature and light condition. In contrast, the two Chenopodium species, C. album and C. aristatum, showed a low germination percentage irrespective of temperature and light conditions, demonstrating that these species were dormant and formed seed banks. Scarification improved the germination percentage of the two Chenopodium species; however, the improvement was mostly nullified under far-red light and dark conditions in C. album. These germination characteristics contributed to the transition of dominant annuals after the drought. Two Chenopodium annuals that were highly dormant and formed seed banks emerged in large numbers during the first year after the drought, and S. collina with high germinability and no seed banks subsequently succeeded the Chenopodium annuals. We concluded that the germination characteristics of the annuals were a key factor driving vegetation dynamics during early recovery after the drought.

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#### 1. Introduction

A drought, defined climatologically as a rainfall deficit, is a naturally occurring disturbance that can seriously damage vegetation in arid grasslands (Albertson and Weaver, 1944; Haddad et al., 2002). Subsequent to the drought, when precipitation is adequate, vegetation starts recovering naturally (Anderson and Inouye, 2001; Tilman and El Haddi, 1992). Post-drought recovery of vegetation is known to depend largely on seedling establishment of ruderal species, mainly annuals (Coupland, 1958; Rosen, 1995; Shinoda et al., 2010). Thus, the composition and productivity of annual communities are important factors determining grassland productivity after the drought.

The Mongolian steppe, located in northeast Asia, is mostly utilized as pastureland by nomadic herders (Nachinshonhor, 2013), and therefore, a sudden reduction in grassland productivity due to

\* Corresponding author. E-mail address: kinugasa@muses.tottori-u.ac.jp (T. Kinugasa). natural disturbances, such as drought, can damage livestock farming in this area (Begzsuren et al., 2004). To facilitate greater stability in livestock farming in this region, an understanding of the process and mechanism of grassland recovery after disturbances is necessary. The Mongolian steppe suffered a severe drought during 2007, during which aboveground biomass decreased to approximately half of that of average years (Nandintsetseg and Shinoda, 2013). We investigated the recovery of vegetation after the 2007 drought in central Mongolia (Kinugasa et al., 2012). Following normal precipitation, the grassland productivity recovered rapidly from less than 5 g m<sup>-2</sup> in 2007 to more than 100 g m<sup>-2</sup> in 2008 due to the emergence of an annual community consisting mainly of two Chenopodiaceae species: Chenopodium album and Chenopodium aristatum. The annual community continued to dominate the vegetation for several years after the drought, and during that period, the dominant annuals shifted from the two Chenopodium annuals to S. collina, although the mechanisms causing the shift remain unclear.

This shift in dominant annuals after the drought may be



ascribed to species-specific germination characteristics. Interannual changes in species composition were observed in a Mediterranean annual pasture; these were attributed to species-specific germination characteristics in combination with inter-annual variation of weather conditions (Espigares and Peco, 1993). However, the inter-specific differences in germination characteristics of annuals in the Mongolian steppe have not been examined. Moreover, the contribution of species-specific germination characteristics to vegetation dynamics during the recovery after the drought has received little attention.

In the current study, we investigated the germination characteristics of annual species in the Mongolian steppe, and examined their contribution to the vegetation dynamics of the annual community that has been observed in the early recovery after the drought. Germination was tested under different light and temperature condition with adequate water availability to evaluate the germination potential under normal precipitation subsequent to the drought. Considering that seed banks in the Mongolian steppe are mostly occupied by Chenopodium annuals (Kinugasa and Oda, 2014), we hypothesized the following germination characteristics which could be the cause of observed vegetation dynamics: 1) seeds of the Chenopodium annuals are highly dormant and lightdemanding at germination, therefore, they form seed banks and emerged in large numbers during the earliest stage of recovery when vegetation is sparse and; 2) seeds of S. collina are highly germinable irrespective of the light environment, therefore, this species becomes dominant by succeeding Chenopodium annuals.

#### 2. Materials and methods

#### 2.1. Seed collection

Five Chenopodiaceae annual species, *Bassia dasyphylla*, *Chenopodium acuminatum*, *C. album*, *C. aristatum*, and *S. collina* were used in the germination tests. These species are common in central Mongolia, although the emergence of *C. acuminatum* and *B. dasyphylla* is relatively sparse. During September 2011, seeds of the aforementioned species were randomly collected from several populations growing on semi-arid grassland near Bayan-Unjuul (47°02.77′N, 105°57.08′E), about 130 km southwest of Ulaanbaatar, the capital of Mongolia. Collected seeds were air-dried and stored in paper bags at 5 °C in a refrigerator until the germination tests were performed.

#### 2.2. Germination test 1

During 2012, germination tests were conducted in a temperature gradient incubator (TG-300CCFL-3LE, EYELA, Tokyo, Japan) using seven temperature treatments (5, 10, 15, 20, 25, 30, and 35 °C), under two continuous light treatments, red (R) and far-red light (FR), and under dark conditions. The R and FR treatments were facilitated by an LED lamp (EP30-A0E0-00, 660 nm; EP30-A0F0-00, 740 nm, EDISON, Taipei, Taiwan).

Before the tests, seeds were sterilized by soaking in 80% ethanol for a minute and rigorously rinsed using distilled water. As *B. dasyphylla, C. acuminatum, C. album*, and *C. aristatum* produce dimorphic seeds of different colors, germination tests were performed separately for seeds according to each color. Two 5 cm Petri dishes filled with 0.75% (w/v) agar (gelling temperature was 30-31 °C, Nacalai Tesque, Japan) were prepared for each treatment in each species, and 50 seeds were sown in each. The numbers of germinated seeds were counted every day in a dark room using a green safe light so as to avoiding induction of germination. Germination was defined as the time when radicles first appear, and germinated seeds were discarded after counting. Germination tests were continued until new germination was not observed for five consecutive days. After the germination tests, the viability of ungerminated seeds was tested by the triphenyl tetrazolium chloride (TTC) staining method. Seeds were cut to expose the embryo, and then soaked in 0.1% (w/v) TTC solution (2,3,5-triphenyltetrazolium chloride, Wako, Japan) under darkness at 30 °C for 24 h. Embryos that turned red were classified as viable.

The germination percentage was calculated by dividing the number of germinated seeds by the sum of germinated seeds and ungerminated viable seeds. In the case of the species with dimorphic seeds of variable colors, overall germination percentage was calculated considering the proportion of each heteromorphic seed against the collected seeds. The proportion of each heteromorphic seed against the collected seeds was determined four times by choosing 50 seeds randomly and calculating the average proportion.

#### 2.3. Germination test 2

Germination tests were conducted at 25 °C after scarification by soaking in 95% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). Germination tests were conducted for the seeds of species exhibiting low germination: the black seeds of *C. album* and *C. acuminatum* and the black and brown seeds of *C. aristatum*. We excluded the brown seeds of *C. album* from this test because they accounted for only 4% of the total seeds and it was difficult to collect an adequate amount for the test. Before the germination tests, soaking treatments were performed for 30 min on the black seeds of *C. album*, 20 min on the black seeds of *C. acuminatum*, and 1 min on the black and brown seeds of *C. aristatum*, as this soaking time has been confirmed to maximize the percentage of germination achieved of these species, in our preliminary examination. For each heteromorphic seed within each species, 50 seeds were used for the test. Other experimental procedures were the same as those in the germination test 1.

#### 2.4. Statistical analysis

Differences in germination percentages between treatments within each heteromorphic seed in each species was tested by Pearson's  $\chi^2$  test using the JMP 7 software (SAS Institute, Cary, NC, USA) followed by sequential Bonferroni correction.

#### 3. Results

Germination percentages were different between heteromorphic seeds of different colors (Fig. 1). Black seeds of *B. dasyphylla* and brown seeds of *C. acuminatum* and *C. album* showed higher germination percentages than the other heteromorphic seeds at any temperature under any light conditions, except *C. album* at 10 °C under far-red light (P < 0.05, Pearson's  $\chi^2$  test). For *C. aristatum*, germination percentages were very low for both heteromorphic seeds. No consistent effects of light condition on germination percentages were found for any species. There was no clear temperature dependency on the germination of heteromorphic seeds within any species.

Overall germination percentage was calculated for each species considering the proportion of each heteromorphic seed against total seeds (Fig. 1). As light conditions did not have a consistent effect on the germination of each heteromorphic seed in each species (Fig. 1), overall germination percentage also did not differ consistently between the light conditions (data not shown). Overall germination percentage under red light was highly variable among species (Fig. 2). *S. collina* along with *B. dasyphylla* demonstrated the highest germination percentages among studied species with more than 90% and 70% germination under any temperature,

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