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Effects of temperature, light, desiccation and cold storage on germination of *Sophora tonkinensis* (Leguminosae) seeds

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Identification of the seed storage behaviour of a target species is essential prior to seed storage so that suitable storage strategies are taken. In the present experiments, germination characteristics, desiccation, and low temperature tolerance of seeds of *Sophora tonkinensis* was studied; a traditional Chinese medicine on the edge of extinction, were investigated for the first time in attempt to interpret their storage behaviour. The results indicate that the temperature optimum for seed germination ranged from 25 to 30°C, the seed germination was non-photoblastic, the seed viability declined with the decrease of moisture content by a silica gel desiccation, and a low temperature storage at -20°C for 3 months had no significant effects on the seeds desiccated to 5.4 ± 0.1% moisture content. These results suggest an intermediate seed storage behaviour. For germplasm long-term conservation purpose, a cryopreservation investigation was proposed.

Key words: Seed, desiccation tolerance, low temperature tolerance, germination, *Sophora tonkinensis*.

INTRODUCTION

The importance of long-term storage of plant genetic resources has been well recognized. *Ex situ* conservation through long-term storage of seed is, in principle, possible for a significant proportion of higher plants. Long-term seed storage, if it is feasible, is generally considered the safest, most inexpensive and most convenient method of plant genetic resources conservation (Hong and Ellis, 1996; Linington and Pritchard, 2001). However, published information on seed storage behaviour, particularly in non-crop species, is meager in the context of the task of *ex situ* biodiversity conservation (Hong and Ellis, 1996).

For seed storage purpose, that is, plant genetic/ germplasm resources conservation, identification of the seed storage behaviour of a target species is essential. Three main categories of seed storage behaviour are now recognized: orthodox, intermediate and recalcitrant (each may be further subdivided) (Roberts, 1973;

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Ellis et al., 1990).

Desiccation tolerance and low temperature tolerance are the two crucial parameter to identify seed storage behaviour. Orthodox seeds undergo maturation desiccation during development, and are shed at a relative lower moisture content, and can be desiccated further to 1-5% moisture content without damage, and can be safely stored for more than decades at a quite low moisture content. Adversely, recalcitrant seeds do not undergo maturation desiccation during development; they are shed at a relative higher moisture content and metabolic activity, and are intolerant to desiccation and sensitive to low temperature. In general, the storage lifespan of recalcitrant seeds at room temperature is only several days to weeks (Smith and Berjak, 1995). While, the storage behaviour of intermediate seeds is between orthodox and recalcitrant seeds, being desiccation-tolerant partially without the increase of storage lifespan by desiccation, being sensitive to low temperature and cannot be conserved in conventional low temperature seed bank for a long-term conservation purpose (Ellis et al., 1990). The majority of seed plants produce orthodox seeds. While, recalcitrant and intermediate seeds mainly produce from mesic plants and tropical and subtropical trees, as well as partial tropical herbs and temperate trees, such as species of Fagaceae (Song et al., 2003).

Orthodox seeds can be maintained satisfactorily *ex situ* over the long term in appropriate environments (5±1 % water content, -18 °C low temperature) (IBPGR, 1976). By contrast, the maintenance of the viability of intermediate and recalcitrant seeds storage behaviour is problematic (Hong and Ellis, 1996). In general, medium-term storage is feasible for intermediate seeds provided the storage environment is well-defined, but short-term storage is usually the best that can be achieved with recalcitrant seeds (Hong and Ellis, 1996). For recalcitrant and intermediate seed species, at present cryopreservation is the only technique available for long-term germplasm conservation (Roberts et al., 1984; Engelmann and Engels, 2002; Hor et al., 2005; Thormann et al., 2006).

Sophora tonkinensis Gagnep (Leguminosae), growing in stone hill and limestone mountain region in Guangxi, uizhou and Yunnan province in Southwest China, as well as Northern Viet Nam, is a shrub whose roots are used as traditional Chinese medicine (Chen et al., 1994; Pharmacopoeia Commission of the Ministry of Health of the People's Republic of China, 2010). Because of excessive digging, at present the wild resources of *S. tonkinensis* is on the edge of extinction (Qin et al., 2006). Therefore, it is extremely significant and urgent to conserve *S. tonkinensis* germplasm resources *ex situ*. During domestication and breeding, it is difficult to store *S. tonkinensis* seeds for a long time (Qin et al., 2011). So far, there is a few available informations on seed storage behaviour of *S. tonkinensis*.

The purpose of the present investigation is to identify the storage behaviour and germination characteristics of *S. tonkinensis* seeds, so that suitable strategies of seed storage and germination are taken for a long-term conservation of *S. tonkinensis* germplasm resources.

MATERIALS AND METHODS

Seeds

Mature *S. tonkinensis* seeds were collected from the cultivation base (23°23'N, 105°57'E; altitude, 1090 m above sea level) in Napo county, Guangxi Zhuang Autonomous Region, China, in October, 2011. The annual mean temperature is 22.1°C. Annual mean rainfall is 1115 mm. After collection, seeds were dried at 20°C and 46% relative humidity (RH) for 72 h. When the moisture content of the seeds was 17.4 ± 0.2% (wet weight basis), seeds were used for germination, desiccation and storage experiments.

Seed viability

To evaluate the potential germinability of freshly-collected seeds, viability of seeds was assessed using a tetrazolium test (Moore, 1973). Three replicates of 50 seeds each were placed on two pieces of filter paper moistened with 5 ml of distilled water, incubated at 10°C for 16 h and then longitudinally cut into halves with a scalpel. The half-seeds were incubated in a 0.1% aqueous solution of 2, 3, 5-triphenyl tetrazolium chloride for 24 h at 30°C in the dark. Only seeds showing a strong red-stained embryo were considered viable (Meulebrouck et al., 2008) and counted.

Determination of seed moisture content

The water content of seeds was determined gravimetrically (Sershen et al., 2012). Four replicates of five seeds each were used, and the moisture content of seeds was expressed as a percentage on a wet weight basis.

Germination test

Four replicates of 50 seeds each were incubated in 9-cm-diameter Petri dishes with moist perlite (water content of perlite, 18.3 ± 0.5%) at several constant temperatures in the dark or in an alternating photoperiod (12 h light/12 h dark; PPFD, 121 μmol m^{-2} s^{-1}). Germinated seeds were counted each day, and then the cumulative germination percentages were calculated. Germination test was terminated when all seeds had germinated except rotten those or no germination was noted for five consecutive days. Radicle protrusion was used as the criterion for germination.

Desiccation and cold storage

Desiccation treatment was performed by mixing seeds with silica gel in a dryer which was placed at an ambient temperature of 20-25°C. The water loss in the seeds was monitored periodically by determining the moisture content of seeds. Seeds desiccated to about 5% moisture content were subjected to a cold storage treatment at -20°C in dark for three months.

Germination test (25°C, 12 h light/day; PPFD, 121 μmol m^{-2} s^{-1}) was executed to seeds that were desiccated to about 12 and 5% moisture content, and to those that were firstly desiccated to about 5% moisture content and followed by a three-month cold storage at -20°C.

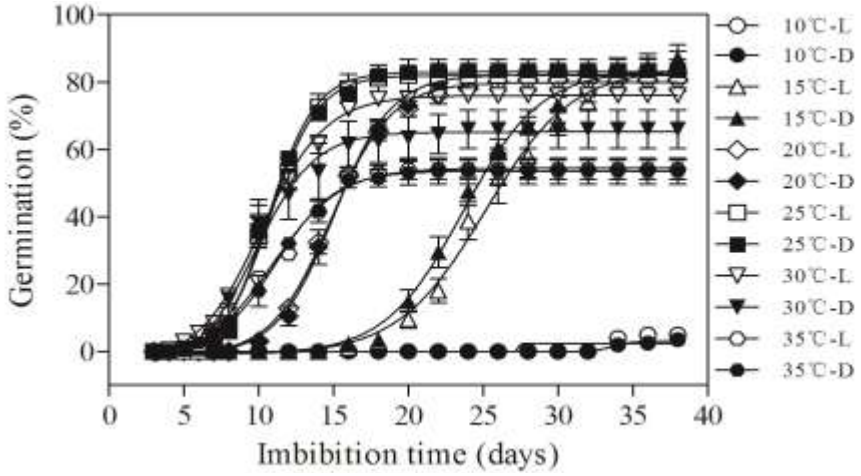

Figure 1. The time courses of germination of *Sophora tonkinensis* seeds under several combinational regimes with different constant temperatures and light. D represents dark and L represents light (12 h light/day). Values are mean ± SE of four replicates of 50 seeds each.

Data analysis

All data were analyzed with the SPSS for Windows 12.0 (SPSS, 2003). Effects of light on germination were analyzed by the independent samples *t*-test at $P = 0.05$, effects of incubation temperature on germination, germination speed and vigor of germination, and effects of desiccation and cold storage on germination were analyzed using a one-way ANOVA followed by the Student-Newman-Keuls (*S-N-K*) multiple comparisons test at $P = 0.05$. The time courses of seed germination at several constant temperature in light were performed a nonlinear regression analysis, and subsequently germination speeds were calculated by regression equations. germination speed was expressed as T_{50}, that is, the time when accumulative germination get to 50% of the final accumulative germination. To stabilize the variances, all germination data were arcsine-transformed prior to statistical analysis. All calculated values were expressed as mean ± SE (standard error).

RESULTS

Seed description and viability

The *S. tonkinensis* fruit is a legume, a pod, including one to three oval seeds. Anatomy experiments showed that a fresh mature seed included a thin black seed coat, an embryo with an axis and two expanded ivory cotyledons and no endosperm. Freshly-collected seeds were 7.7 ± 0.2 mm long, 5.9 ± 0.1 mm wide and 5.7 ± 0.1 mm thick (n = 30). The thousand seed weight (TSW) was 143.3 ± 1.0 g (n = 4). The moisture content was 17.4 ± 0.2% (n = 4). Seed viability by a TTC staining was 93.0 ± 1.3%, indicating that these seeds had a high germination potential.

Effects of light and temperature on seed germination

Light had no significant effect on seed germination at several constant temperatures ($P > 0.05$, Figure 1). In terms of final mean germination percentage, there was no significant difference between 15, 20, 25 and 30°C ($P > 0.05$), being significant higher than those at 35 and 10°C ($P < 0.05$), and the final mean germination percentage at 35°C was significant higher than those at 10°C ($P < 0.05$, Figure 1).

By a nonlinear regression analysis, mean germination speed (T_{50}) was calculated. The T_{50} at 10, 15, 20, 25, 30 and 35°C in light was 33.0 ± 0, 25.3 ± 1.0, 14.9 ± 0.1, 10.7 ± 0.2, 10.2 ± 0.4 and 11.0 ± 0.2 days, respectively. The mean germination speeds between 25, 30 and 35°C had no significant differences ($P > 0.05$), being significantly higher than those of others ($P < 0.05$, Figure 1). Vigors of germination, mean accumulative germination percentages after a 10-day incubation when the number of germinated seeds in a single day reached the maximum under most constant temperature regimes, at 10, 15, 20, 25, 30 and 35°C in 12 h/day light were 0, 0, 3.0 ± 1.3%, 34.0 ± 3.3%, 39.0 ± 5.0% and 22.0 ± 1.8%, respectively. Vigors of germination between 25 and 30°C had no significant differences ($P > 0.05$) and were significantly higher than those of others ($P < 0.05$).

Effects of desiccation and cold storage on seed germination

Initial germination of freshly-collected seeds with 17.4 ± 0.2% moisture content was 82.0 ± 4.6%. While seeds were desiccated to 8.4 ± 0.4% moisture content, the mean germination percentage significantly declined to 58.5 ± 5.6%, and 5.4 ± 0.1% moisture content, 36.0 ± 3.2% mean germination percentage ($P < 0.05$, Figure 2). A three-month cold storage at -20°C had no significant effect on seeds desiccated to 5.4 ± 0.1% moisture

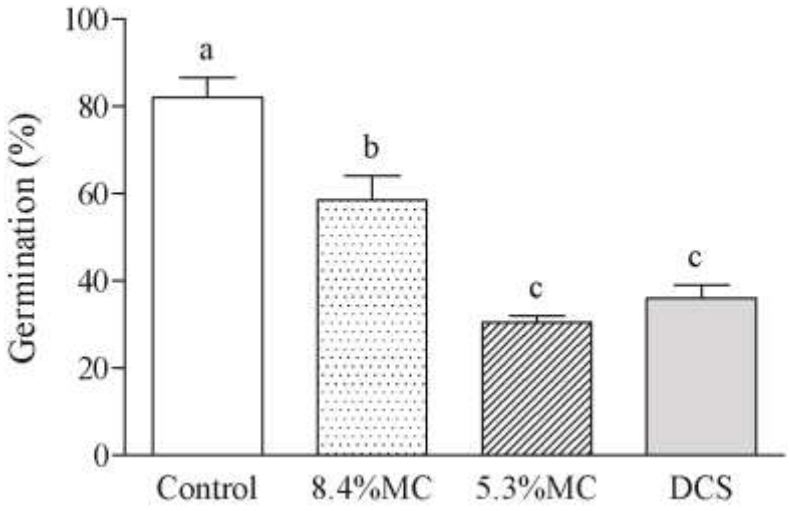

Figure 2. Effects of desiccation and cold storage on *Sophora tonkinensis* seed germination. MC, moisture content; DCS, desiccation and cold storage, that is, a desiccation by 5.4 ± 0.1% moisture content followed by a cold storage at -20°C for three months; Control represents freshly-collected seeds with 17.4 ± 0.2% moisture content. There is no significant difference between columns marked with the same lowercase letters (*S-N-K*, $P = 0.05$). Values are mean ± SE of four replicates of 50 seeds each.

content ($P > 0.05$, Figure 2).

DISCUSSION

Seeds between 30 and 13 000 g TSW may show orthodox, intermediate or recalcitrant storage behaviour. For species with recalcitrant seed storage behaviour their shedding moisture contents are distributed between 36 and 90%, for intermediate between 23 and 55%, and for orthodox between <20 and 50% (Hong and Ellis, 1996). In the present investigation, the TSW of freshly-collected *S. tonkinensis* seeds was 143.3 ± 1.0 g with 17.4±0.2% moisture content. As far as TSW and moisture content were concerned, *S. tonkinensis* seeds seemed to be orthodox.

Light is ecologically of great importance for seed germination (Bewley and Black, 1982). According to Evenari (1956), seeds are divided into three groups in terms of sensitivity of germination to white light: 1) positively photoblastic seeds whose germination is promoted by white light; 2) negatively photoblastic seeds whose germination is inhibited by white light; 3) non-photoblastic seeds whose germination is insensitive to white light. In the present investigation, white light irradiation (12 h/day) had no significant effect on seed germination under several constant temperature regimes ($P > 0.05$, Figure 1), suggesting that *S. tonkinensis* seeds might be non-photoblastic. Taking into account final mean germination percentages, mean germination speeds and vigors of germination, the temperature optimum for seed germination of *S. tonkinensis* ranged from 25 to 30°C.

Seed moisture is the most important factor in maintaining viability during storage; it is the primary control of all activities. Metabolic rates can be minimized by keeping seeds in a dry state (Bonner, 2008). Seeds that are killed by desiccation to 10-12% moisture content are likely to be recalcitrant; seeds that tolerate desiccation to about 10-12% moisture content, but whose viability is reduced when subjected to further desiccation to a lower moisture content, are likely to show intermediate seed storage behavior; seeds that tolerate desiccation (show no loss in viability) to 5% moisture content or below are likely to show orthodox seed storage behavior (Rao et al., 2006). In the present experiments, the initial germination of freshly-collected seeds was 82.0 ± 4.6% with 17.4 ± 0.2% moisture content, while desiccation treatments significantly decreased the mean germination of *S. tonkinensis* seeds, 8.4 ± 0.4% moisture content, 58.5 ± 5.6% mean germination percentage, and 5.4 ± 0.1% moisture content, 36.0 ± 3.2% mean germination percentage ($P < 0.05$, Figure 2). These results suggested that the storage behaviour of *S. tonkinensis* seeds were likely to be intermediate, according to Hong and Ellis (1996).

Low temperatures can also minimize metabolic rates of stored seeds. Meanwhile, the storage moisture content determines how low temperatures can be suitable for seed storage (Berjak and Pammenter, 2002; Bonner, 2008). In the present investigation, a cold storage at -20°C for 3 months had no significant effects on viability of seeds desiccated to 5.4 ± 0.1% moisture content ($P >$

0.05, Figure 2). According to Hong and Ellis (1996), together with desiccation tolerance, *S. tonkinensis* seeds were likely to be intermediate. So, for a purpose of long-term storage of *S. tonkinensis* seeds, an investigation of cryopreservation (liquid nitrogen conservation) is required.

According to a compendium (Hong et al., 1996), among investigated 1088 species within Leguminosae an overwhelming majority showed orthodox seed storage behaviour, and only a minority showed recalcitrant seed storage behaviour, and none showed intermediate seed storage behaviour. So far, intermediate seed storage behaviour in Leguminosae species was discovered for the first time.

Conflict of interests

The authors did not declare any conflict of interest.

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