Preliminary and Regional Reports

Effects of Desiccation and Storage Temperature on Seed Germination in Kapok

YanLing Zheng, GaoJuan Zhao, and HuanCheng Ma[1]

ADDITIONAL INDEX WORDS. *Bombax ceiba*, seed maturity, seed storage behavior, seed vigor, seedling growth

SUMMARY. Kapok (*Bombax ceiba*) is a deciduous tree that can grow in the dry-hot valley of southwestern China where its natural regeneration by seedlings is difficult. As mature fruit split open and seeds disperse by wind, it is difficult to collect fully mature seeds. The effects of seed moisture content (MC) and storage temperatures on seed germination of dark-brown seeds collected from split fruit and light-brown seeds collected ≈10–15 days earlier than the time of fruit split were studied to determine the effective germplasm preservation via the seeds. Dark-brown mature seeds could tolerate desiccation to less than 5% MC and could tolerate −20 and −80 °C. Seeds of kapok showed orthodox storage behavior. They can be stored at subzero temperatures with low MC for a long time. For light-brown seeds, germination percentage (GP), germination index (GI), seedling fresh weight (SFW), and vigor index (VI) decreased significantly after seed desiccation. Germination percentage of light-brown seeds with different MC increased to a different extent after being stored at different temperatures for 1 year (76% to 99%), compared with the fresh seeds (73%). Storing fresh seeds at 4 °C was most favorable to keep seed viability and seed vigor of light-brown seeds. Seed collection could be done several days earlier than the time of fruit burst to ensure increased quantity of collected seeds.

Kapok is a tall tree buttressed at the base, widely found throughout India and other parts of tropical and subtropical Asia, Australia, and Africa (Flora of China Editorial Committee, 1984; Verma et al., 2011). Kapok can grow in the southwestern China where the adult trees of the species produce many seeds; however, few seedlings are found around the mature trees (Zheng et al., 2013; Zheng and Ma, 2014). The research by Ballabha et al. (2013) also showed poor regeneration of kapok in India. Koenig and Griffiths (2012) reported that seeds of kapok did not survive in the soil past the wet season to form a seed bank. Therefore, germplasm preservation through seed storage is necessary for kapok. Understanding storage behavior of seeds is essential to determine the most suitable storage environment, and the likely duration of successful storage (Hong and Ellis, 1996). However, the storage behavior of this species has not previously been studied in detail. Although Nakar and Jadeja (2014) suggested that seeds of kapok were recalcitrant type, their classification was simply based on seed viability. The Seed Information Database [SID (Royal Botanic Gardens, Kew, 2015)] lists it as “probably orthodox.”

In the wild, kapok grows to an average of 20 m tall, with old trees up to 60 m in wet tropical weather. The brown, mature fruit of kapok split open on the tree and the seeds disperse by wind making it difficult to collect fully mature seeds. Generally, unsplit fruit, which can be brown or green can be collected and placed at room temperature and seeds can be collected once the fruit burst. However, based on several years of our observation, the color of these seeds is lighter (light brown) than that of the normal mature seeds (dark brown), which are collected after the fruit split on the trees. We suspect that light-brown and dark-brown seeds may possess different maturity and thus might show different physiological responses to desiccation and subzero temperatures. In practice, most of the collected kapok fruit are the unsplit fruit. So far, the effects of storage on seed germination of light-brown seeds are not clear, and this is a disadvantage to the reforestation and restoration projects in the region.

In the present study, the effects of desiccation and storage temperature on seed germination of dark-brown and light-brown seeds in kapok were tested to determine the seed storage behavior and to determine if dark-brown and light-brown seeds possess different sensitivity to desiccation and low temperatures. The results can provide guidelines for seed storage and seed collection timing.

Materials and methods

Fruit and seeds of kapok were collected from Lujiangba, Baoshan, Yunnan Province (lat. 24°54′N, long. 98°53′E) before fruit burst in

Key Laboratory of Biodiversity Conservation in Southwest China, State Forestry Administration, Southwest Forestry University, Kunming 650204, China

This research was funded by the National Natural Science Foundation of China (31260175).

We thank Rick Zenn and Chandalin Bennett for the English correction.

[1]Corresponding author. E-mail: flashingzyl@163.com.

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
28.3495	oz	g	0.0353
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

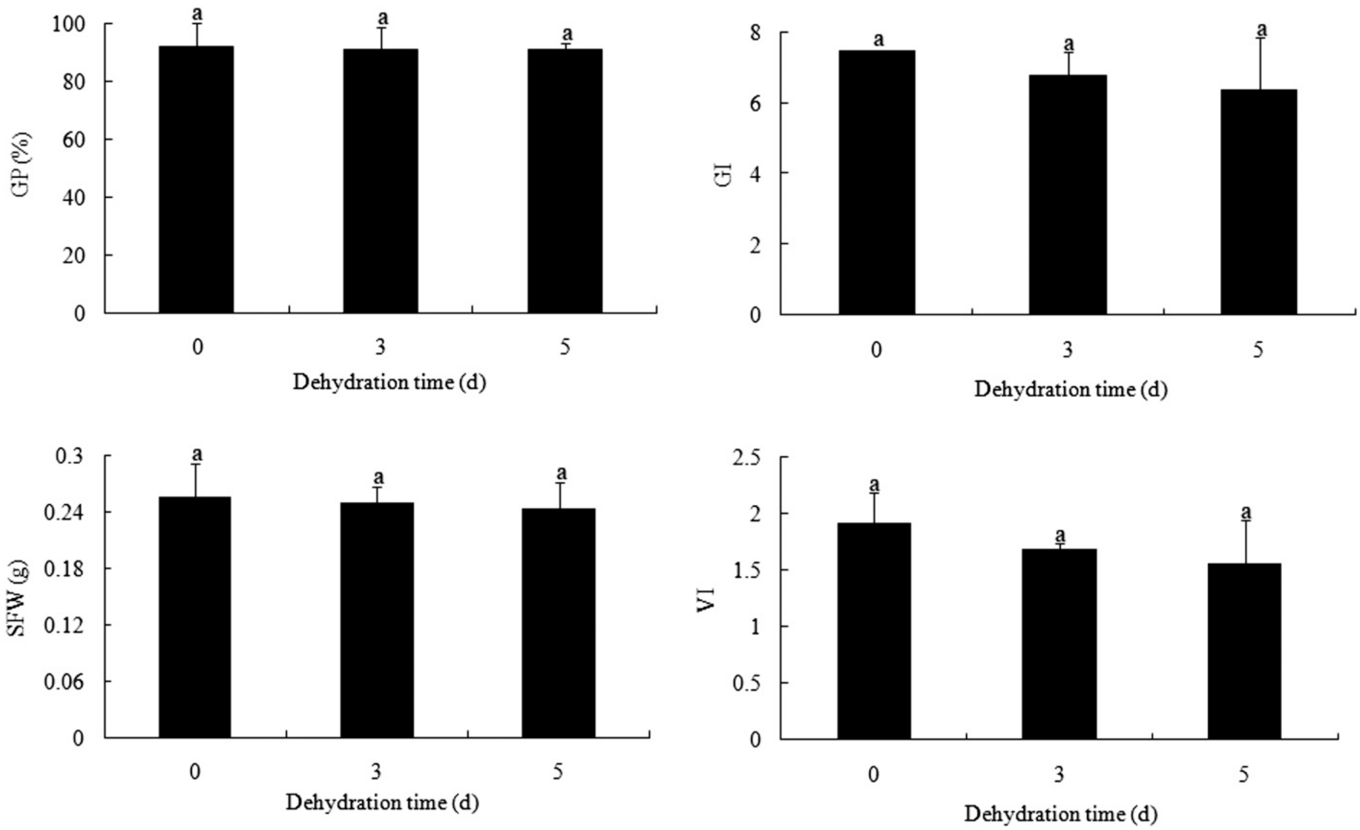

Fig. 1. The effect of desiccation on germination percentage (GP), germination index (GI), seedling fresh weight (SFW), and vigor index (VI) of dark-brown mature seeds (collected from split fruit in 2013) of kapok. The moisture content of seeds desiccated for 0, 3, and 5 d was 11.10%, 6.35%, and 4.98%, respectively. Data points represent means of four replicates ±SD. The bars indicated by different lowercase letters are significantly different by Fisher's least significant difference at $P < 0.05$; GI = $\sum(G_t/D_t)$, where G_t is the number of seeds germinating on the t^{th} day of incubation and D_t is the number of days of seed incubation; VI = GI × SFW; 1 g = 0.0353 oz.

Apr. 2012 (≈10–15 d earlier than the time of fruit burst) and after fruit burst in May 2013. Lujiangba is in the south subtropical dry and hot river valley areas. Mean temperature of the coldest and warmest months are 14.7 and 26.3 °C, respectively. The mean annual precipitation is 755 mm, with most rainfall concentrated between June and October (Li et al., 2009). The unsplit fruit were put at room temperature until they split open. Seeds were selected from the split fruit manually and used immediately for the experiment. The color of seeds collected in April (light brown) was obviously lighter than that of normal mature seeds collected in May, which were dark brown.

Desiccation tolerance was compared between light-brown and dark-brown seeds. To determine seed tolerance to desiccation, light-brown and dark-brown seeds were dried to different MCs in sealed containers with silica gel at room temperature (mean temperature in Apr. 2012 and May 2013 of 20 ± 2 °C). Seeds were removed and MC and germination were tested after 0, 1, 2, and 5 d of drying for light-brown seeds and 0, 3, and 5 d of drying for dark-brown seeds. Moisture content of 20 seeds in four replications was determined on a fresh weight basis after oven drying for 17 h at 103 °C. To determine the effects of seed MC on seed viability and seed vigor of light-brown seeds stored at different temperatures, seeds desiccated for 0, 1, 2, and 5 d were sealed in aluminum foil bags and stored at room temperature (mean annual temperature of 14.7 °C), 4, and –20 °C for 1 year, respectively. To determine the tolerance of dark-brown seeds to low temperature, seeds desiccated for 5 d were sealed in aluminum foil bags and stored at –20 and –80 °C for 3 months. The germination characteristics of fresh dark-brown seeds were used as the control. Germination was determined at the end of each storage time.

For germination, four replicates of 25 seeds for each treatment were randomly sampled and placed on top of two layers of qualitative filter papers (Xinxing brand; Hangzhou Special Paper Corp., Hangzhou, China), moistened with distilled water in petri dishes (10 cm diameter). The petri dishes were placed in a germination chamber set at 30 °C under a 12-h photoperiod. The germination conditions were chosen according to Zheng et al. (2013). The filter papers were kept moist, and germinating seeds were counted every day until no further germination was observed. Seeds were considered as germinated when the radicle protruded 2 mm. After 10 d, SFW was determined. Germination percentage, VI, and GI indicating germination rate were determined. VI and GI were calculated according to Zheng and Sun (2008):

$$GI = \sum (G_t/D_t) \qquad [1]$$

where G_t is the number of seeds germinating on the t^{th} dayof incubation and D_t is the number of days of seed incubation.

$$VI = GI \times SFW \qquad [2]$$

Statistical analyses were performed using SPSS 15.0 (IBM Corp., Armonk, NY). Germination percentage was subjected to arcsin transformation before statistical analysis. GP, GI, SFW, and VI of seeds desiccated for different periods or stored at different temperatures were subjected to one-way analysis of variance for dark-brown and light-brown seeds, respectively. Significant differences between means were tested by Fisher's least significant difference at 0.05 level of probability.

Results and discussion

Moisture content of dark-brown mature (collected May 2013) seeds was 11.10% and it decreased to 6.35% and 4.98% after 3 and 5 d of desiccation, respectively. GP, GI, SFW, and VI of the fresh dark-brown seeds were 92.00%, 7.50, 0.26 g, and 1.92, respectively. Desiccation did not significantly affect germination-related indexes mentioned above (Fig. 1). It showed that dark-brown mature seeds were tolerant to desiccation. After 3 months storage at –20 and –80 °C, GP and SFW of mature seeds with MC of 4.98% did not change significantly compared with the control. However, GI decreased significantly by 26.67% and 29.33%, respectively, and VI also decreased significantly by 30.73% and 35.42%, respectively (Fig. 2). GI reflects the rate of seed germination and its decrease was mainly due to the slow metabolic rate of seeds that were immediately retrieved from storage at subfreezing temperatures. As SFW did not decrease, decrease of VI also occurred because of the slow metabolic rate of seeds.

Three categories of seed storage behavior have been identified: orthodox, intermediate, and recalcitrant (Hong and Ellis, 1996). Orthodox seeds can be dried to a MC of 5% or less (fresh weight basis) and stored at subzero temperatures for a long period. On the other hand, recalcitrant seeds cannot be dried to a MC less than 15% to 30%, depending on species, or stored at subzero temperatures without losing viability. Intermediate seeds can tolerate drying to ≈7% to 12% MC, but further drying and storage at subzero temperatures; e.g., –20 °C, reduces seed longevity (Chen et al., 2008; Hong and Ellis, 1996). On the basis of the tolerance of mature seeds to desiccation and subzero temperatures, seeds of kapok are orthodox seeds (Hong and Ellis, 1996). However, Nakar and Jadeja (2014) suggested that seeds of kapok were recalcitrant type. As the classification adopted by Nakar and Jadeja (2014) was simply based on seed viability (lack of seed dormancy) not on the seed sensitivity to desiccation and low temperatures, we think their conclusion was arbitrary. Seed potential longevity is affected by genotype and provenance. And how much of the potential longevity is

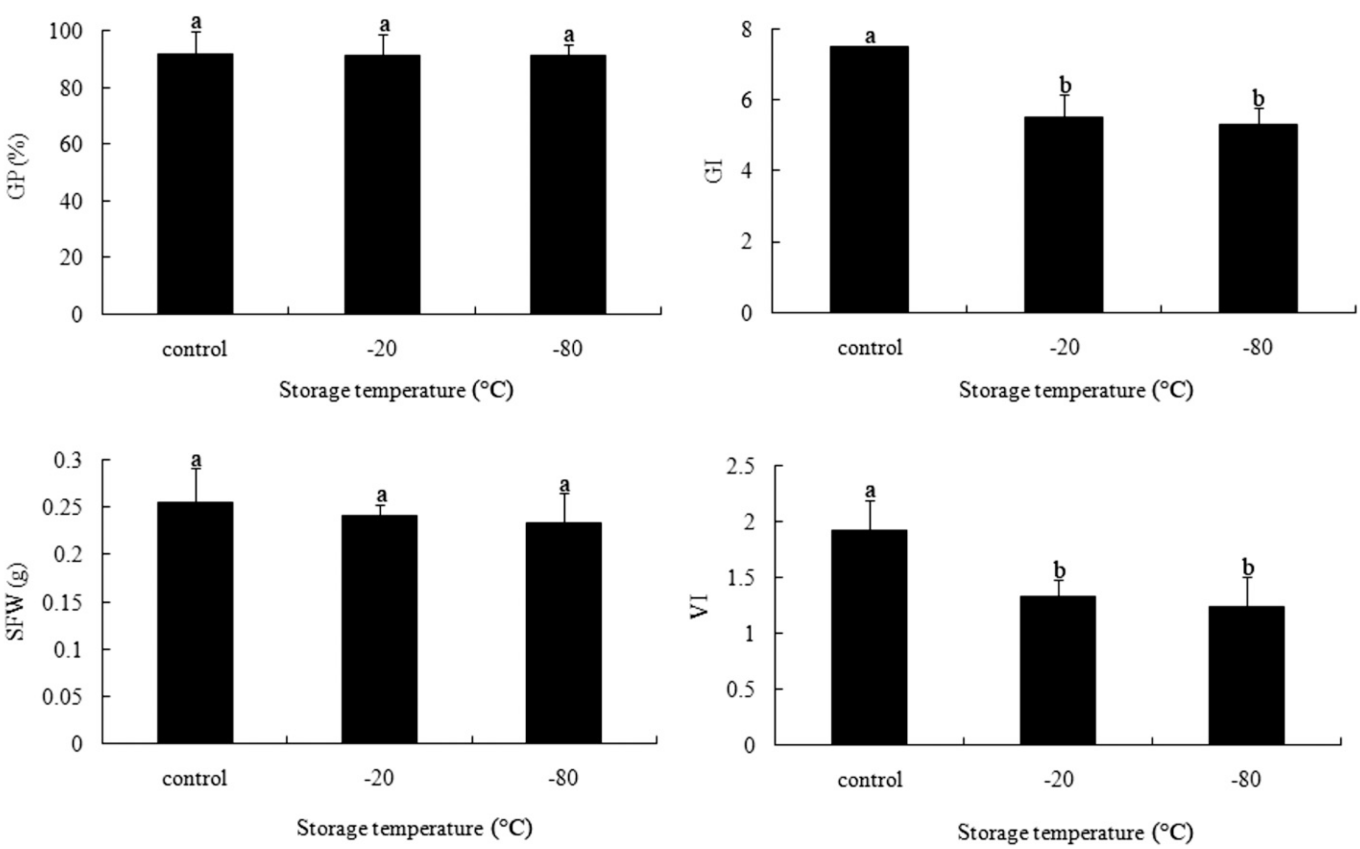

Fig. 2. Germination percentage (GP), germination index (GI), seedling fresh weight (SFW), and vigor index (VI) of dark-brown mature seeds (collected from split fruit in 2013) of kapok with moisture content of 4.98% after storage at −20 and −80 °C for 3 months. Data points represent means of four replicates ± SD. The bars indicated by different lowercase letters are significantly different by Fisher's least significant difference at $P < 0.05$; GI = $\sum(G_t/D_t)$, where G_t is the number of seeds germinating on the t^{th} day of incubation and D_t is the number of days of seed incubation; VI = GI × SFW; (1.8 × °C) + 32 = °F, 1 g = 0.0353 oz.

realized depends on subsequent storage conditions (Hong and Ellis, 1996). Species of trees with orthodox seed storage characteristics can be stored for long periods and up to hundreds of years in some cases (Bewley et al., 2013; Simpson et al., 2004). Thus, seeds of kapok can be stored at subzero temperatures with low MC for a long time. In the wild, seeds of kapok did not survive in the soil past the wet season to form a seed bank (Koenig and Griffiths, 2012) and the regeneration of this species is poor (Ballabha et al., 2013; Zheng et al., 2013). Thus, seed storage is an effective method for long-term conservation of the genetic resources of kapok.

Moisture content of light-brown seeds was 12.8% and it decreased to 7.48%, 5.51%, and 4% after 1, 2, and 5 d of desiccation, respectively. GP, GI, SFW, and VI of fresh light-brown seeds were 73%, 5.58, 0.27 g, and 1.53, respectively. The corresponding GIs decreased significantly with the decreasing of seed MC, decreasing by 65.75%, 76.70%, 51.85%, and 88.89%, respectively, for the seeds with MC of 4% (Fig. 3). This indicated that light-brown seeds of kapok were sensitive to desiccation and may not be fully mature. As mature seeds of kapok are tolerant to desiccation but the light-brown (immature) seeds are not. The desiccation tolerance of kapok seeds appears to be related to seed maturity. Similar results have also been reported in many plants, in which immature seeds/embryos are desiccation sensitive and mature seeds/embryos are desiccation tolerant (Huang et al., 2011; Kalemba et al., 2009). It has been reported that at later stages of seed development such as at the end of the seed-filling phase many orthodox species may still show damage on desiccation to very low MC levels (Ellis and Hong, 1994; Hong and Ellis, 1996). It has been well studied that orthodox seeds undergo maturation drying on the mother plant and a number of mechanisms are involved in the acquisition of desiccation tolerance, such as antioxidant systems, late embryogenesis–abundant proteins, carbohydrates, and subcellular organization (Connor and Sowa, 2003; Farrant et al., 1997).

Light-brown seeds with different MC were stored at different temperatures for 1 year. For seeds stored at room temperature, GP did not vary significantly among seeds with different MC [total average 90.75% (Fig. 4)]. GI (mean 4.76) and VI (mean 0.99) of seeds with MC of 4% were significantly higher than that of seeds with higher MC. SFW of seeds with MC of 4% was significantly higher than that of seeds with MC of 12.8%. For seeds stored at –4 °C, GP did not vary significantly among seeds with different MC (total average 96.75%). GI (mean 8.78) and VI (mean 2.02) of seeds with MC of 12.8% were significantly higher than that of seeds with relatively low MC.

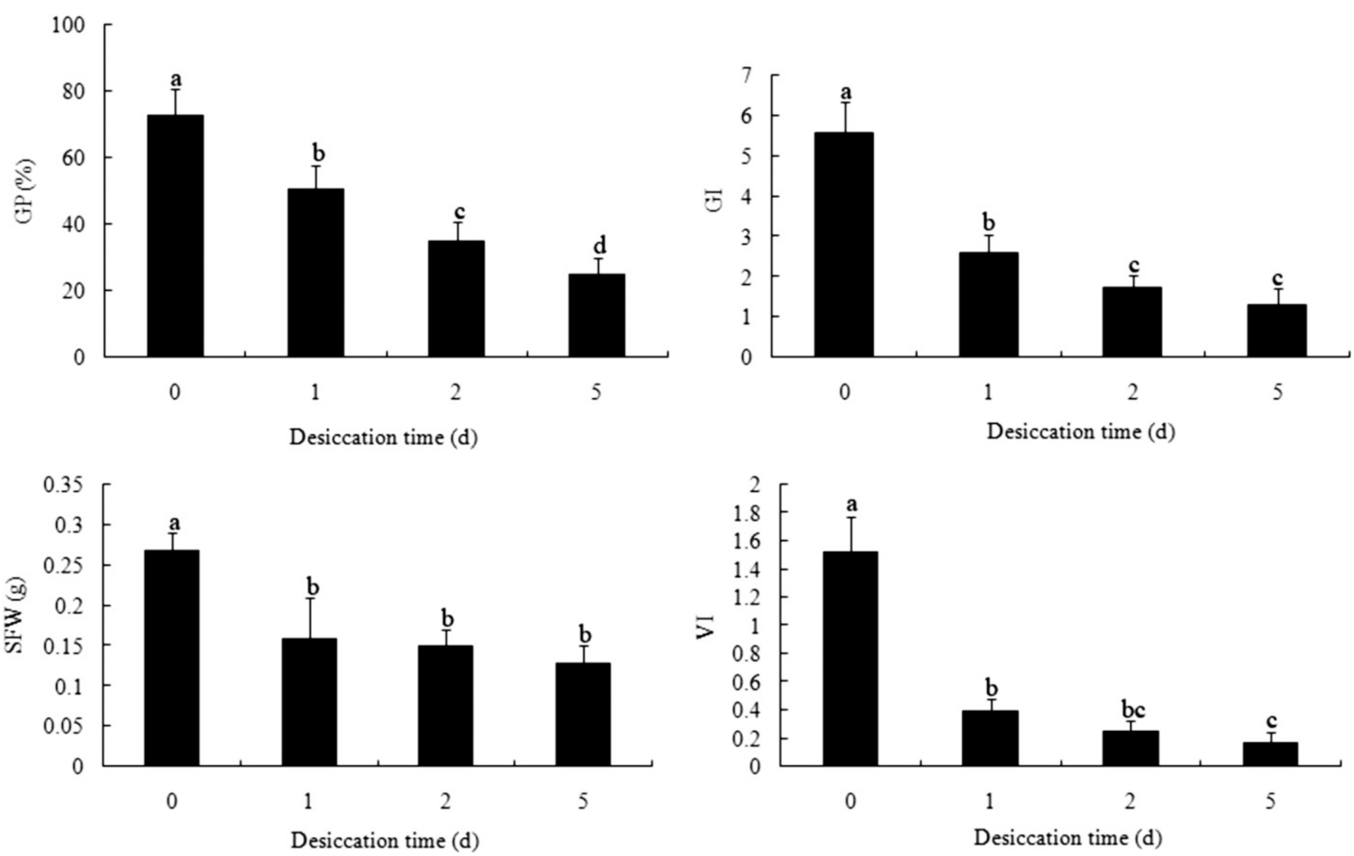

Fig. 3. The effect of desiccation on seed germination percentage (GP), germination index (GI), seedling fresh weight (SFW), and vigor index (VI) of light-brown of kapok (collected ≈10–15 d earlier than the time of fruit burst in 2012). The moisture content of seeds desiccated for 0, 1, 2, and 5 d was 12.80%, 7.48%, 5.51%, and 4.00%, respectively. Data points represent means of four replicates ± SD. The bars indicated by different lowercase letters are significantly different by Fisher's least significant difference at $P < 0.05$; GI = $\sum(G_t/D_t)$, where G_t is the number of seeds germinating on the t[th] day of incubation and D_t is the number of days of seed incubation; VI = GI × SFW; 1 g = 0.0353 oz.

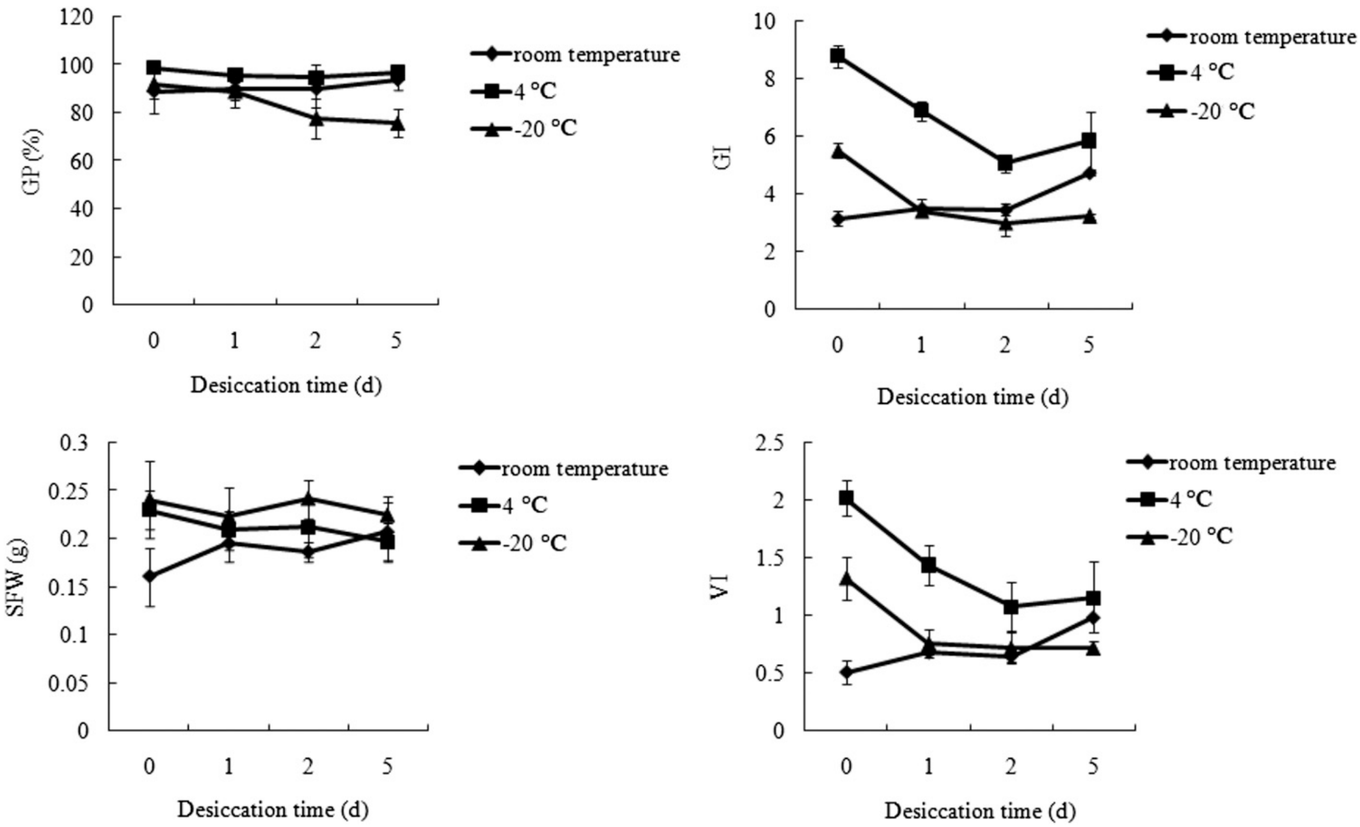

Fig. 4. Seed germination percentage (GP), germination index (GI), seedling fresh weight (SFW), and vigor index (VI) of light-brown seeds (collected ≈10–15 d earlier than the time of fruit burst in 2012) of kapok with different moisture content after storage at different temperatures for 1 year. The moisture content of seeds desiccated for 0, 1, 2, and 5 d was 12.80%, 7.48%, 5.51%, and 4.00%, respectively. Data points represent means of four replicates ± SD. GI = $\sum(G_t/D_t)$, where G_t is the number of seeds germinating on the t^th^ day of incubation and D_t is the number of days of seed incubation; VI = GI × SFW; (1.8 × °C) + 32 = °F, 1 g = 0.0353 oz.

SFW of seeds with MC of 12.8% (mean 0.23 g) was significantly higher than that of seeds with MC of 4% (mean 0.20 g). For seeds stored at –20 °C, GP of seeds with MC of 12.8% and 7.48% (92% and 89%, respectively) was significantly higher than that of seeds with MC of 5.51% and 4% (78% and 76%, respectively). GI (mean 5.53), and VI (mean 1.33) of seeds with MC of 12.8% were significantly higher than that of seeds with relatively low MC. SFW did not vary significantly among seeds with different MC (total average 0.23 g). This showed that relatively high seed MC was more conducive to maintain seed viability for seeds stored at 4 and –20 °C and storing fresh seeds at 4 °C was most favorable to keep seed viability and seed vigor. Studies on other species have also suggested that there is an optimum water content for each storage temperature, which increases as storage temperature decreases (Buitink et al., 2000; Vertucci et al., 1994). Compared with the fresh seeds, GP of kapok seeds with different MCs increased to different extents after being stored for 1 year. Therefore, light-brown seeds of kapok collected from unsplit fruit on the trees can be stored to keep their germinability. This is important in practice to the reforestation and restoration projects. Our laboratory work also showed that seed viability and seed vigor of kapok seeds with high MC (collected from unsplit fruit) were improved after some days of slow-rate desiccation at room temperatures (data not shown). It is probable that fresh light-brown seeds of kapok may exhibit dormancy characteristics, and some changes including physiological processes as well as gene expression and metabolic programs may occur during the period of desiccation and storage. This needs further work to be confirmed.

For many species, mature seeds are used to store germplasm and propagate seedlings. However, for some species with dormant seeds, higher frequencies of germination have been achieved by culturing immature seeds rather than by culturing mature seeds (Light and MacConaill, 1998). It has been postulated that dormancy is induced by accumulation of some inhibitory substances or by increasing impermeability of the embryos during seed maturation (van der Kinderen, 1987; Yamazaki and Miyoshi, 2006). For kapok, as mature fruit split open and seeds disperse by wind, it is difficult to collect fully mature seeds. In addition, as light-brown seeds of kapok have relatively high GP and appropriate storage conditions could improve the seed viability and seed vigor, light-brown seeds could be collected several days earlier than the time of fruit burst on the trees to increase the quantity of collected seeds.

Literature cited

Ballabha, R., J.K. Tiwari, and P. Tiwari. 2013. Regeneration of tree species in the subtropical forest of Alaknanda Valley, Garhwal Himalaya, India. For. Sci. Practice 15:89–97.

Bewley, J.D., K.J. Bradford, H.W.M. Hihorst, and H. Nonogaki. 2013. Seeds: Physiology of development, germination and dormancy. 3rd ed. Springer, New York, NY.

Buitink, J., O. Leprince, M.A. Hemmings, and F.A. Hoekstra. 2000. Molecular mobility in the cytoplasm: An approach to describe and predict lifespan of dry germplasm. Proc. Natl. Acad. Sci. USA 97:2385–2390.

Chen, S.Y., S.Y. Lu, and C.T. Chien. 2008. Germination and storage of *Trema cannabina* seeds. Seed Sci. Technol. 36:105–113.

Connor, K.F. and S. Sowa. 2003. Effects of desiccation on the physiology and biochemistry of *Quercus alba* acorns. Tree Physiol. 23:1147–1152.

Ellis, R.H. and T.D. Hong. 1994. Desiccation tolerance and potential longevity of developing seeds of rice (*Oryza sativa* L.). Ann. Bot. (Lond.) 73:501–506.

Farrant, J.M., N.W. Pammenter, P. Berjak, and C. Walters. 1997. Subcellular organization and metabolic activity during the development of seeds that attain different levels of desiccation tolerance. Seed Sci. Res. 7:135–144.

Flora of China Editorial Committee. 1984. Flora of China. Science Press, Beijing, China.

Hong, T.D. and R.H. Ellis. 1996. A protocol to determine seed storage behaviour. Intl. Plant Genet. Resources Inst. (IPGRI) Tech. Bul. No. 1, Rome, Italy.

Huang, H., I.M. Møller, and S.Q. Song. 2011. Proteomics of desiccation tolerance during development and germination of maize embryo. J. Proteomics 75:1247–1262.

Kalemba, E.M., F. Janowiak, and S. Pukacka. 2009. Desiccation tolerance acquisition in developing beech (*Fagus sylvatica* L.) seeds: The contribution of dehydrin-like protein. Trees (Berl.) 23:305–315.

Koenig, J. and A. Griffiths. 2012. The population ecology of two tropical trees, *Brachychiton diversifolius* (Malvaceae) and *Bombax ceiba* (Bombaceae), harvested by indigenous woodcarvers in Arnhem Land, Australia. Environ. Manage. 50:555–565.

Li, K., F.Y. Liu, and C.H. Zhang. 2009. Essential characteristic of climate and plant recovery in dry-hot valley. World For. Res. 22:24–27.

Light, M.H.S. and M. MacConaill. 1998. Factors affecting germinable seed yield in *Cypripedium calceolus* var. *pubescens* (Wild.) Correll and *Epipactis helleborine* (L.) Crantz (Orchidaceae). Bot. J. Linn. Soc. 126:3–26.

Nakar, R.N. and B.A. Jadeja. 2014. Seed pattern, germination and viability of some tree species seeds from Girnar Reserve Forest of Gujarat. Indian J. Plant. Physiol. 19:57–64.

Royal Botanic Gardens, Kew. 2015. Seed Information Database (SID), Version 7.1. 3 Dec. 2015. <http://data.kew.org/sid/>.

Simpson, J.D., B.S.P. Wang, and B.I. Daigle. 2004. Long-term seed storage of various Canadian hardwoods and conifers. Seed Sci. Technol. 32:561–572.

van der Kinderen, G. 1987. Absisic acid in terrestrial orchid seeds: A possible impact on their germination. Lindleyana 2:84–87.

Verma, V., S.S. Jalalpure, A. Sahu, L.K. Bhardwaj, and Y. Prakesh. 2011. *Bombax ceiba* Linn: Pharmacognostical, phytochemistry, ethnobotany, and pharmacology studies. Internationale Pharmaceutica Sciencia 1:62–68.

Vertucci, C.W., E.E. Roos, and J. Crane. 1994. Theoretical basis of protocols for seed storage III. Optimum moisture contents for pea seeds stored at different temperatures. Ann. Bot. (Lond.) 74:531–540.

Yamazaki, J. and K. Miyoshi. 2006. In vitro asymbiotic germination of immature seed and formation of protocorm by *Cephalanthera falcata* (Orchidaceae). Ann. Bot. (Lond.) 98:1197–1206.

Zheng, Y.L. and H.C. Ma. 2014. Effects of seed aging on seed germination and seed reserve utilization in mumian. HortTechnology 24:471–474.

Zheng, Y.L., H.C. Ma, R. Scheller, G.J. Zhao, and Y. Zheng. 2013. Influence of environmental factors on seed germination of *Bombax malabaricum* DC. Acta Ecologica Sinica 33:0382–0388.

Zheng, Y.L. and W.B. Sun. 2008. Variation in morphophysiological characters of fruits of *Trigonobalanus doichangensis* (Fagaceae) according to individual trees, populations and years. Euphytica 164:231–238.