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Effects of Seed Aging on Seed Germination and Seed Reserve Utilization in Mumian

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SUMMARY. Mumian (*Bombax ceiba*) is a deciduous tree that can grow in the dry-hot valley of southwestern China. However, natural regeneration of mumian is significantly inhibited by some factors such as high temperature that can accelerate seed deterioration. The objective of this study was to identify the change of seedling growth component(s) in response to seed aging at 45 °C either at low relative humidity (15%) or at high relative humidity (100%) for 0, 1, 2, 3, and 4 days. Germination assay results at 30 °C in darkness showed an interaction between relative humidity and aging duration. At high temperature and low relative humidity seedling growth, weight of used seed reserve and fraction of seed reserve depletion significantly decreased with increasing of aging duration. But the conversion efficiency of mobilized reserve to seedling tissues did not change significantly. In contrast, at high temperature and high relative humidity, seedling growth and conversion efficiency of seed reserve utilization decreased significantly, but weight of used seed reserve and fraction of seed reserve depletion did not change significantly until seeds were dead. It was concluded that there existed a different pattern of seed reserve utilization for seeds aged at low and high relative humidity, and humidification of seeds at 45 °C for 1 day is a possible way to increase germination of mumian.

Seed deterioration is expressed as the loss of quality, viability, and vigor during aging. Several comprehensive reviews have identified free radical-mediated lipid peroxidation, enzyme inactivation or protein degradation, disruption of cellular membranes, and damage to genetic (nucleic acids) integrity as major causes of seed deterioration (McDonald, 1999; Walters, 1998). The rate of deterioration is determined by seed moisture, storage temperature, and seed traits that are influenced by genetic and environmental interactions during seed maturation and harvest (Walters et al.,

2010). Among these factors temperature and seed moisture content and relative humidity are well studied (Ellis et al., 1992). Accelerated aging provides a simple method for studying the mechanisms of deterioration over short periods, and it generally is done by subjecting seeds to elevated temperature and high relative humidity (Kapoor et al., 2011). Aging is evidenced by delayed germination and emergence, slower growth, increased susceptibility

to stress, and finally a decline in germinability compared with nonstressed seeds (Al-Maskri et al., 2003; Byrd and Delouche, 1971; McDonald, 1975; Woodstock, 1973). Moreover, the fraction of seed reserve mobilization decreases with aging duration (Mohammadi et al., 2011; Nik et al., 2011).

Mumian is a tall tree buttressed at the base, widely found throughout India and other parts of tropical and subtropical Asia, Australia, and Africa (Chaudhary and Khadabadi, 2012; Verma et al., 2011). Mumian is a multipurpose tree species providing food, fodder, and fiber (Jain et al., 2011), and almost all plant components are of medicinal importance (Chaudhary and Khadabadi, 2012). The trees produce oblong-oval capsules ≈10–18 cm in length. The seeds, located within capsules, are rich in lipids, 6–9 mm long, and weigh 35–50 mg. Seeds of mumian mature and disperse in April and May. Mumian can grow in the dry-hot valley of southwestern China where the adult trees of the species produce many seeds; however, few seedlings are found around the mature trees. The annual average temperature in the dry-hot valley is above 20 °C and the extreme temperature can reach above 40 °C. The seasonal distribution of precipitation is uneven and concentrated in the period from June to October. High temperature can accelerate seed deterioration that consequently affects seed viability and vigor (Walters, 1998; Walters et al., 2010). Our previous studies show that drought (Zheng et al., 2013) and sticky snake-root [*Eupatorium adenophorum* (unpublished data)] could inhibit seed germination of mumian. However, seedling regeneration is still impaired around the mature trees even though no sticky snakeroot exists at some distribution sites. Besides, high temperature is a limiting factor of seed germination in mumian, and germination is significantly inhibited at ≥40 °C (Zheng et al., 2013). As mechanisms of seed aging under different conditions may vary (Murthy et al., 2003;

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
28,350	oz	mg	3.5274×10^{-5}
$(^{\circ}\text{F} - 32) \div 1.8$	$^{\circ}\text{F}$	$^{\circ}\text{C}$	$(^{\circ}\text{C} \times 1.8) + 32$

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Walters, 1998), and patterns of seed reserve utilization vary with plant species and adverse conditions (Gholami et al., 2010; Mohammadi, 2013; Mohammadi et al., 2011), this study was conducted to analyze seed reserve utilization of mumian seeds aged at high temperature with low and high relative humidity.

Materials and methods

Dry fruits (capsules) containing mature seeds of mumian were collected from Lujiangba, Baoshan, Yunnan province, China (lat. 24°54'N, long. 98°53' E) in Apr. 2012. 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 capsules were randomly harvested from 17 trees and brought back to the laboratory within 5 d. The seeds were selected from the capsules manually and used immediately for the experiment. To create different aging treatments, two aging conditions were chosen. The first was high temperature with high relative humidity (100%) by putting seeds in muslin cloth bags and placing bags over sieves in containers holding water at the bottom at 45 °C. Seeds were removed at 1, 2, 3, and 4 d after incubation. The second was high temperature with low relative humidity (≈15%) by sealing seeds in aluminum foil bags and incubating them at 45 °C. The seeds were removed at 1, 2, 3, and 4 d after incubation. The 0 d-aged seeds were control seeds.

A randomized complete block design with four replications was used to compare the aging treatments in both experiments. To determine seed dry weight and seed moisture content, 20 seeds from each treatment in three replications were weighed (W1), dried at 105 °C for 24 h and then weighed again (W2). Seed water content (WC) was calculated as $[(W1 - W2)/W2] \times 100$ (Mohammadi, 2013).

Twenty-five seeds per treatment were randomly sampled and placed on top of two layers of qualitative filter papers, moistened with distilled water in petri dishes (10 cm diameter). The petri dishes were placed in a dark germination chamber set at 30 °C and kept moist. The germination conditions were chosen according to

Zheng et al. (2013). 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, weight of seedlings and seed remnant were determined after oven drying at 75 °C for 24 h. Germination percentage (G) and germination index (GI) indicating germination rate were determined. GI was calculated according to Zheng and Sunday (2008): $GI = \sum(G_t/D_t)$, where G_t is the number of seeds germinating on the t^{th} day of incubation and D_t is number of days of seed incubation.

Seedling growth components were calculated as outlined by Soltani et al. (2006). The weight of used (mobilized) seed reserve was calculated as the dry weight of the original seed minus the dry weight of the seed remnant. Conversion efficiency of mobilized seed reserve into plant tissue was estimated by dividing seedling dry weight by the used seed reserve. The ratio of used seed reserve to initial seed dry weight was considered to be seed reserve depletion percentage.

The experimental design was two factors arranged in a completely randomized design with four replicates. The first factor was the relative humidity (low and high) and the second was aging duration (0, 1, 2, 3, 4 d). All data were analyzed statistically by analysis of variance using SPSS 11.0 software (IBM Corp., Armonk, NY). Significant differences between means were tested by Fisher's least significant difference test.

Results and discussion

The interaction between relative humidity and aging duration was significant for seed WC (Table 1). Seed WC decreased significantly for seeds aged for 3–4 d at low relative humidity. However, it increased significantly with increased aging duration at high relative humidity, reaching

to 66.2% after 4 d of aging (Fig. 1). Because seeds aged at high temperature and high relative humidity for 4 d died, variance in GI, seedling dry weight, weight of used seed reserve, seed reserve depletion, and seed reserve utilization efficiency of seeds aged at high relative humidity were analyzed with data only up to 3 d of accelerated aging.

The interaction between relative humidity and aging duration was significant for seed germination and GI (Table 1). Seed aging duration at high temperature and low relative humidity had no significant effect on seed germination percentage (mean 73%), but GI and seedling dry weight significantly decreased with seed aging (Fig. 1). In contrast, at high temperature and high relative humidity, the variation in seed germination percentage, GI, and seedling dry weight among seeds subjected to different aging duration were all significant. Generally, seed viability and vigor decreased with the process of aging at high temperature and high relative humidity (Al-Maskri et al., 2003; Kaewnaree et al., 2011). However, in this study, germination percentage and GI of seeds aged at high temperature and high relative humidity increased significantly after 1-d treatment compared with the control, increasing by 12% and 8.5%, respectively. This may be due to the effect of humidification, which increased seed WC by ≈10% in seeds aged at high relative humidity for 1 d. Humidification is a presowing hydration treatment in which seeds are equilibrated under conditions of high humidity (Basra et al., 2002; Suzuki and Khan, 2001). Khan et al. (2003) also found that humidification at high temperature (45 °C) and high relative humidity (100%) for 3–7 d greatly increased seed germination percentage and germination speed in cockscomb (*Celosia argentea* var. *cristata*). However, cotton (*Gossypium hirsutum*) seed

Table 1. Analysis of variance for seed water content (WC), germination percentage (G), germination index (GI), seedling dry weight (SLDW), weight of used seed reserve (WUSR), seed reserve depletion percentage (SRD), and seed reserve utilization efficiency (SRUE) of mumian.

	WC	G	GI	SLDW	WUSR	SRD	SRUE
Relative humidity (RH)	**	**	**	**	**	**	NS
Aging duration (AD)	**	**	**	**	**	**	NS
RH × AD	**	**	**	NS	**	**	**

**, *Significantly different at $P < 0.01$, not significantly different at $P < 0.05$.

invigoration could not be achieved by humidification at 95% to 100% relative humidity and 24 to 26 °C for 1–3 d (Basra et al., 2002). The differential

effects of humidification may be because the success of humidification may reflect the complex interaction of factors including plant species, duration

of treatment, temperature, and storage conditions of the seeds. For conservation and reforestation efforts in mumian, 1-d high temperature

Fig. 1. Effect of seed aging on germination percentage, germination index, and seedling dry weight of mumian at 45 °C (113.0 °F) with low relative humidity (15%) or high relative humidity (100%). Error bars represent $\pm$ SD; LSD = significant differences between means via Fisher's least significant difference test at $P < 0.05$; 1 mg = 3.5274×10^{-5} oz.

Fig. 2. Effect of seed aging on weight of used seed reserve, seed reserve depletion, and seed reserve conversion efficiency of mumian at 45 °C (113.0 °F) with low relative humidity (15%) or high relative humidity (100%). Error bars represent $\pm$ SD; LSD = significant differences between means via Fisher's least significant difference test at $P < 0.05$; 1 mg = 3.5274×10^{-5} oz.

humidification could be an option to increase seed germination and seedling emergence.

There was a significant interaction between relative humidity and aging duration for characteristics of seed reserve utilization (Table 1). Weight of used seed reserve and seed reserve depletion percentage decreased significantly in seeds treated at low relative humidity, decreasing by 23.7% and 24.5% after 4 d of aging, respectively (Fig. 2). However, both the traits mentioned above did not change significantly until seeds were dead aging for 4 d at high relative humidity. Seed reserve utilization efficiency did not change significantly for seeds aged at low relative humidity; however, it decreased significantly for seeds aged at high relative humidity up to 3 d of treatment. Gholami et al. (2010) reported that seedling growth, fraction of seed reserve utilization and weight of mobilized seed reserve of five crop species decreased with increasing drought and salt intensity, but drought and salinity stresses had no effect on the conversion efficiency of five crop species. Mohammadi et al. (2011) reported seedling growth and fraction of seed reserve mobilization of soybean (*Glycine max*) significantly decreased with the advance of deterioration, but the conversion efficiency of mobilized reserves to seedling tissues was not significant. In other research, Mohammadi (2013) observed that although the fraction of seed reserve depletion improved with drought in seeds subjected to osmopriming, seed reserve utilization efficiency declined. The different change pattern of seed reserve utilization may be related to plant species and treatment conditions.

In conclusion, seed aging resulted in reduced seedling growth, which was a consequence of decline in seed reserve depletion percentage at low relative humidity but decline in seed reserve utilization efficiency at high relative humidity before seeds were dead. It showed that there is a different pattern of seed reserve utilization in seeds aged at high relative humidity to those aged at low relative humidity. Whether mechanisms of cell death during seed aging at different relative humidity differ need to be explored. Additionally, humidification of seeds at 45 °C for 1 d

is a possible way to increase germination of mumian.

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