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Germination of recalcitrant *Baccaurea ramiflora* seeds

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ABSTRACT: Tolerance to desiccation in recalcitrant seeds depends on the genetic background and the developmental conditions of the seeds, both of which are relevant to harvest and storage for conservation purposes. The tropical south and south-east Asian species, *Baccaurea ramiflora* Lour., has recalcitrant seeds and is a locally important resource. We measured germination success and desiccation tolerance of *B. ramiflora* at different temperatures and light conditions for 76, 86, 96, and 106 days after anthesis to describe seed germination requirements and the relationship between seed development and desiccation tolerance of seeds for storage. We found that light is not an important factor in *B. ramiflora* seed germination, and that optimal seed germination conditions occur at 30/20 °C. During their development from 76–106 days after anthesis, seeds and their embryonic axes undergo changes in morphological characteristics, germination ability, moisture content, and desiccation tolerance. The best time for collection of *B. ramiflora* seeds for storage is around 96 days after anthesis, when desiccation tolerance is maximal.

KEYWORDS: Burmese grape, tropical forest tree

INTRODUCTION

Seeds are traditionally categorized as either recalcitrant or orthodox[1]. Orthodox seeds can be dried to low moisture content (2–5%) and stored at low temperatures. Recalcitrant seeds have a high moisture content (often $>$ 30–50%) at maturity, and are sensitive to desiccation at moisture contents below 12–30%, depending on the species. Recalcitrant seeds have a short storage potential and can rapidly lose viability under storage conditions[2]. It is believed that desiccation tolerance depends not only on the inherent characteristics of the species, but also on the developmental status of the seeds, and the environmental conditions in which they are dried, particularly the rate of dehydration[3].

Baccaurea ramiflora Lour., a member of the Euphorbiaceae family, is found in the tropical forests of south and south-east Asia, and is an important tree for local people and forest ecology. The fruits of *B. ramiflora* are a food source, and the hard and durable wood is often used in furniture production[4]. Research on *B. ramiflora* has included its ethnobotanical uses, seed biology, and chemical constituents of essential oils[5–7].

In tropical forests, light is one of the most important abiotic resources, and can influence the timing of seed germination[8,9]. *B. ramiflora* seeds shed in summer and like other species with recalcitrant seeds germinate quickly in the field independent of the light conditions[10]. Once they are shed from the parent plant, recalcitrant seeds lose viability when exposed to extreme dry or cold conditions. Unlike orthodox seeds, recalcitrant seeds cannot be stored for a long period at low temperatures. Thus it is challenging to maintain a regular supply of these seeds for genetic conservation purposes[3,11]. At the moment, cryopreservation is considered one of the best alternatives for long-term conservation of plant germplasm[12]. However, the application of exogenous CO or NO markedly improved the tolerance of *B. ramiflora* seeds to low-temperature stress[13]. Importantly, desiccation tolerance in recalcitrant seeds differs at each developmental stage[3].

Suitable temperature and light, are important for seed germination. The objectives of the present study were to determine (1) the light and temperature conditions best suited for seed germination of *B. ramiflora*, and (2) the time needed for *B. ramiflora* seeds to reach physiological maturity for seed harvest collection and further study of seed storage.

MATERIALS AND METHODS

Materials

Seeds of *B. ramiflora* at different developmental stages were collected from trees grown in Xishuangbanna Tropical Botanical Garden of the Chinese Academy of Sciences, Yunnan, Southwest China.

Table 1 Physical changes of *B. ramiflora* seeds and embryos during development.

	Days after anthesis (DAA)			
	76	86	96	106
seed fresh weight (mg)	58.5 ± 8.9	63.7 ± 1.9	64.7 ± 3.8	56.6 ± 2.8
seed dry weight (mg)	23.0 ± 4.9	25.53 ± 0.80	28.5 ± 1.6	23.43 ± 0.91
fruit size (cm^3)	10.20 ± 0.09	15.00 ± 0.30	17.90 ± 0.19	15.44 ± 0.12
embryo fresh weight (mg)	25.8 ± 6.6	30.3 ± 1.3	31.7 ± 6.5	28.7 ± 2.0
embryo dry weight (mg)	8.5 ± 2.0	10.54 ± 0.52	14.0 ± 4.9	9.33 ± 0.72
thousand seed weight (g)	299 ± 17	318 ± 22	330 ± 37	283 ± 32

Values are mean ± SEM, 5 replicates of 3 seeds and 5 replicates of 2 embryos for the fresh weight and dry weight, 10 replicated with 1 fruit for the fruit size.

Seed age was monitored by tagging at anthesis (on the day that most of the flowers on the tree had bloomed) and fruits were harvested at intervals of 10 days from 76–106 days after anthesis (DAA). The final collection day was the time at which most seeds were naturally shed from the tree.

Measurement of seed weight, moisture content and fruit volume

Seeds and their embryos collected at different developmental stages were weighed for fresh weight, then dried at 103 °C for 17 h and reweighed to determine dry weight and moisture content (MC) in 5 replicates of 3 seeds and 5 replicates of 5 embryos. MC was expressed in g per dry weight (g H_2O/g DW). A thousand seed weight was determined with 5 replicates. Fruit volume was measured by a single fruit (10 replicates) with water excluded in a 200 ml volumetric cylinder.

Germination requirement test

Four replicates, each of twenty seeds were sown on moist filter paper in closed Petri dishes and placed in an incubator with 14 h light and 10 h darkness with temperatures of 15 °C, 20 °C, 25 °C, 30 °C, and 20/30 °C (light/darkness). The number of seeds germinated was recorded every 2 days over 30 days. The seeds were considered as germinated when the radicle length was larger than 2 mm [14].

Desiccation treatment

Seeds from each developmental stage were desiccated in a closed glass container (26 cm in diameter and 17 cm in height) over activated silica gel at 25 °C for 1 h, 2 h, 4 h, 6 h, 8 h, and 12 h. The ratio of silica gel to seed was 15:1 (v/v) and silica gel was regenerated every 24 h. Germination tests were performed in darkness at 25 °C by sowing 4 replicates of 15 seeds or 10 embryos on moist filter paper in closed Petri dishes in an incubator for 30 days.

RESULTS

Physical changes in seeds and embryos during development

Changes in fresh weight, dry weight, moisture content, and size of *B. ramiflora* seeds and fruits throughout their development are shown in Table 1. The fresh and dry weights of seeds and excised embryos exhibited similar changes: a gradual increase during the early stage of development, reaching a maximum at 96 DAA, followed by a slight decrease. From 76–96 DAA, fruits grew from 10.2 cm^3 to a maximum of 17.9 cm^3 in volume. Development of *B. ramiflora* seeds was completed at 106 DAA, when the seeds were naturally shed (Table 1). The moisture content of seeds was high (above 1.73 g H_2O/g DW) prior to 76 DAA, then decreased to the minimum at 96 DAA (1.27 g H_2O/g DW), and increased by the final collection. Embryo maximum moisture content occurred at 86 DAA (2.14 g H_2O/g DW) and decreased to 1.84 g H_2O/g DW by 96 DAA, but increased to 2.10 g H_2O/g DW by 106 DAA (Fig. 1).

Effects of light and temperature on seed germination

Very few of the seeds incubated at 15 °C in the light for 30 days germinated (only 1%), and the best germination rate was at 25 °C and 30/20 °C. Temperature ($F = 26.48$, $p < 0.01$) and temperature-illumination interaction ($F = 4.00$, $p < 0.05$) had significant influence on seed germination (Fig. 2); but illumination alone was not significant ($F = 0.04$, $p > 0.05$). There were significant differences in the proportion of germinated seeds between different temperatures, except between 25 °C and 30/20 °C. The germination rate at 30/20 °C was slightly better than at 25 °C, although not statistically significant ($t = 0.13$,

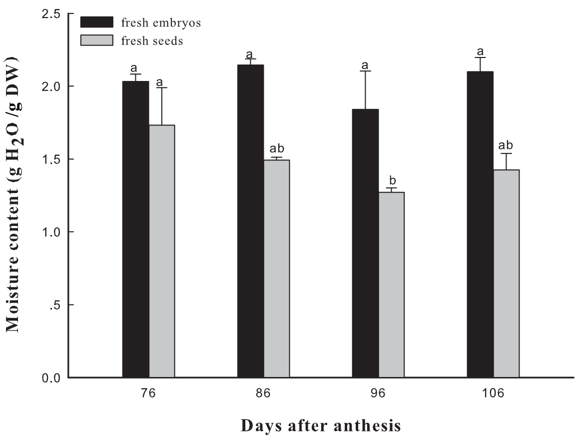

Fig. 1 Changes in moisture contents of fresh *B. ramiflora* seeds and embryos during development. Error bars indicate mean ± SE, 5 replicates of 3 seeds and 5 replicates of 5 embryos. The same letters indicate that there are no significant differences between data; different letters indicate a statistically significant difference (ANOVA, $p < 0.05$).

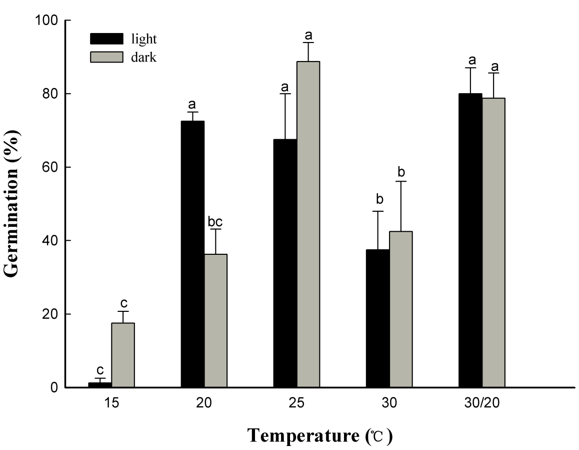

Fig. 2 Effects of light and temperature on seed germination of *B. ramiflora*. Error bars indicate mean ± SE. Each data point is the mean of 4 replicates of 20 seeds. The same letters indicate that there are no significant differences between data; different letters indicate a statistically significant difference (ANOVA, $p < 0.05$).

$p > 0.05$). Light had no significant effect on seed germination ($t = 0.10$, $p > 0.05$). These results suggested that the optimum condition for germination of *B. ramiflora* was at 30/20 °C in either dark or light condition (Fig. 2).

Changes in germinability

Germination capability of *B. ramiflora* seeds depended on their developmental stage. During early development (before 76 DAA), seeds did not germinate, but 73% of intact seeds germinated at 76 DAA. The number of germinated seeds decreased sharply at 86 DAA (27%), then decreased to the minimum at 96 DAA (22%), and increased again at 106 DAA (55%). Removal of the testa significantly increased the number of germinated seeds ($t = 5.21$, $p < 0.01$). For example, the germination percentage of 76 DAA embryos reached 96% (Fig. 3). The one-way ANOVA showed that the changes in germination percentage for seeds during development were significant ($F = 7.62$, $p < 0.01$), while that for embryos were not ($F = 1.46$, $p > 0.05$).

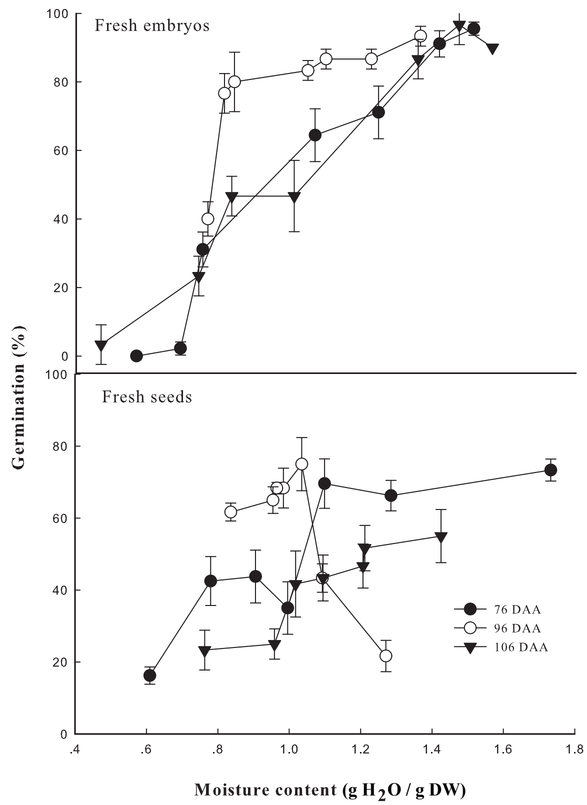

Fig. 3 Changes in germination percentage of *B. ramiflora* seeds and embryos during development following rapid dehydration to different moisture contents. Error bars indicate mean ± SE, 4 replicates of 15 seeds and 3 replicates of 10 embryos.

Desiccation tolerance

Moisture content of intact seeds and embryos were highest at 76 DAA and 86 DAA, respectively, (Fig. 1) and dehydration rates were most rapid at 76 DAA for both intact seeds and embryos. After 12 h of rapid drying, the moisture content of seeds and

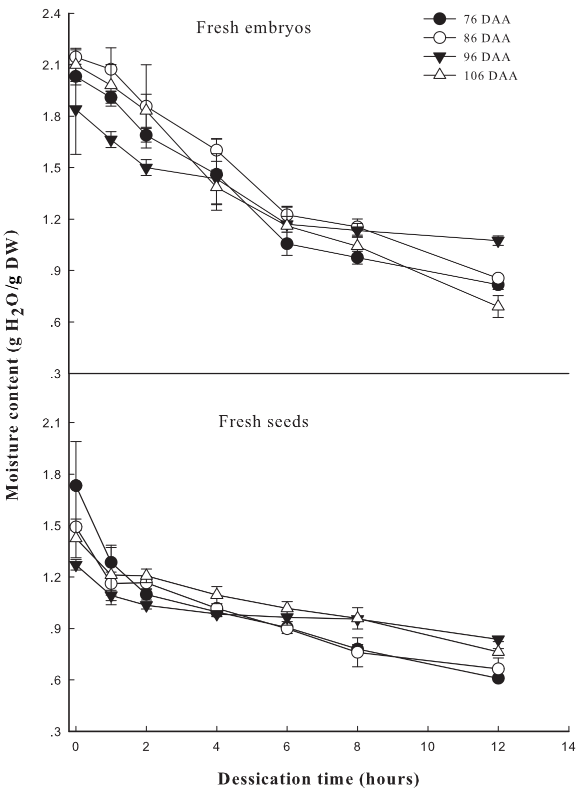

Fig. 4 Changes in moisture contents of *B. ramiflora* seeds and embryos during development following rapid dehydration. Error bars indicate mean ± SE, 5 replicates of 3 seeds and 5 replicates of 5 embryos.

embryos at 76 DAA declined to minimum values of about 0.61 g H_2O/g DW and 0.82 g H_2O/g DW, respectively. During development, the desiccation tolerance of seeds and their embryos increased significantly ($F_{\text{seed}} = 4.86$, $p < 0.05$ and $F_{\text{embryo}} = 4.26$, $p < 0.05$) and were greatest at 96 DAA. After that time, both seeds and embryos exhibited similar reductions in desiccation tolerance (Fig. 4). However, significant differences in seed desiccation tolerance only occurred at between 76 DAA and 96 DAA ($p = 0.002$), 76 DAA and 106 DAA ($p = 0.025$); but for embryos, the greatest tolerance to desiccation was at 96 DAA, and was significantly different from that at 76 DAA ($p = 0.034$), 86 DAA ($p = 0.014$), and 106 DAA ($p = 0.004$). For example, after 12 h of rapid drying, the germination percentage of embryos dropped to 0% at 76 DAA and 106 DAA, but 40% germination percentage remained at 96 DAA. The germination percentage of seeds had the same tendency, but the descend range was smaller than embryos' because of testa (Fig. 3).

DISCUSSION

A previous study also showed no significant difference in *B. ramiflora* seed germination between light and dark conditions in the field[10]. Under our laboratory conditions, the seeds of *B. ramiflora* germinated at temperatures between 20 °C and 30 °C. The optimal germination condition was at a 30/20 °C (light/darkness) cycle in either light or darkness, which reflects its natural growing environment. In its natural habitat in the tropical rain forests of Xishuangbanna, *B. ramiflora* seeds usually germinate immediately after shedding from the trees in July when day and night temperatures average around 31 °C and 23 °C, respectively.

Physiological maturity denotes the stage of development when a seed reaches its maximum dry weight and marks the end of the seed-filling period[15]. *B. ramiflora* seeds appear to reach physiological maturity at 96 DAA, when both seeds and embryos attained their maximum fresh weights of 64.7 ± 3.8 mg, 31.7 ± 6.5 mg and dry weights of 28.5 ± 1.6 mg, 14.0 ± 4.9 mg, respectively. In other studies, germination ability increased with seed development[16–18], but in this study most seeds germinated at 76 DAA, and germination rate decreased with further development with fewest seeds germinating at 96 DAA (22%), but the excised embryo reached 93% (Fig. 3), and we observed the same pattern at other stages. Apparently, the tissues enclosing the embryonic axis, such as pericarp, cotyledon, and testa, may inhibit growth elongation along the embryonic axis under conditions conducive to germination, which may have an important role in preventing viviparous germination[18]. Alternatively, the testa of *B. ramiflora* would prevent moisture loss in seeds and maintain longer viability, especially in the case of continuous high temperature. Rapid dehydration treatment can weaken this inhibitory effect on germination along with seed development, and the most significant stage was at 96 DAA.

In summary, like Yu et al[10], we found that temperature but not light is an important factor for *B. ramiflora* seed germination, and seeds almost could not germinate below 20 °C. Optimal seed germination occurs at 30/20 °C in either light or darkness. Desiccation tolerance depends not only on the inherent characteristics of the species, but also on the developmental status of the seeds, which is the key factor for long-term storage of recalcitrant seeds. Based on our results, the best time for collection of *B. ramiflora* seeds for storage is around 96 DAA when desiccation tolerance is maximal.

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