Title page

Article title: Effect of stratification and gibberellic acid on epicotyl dormancy release in seeds of *Yunnanopilia longistaminea* (Opiliaceae)

Types of contribution: Notes and Comments

Running title: Seed germination of *Y. longistaminea*

Authors: Guan-Song Yang, Liu Yang, Yue-Hua Wang[*], Shi-Kang Shen[*]

[*] School of life sciences, Yunnan University, Kunming, 650091, the People's Republic of China

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Address for Correspondence: Yue-Hua Wang: Professor of Botany, School of Life Sciences, Yunnan University, No. 2 Green lake North road Kunming, Yunnan, 650091, the People's Republic of China.

Telephone: +86-871-65031491; Fax: +86-871-65031491;

E-mail: ygsyang@sohu.com (**Yue-Hua Wang**)

Abstract:

Yunnanopilia longistaminea is an endangered monotypic species belonging to Opiliaceae. This edible plant is an important germplasm source with a high economic value in China. The seed dormancy and germination of Opiliaceae species have been rarely investigated. This study examined the effects of scarification, soaking in gibberellic acid, and dehydration on the seed germination of *Y. longistaminea*. Results indicated that the seed germination of this species involves two stages: radicle emergence and epicotyls (shoot) emergence. During radicle emergence, the optimum temperatures were 28 °C and 28 °C/20 °C. Seed moisture content and viability decreased as dehydration occurred. Thus, the seeds may be recalcitrant. The optimum GA_3 solution for the seeds undergoing shoot emergence was 100 mg·L^{-1}. The percentages of shoot emergence in 7 and 14 days of stratification at 5 °C were slightly higher than those in other groups. This study is the first to describe epicotyl dormancy in *Y. longistaminea* seeds. From the seed grow to the seedling of Y. longistaminea subjected to a autumn→winter→spring temperature process in nature conditions. Warm and cold stratification can alleviate radicle and epicotyl dormancy, respectively. The duration of cold stratification also significantly affects the epicotyl dormancy release of Y. longistaminea. The researches on the seeds breaking methods: warm(28°C/20°C)→cold(5°C)→GA3(100mg·L−1)→warm(28°C/20°C).

Key words: *Yunnanopilia longistaminea*, epicotyl dormancy, radicle emergence, endangered plant, stratification

Introduction

Seed dormancy is a survival mechanism through which germination time and distribution area are adjusted on the basis of different environmental conditions (Copete et al., 2011). Temperate plant seeds are typically dormant in the seed formation period; as such, these seeds require an appropriate temperature to germinate and for the seedlings to grow (Baskin and Baskin, 2004; Baskin et al., 2000). Dormancy ensures seed germination at appropriate temperature or rainfall conditions; this phenomenon also results in a high probability of successful seedling establishment (Bewley et al., 2006).

Yunnanopilia longistaminea (W. Z. Li) C. Y. Wu et D. Z. Li, a monotypic species belonging to Opiliaceae, is a vulnerable plant endemic to Red River Valley, Yunnan Province, Southwest China. As the sole member of a geographically isolated genus, *Y. longistaminea* plays a significant part in the phylogeny and evolution of Opiliaceae. Furthermore, *Y. longistaminea* is an important resource plant consumed as a delicious wild woody vegetable. However, this species is endangered because of excess utilization and habitat destruction. As a key component of germplasm conservation and access to wild plant resource, artificial propagation, including seed germination and seedling establishment, is an essential step in conservation procedure plans (Shen et al., 2015; Khanna et al., 2013). However, the germination characteristics of *Y. longistaminea* have yet to be investigated.

Limited information on seed dormancy and germination in Opiliaceae has been published. Willis et al. (2014) presented the phylogeny of seed plant families with current dormancy classes and proposed that Opiliaceae seeds exhibit morphophysiological dormancy (MPD). A common type of this condition is deep simple epicotyl MPD, generally referred to as epicotyl dormancy. Epicotyl dormancy occurs in many species growing in various vegetation types. In this case, various temperature conditions, in addition to the epicotyl, can break radicle dormancy; warm and cold temperatures independently alleviate radicle and epicotyl dormancy (Baskin and Baskin, 1998; Barton, 1933; Nikolaeva, 1977). We hypothesized that *Y. longistaminea* seeds may undergo epicotyl dormancy. This study generally aimed to characterize the germination requirements for radicle and epicotyls (shoot) emergence of *Y. longistaminea*. The desiccation tolerance of *Y. longistaminea* seeds was also examined because large seeds are sensitive to dehydration.

Materials and methods

Seed material and seed collection

Y. longistaminea is mainly distributed in the Red River Valley in Yunnan, Southwest China. All individual plants were distributed vertically from 1,500 m to 3,000 m in elevation. The annual mean temperature was 18 °C with a minimum of 5.2 °C in January and a maximum of 35.8 °C in July. The annual average rainfall was approximately 2000 mm, which is mostly recorded between June and September. The region is characterized by a typical high humidity geographical environment with an annual mean relative humidity of more than 80%. There were two stages in Y. longistaminea of seeds seedlings, the first stages was radicle-emergence; the second stages was shoot emergence (epicotyl-plumule emergence). Radicle-emergence procedure includes four processes: embryo growth, testa rupture, endosperm rupture. In the study, radicles grown to $\geqslant$ 2 mm, shoot grown to $\geqslant$ 5 mm were considered as radicle-emergence and epicotyl-plumule emergence. All experiments were finished when the surplus seeds (no germinated seeds) were for a consecutive one month until eventually rotted out.

In the nature, Y. longistaminea drupes reach maturity between June to July, and seedlings emerge in spring next year. In this study, mature Y. longistaminea fruits were manually harvested in germination tests started one week after collection. 2015. Y. longistaminea drupes were full grow, it got a yellow tone on the skin. The drupes yellow were selected within the sarcocarp to softening. The seeds were manually separated from the drupes and divided into two portions. One portion was reserved in paper bags for germination tests; the other portion was immediately placed under moisture-controlled condition for desiccation tolerance tests (Kunming, Yunnan Province).

Seed morphology, weight, and moisture content

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The dimensions (breadth and length) of the seeds were measured by using a vernier caliper in triplicates and the 1000-seed-weight was determined. Moisture content was examined gravimetrically at 103 °C ± 2 °C for 17 h in accordance with standard procedures. The final moisture content was expressed as dry weight basis (%) (Shen et al., 2010). Four replicates were used to determine moisture content expressed as dry mass basis.

Effect of stratification and desiccation on radicle-emergence

Stratification

Seeds were surface-sterilized with 0.85% sodium hypochlorite for 1 min and washed with distilled water. Fresh seeds were placed on moist autoclaved fine sand in a plastic box (25 × 10 × 8 cm length, width, height). The seeds were incubated within a 12 h/12 h photoperiod at daily alternating temperatures of 28 °C/15 °C and 28 °C/20 °C and at constant temperatures of 5 °C, 15 °C, 20 °C, 25 °C, 28 °C, and 30 °C. All temperatures were set in four replicates with 10 seeds in each replicate. The seeds were then checked for radicle emergence every 5 days throughout each treatment duration. Distilled water was added as needed to ensure that moisture was non-limiting during germination.

Desiccation

The seeds were dried for 0, 6, 9, 12, 24, and 36 h in an airtight glass desiccator overlaid with activated silica gel. The moisture content of the seeds was determined in weight in each drying treatment. Afterward, four replicates of dried seeds were tested for germination at 28 °C/20 °C in daylight for 12 h.

Effect of GA_3 treatment and cold stratification on the epicotyl dormancy release of radicle-emerged seeds

GA_3

This experiment aimed to determine whether GA_3 eliminates the epicotyl dormancy of radicle-emerged *Y. longistaminea* seeds. The seeds with the same radicle lengths (about 5 cm) were independently soaked in 0, 50, 100, and 200 mg·L^{-1} GA_3 (95% purity, Sigma) for 10 h at the same temperature set to test the epicotyl plumule emergence. The test conditions were the same as described above.

Cold stratification

The seeds with similar radicle lengths (about 5 cm) were stratified at 5 °C. During stratification, Four replicates of 10 seeds were removed and monitored for epicotyl-plumule emergence after 0, 7, 14, 21, and 35 days. The seeds with observed epicotyl-plumule emergence were transferred into a temperature- and light-controlled incubator (BSG-400 Illuminating Incubators, Shanghai, China) and incubated at an alternating temperature of 28 °C/20 °C in the plastic boxes. Epicotyl dormancy was considered broken when the cotyledons emerged partly out of the seed coat (Hidayati et al., 2005).

Statistical analysis

The shoot emergence percentage (SP) and the final radicle emergence percentage (REP) were determined as follows:

$$\text{Radicle emergence percentage } (\%) = \left(\frac{\text{number of radicle emerged seeds}}{\text{number of seeds per sample}}\right) \times 100$$

$$\text{Shoot emergence percentage } (\%) = \left(\frac{\text{number of shoot emerged seeds}}{\text{number of radicle has emerged}}\right) \times 100$$

Arcsine transformation was applied for REP and SP data before statistical analysis was conducted to ensure variance homogeneity. Data given in figures were not transformed. The factors influencing REP and SP were analyzed through ANOVA. If a significant difference was detected by ANOVA, Fisher's least significant difference (LSD) was applied to accomplish multiple comparison tests. Statistical analyses were performed in SPSS 18.0.1. The critical level of significance was $P = 0.05$ in all of the tests.

RESULTS

Characteristics of Y. longistaminea seeds

Y. longistaminea seeds generally consist of a seed coat, an embryo, an endosperm, and a cotyledon. The fresh seeds were large with 19.12 ± 1.22 mm (average ± SE, *n* = 10) in length and 12.68 ± 0.63 mm (average ± SE, *n* = 10) in width. The 1000-fresh seed weight was 2580 ± 108 g. Indeed, *Y. longistaminea* seeds are large.

Effects of stratification and desiccation on radicle growth

Stratification

The radicle emergence of *Y. longistaminea* seeds is sensitive to temperature. Radicles did not emerge from any of the newly matured seeds at 5 °C. The optimum temperatures for radicle emergence were 28 °C and 28 °C/20 °C. High and low temperatures significantly decreased the REP (Fig.1).

Desiccation treatments

The moisture content of *Y. longistaminea* seeds steadily decreased for 24 h of drying, and 19.23 ± 1.45% moisture was reached after 24 h of dehydration. The REP of the seeds decreased during dehydration. The REP decreased from 38.89 ± 4.81% to 0 ± 0% when the seed moisture content decreased from 25.6 ± 1.55% to 19.23 ± 1.45% (Fig. 2). This finding indicated that REP is sensitive to dehydration.

Effect of GA_3 treatment and stratification on the epicotyl dormancy release of radicle-emerged seeds

GA_3

The optimum GA_3 solution for the shoot emergence of radicle-emerged seeds was 100 mg·L^{-1}. Although 50, 200, and 0 mg·L^{-1} (control) did not significantly differ, the SP of the seeds with emerged radicles was improved by the GA_3 treatment (Fig. 3). The mean germination time decreased rapidly.

Cold stratification

After 7, 14, and 21 days of cold stratification at 5 °C, the shoot emergence was significantly increased to 86.67 ± 5.77%, 80 ± 10%, and 66.67 ± 5.77%, respectively. By contrast, SP declined when the duration of cold stratification was prolonged (Fig. 4).

Discussion

The seed germination of *Y. longistaminea* involves two stages: radicle and epicotyl emergence. Warm stratification can break radicle emergence and cold stratification can stimulate the epicotyl emergence of radicle-emerged seeds. GA_3 treatments can also improve both radicle and epicotyl emergence. According to seed dormancy classification criteria, *Y. longistaminea* seeds undergo epicotyl dormancy. Numerous species in the Araceae, Araliaceae, Aristolochiaceae, Berberidaceae, Gentianaceae, Liliaceae, Papaveraceae, and Ranunculaceae families also experience epicotyl dormancy (Baskin et al., 2004; Baskin and Baskin, 2005; Adams et al., 2003; Baskin et al., 1993; Baskin and Baskin, 2004; Kondo et al., 2004; Karlsson et al., 2003; Walck et al., 2000). However, this study is the first to report an Opiliacea species with epicotyl dormancy.

Y. longistaminea seeds that dispersed in June-July germinated in the field in the following spring. The drupes reach maturity between June to July, and seedlings emerge in spring next year in the nature conditions, the process time is about 10 months. The time of Y. longistaminea seeds germination was shorter after stratification and GA_3 treatment. In this case, they were exposed to a sufficient warm stratification period after they were dispersed for radicle emergence. They also received cold stratification in winter for epicotyl emergence. This finding is similar to those observed in other species with epicotyl dormancy (Hidayati et al., 2005). However, the embryonic growth in *Y. longistaminea* seeds during radicle and epicotyl emergence should be further investigated.

Dehydration-sensitive seeds typically grow in large, broadleaf evergreen tropical or subtropical zones and then mature in the rainy season (Pammenter and Berjak, 2000; Shen et al., 2015). In our study, *Y. longistaminea* seeds matured in the rainy season (June) at >25 °C in their natural habitat. In the dehydration test, the seed moisture content of *Y. longistaminea* decreased gradually during desiccation. Seed viability also declined as the duration of dehydration was prolonged. Thus, *Y. longistaminea* seeds are recalcitrant.

In summary, this study is the first to demonstrate that *Y. longistaminea* seeds undergo epicotyl dormancy and exhibit recalcitrance. Considering the high frequency of human disturbances in the natural habitat of this endangered species, we speculate that the prolonged duration of *Y. longistaminea* seed germination may affect its natural regeneration. Thus, we recommend the artificial promotion of dormancy release and germination for its conservation and management. The studies should be proceed with GA_3 and temperature for embryo becoming coarser in the future.

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Figure legends

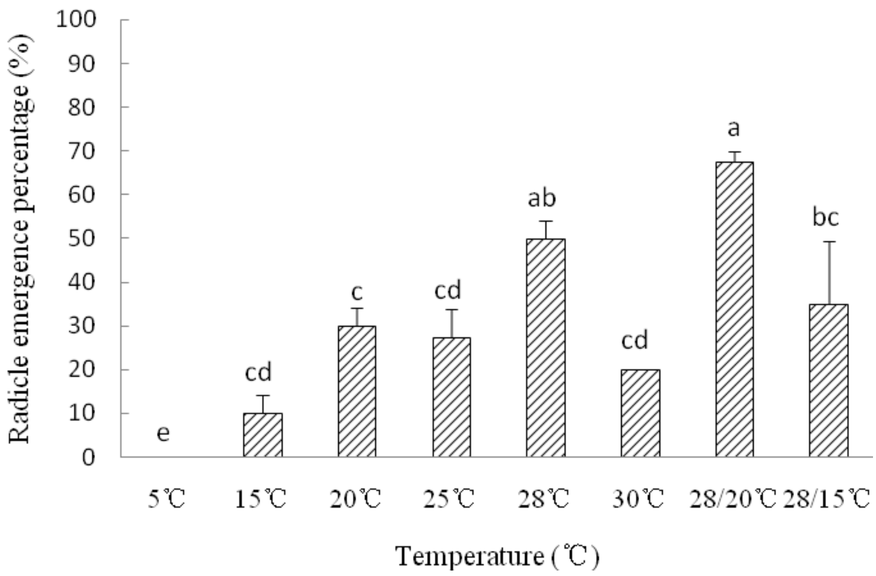

Fig. 1. Seed radicle emergence of *Y. longistaminea* at different temperatures and a photoperiod of 12/12 h day/night. Values are mean ± standard error(n = 3). Different letters indicate significant differences between the temperatures ($P < 0.05$).

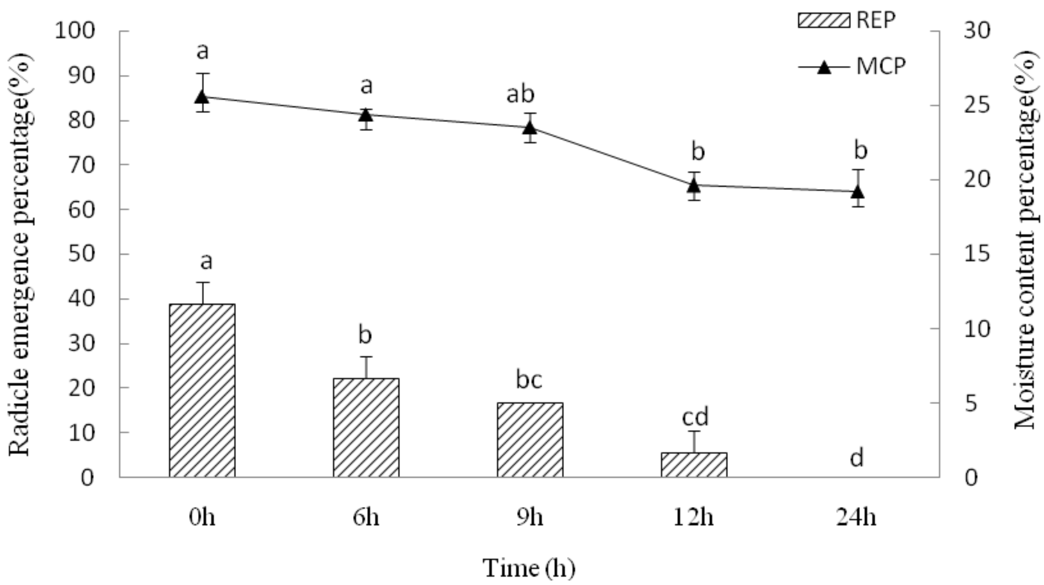

Fig. 2. Seed radicle emergence of *Y. longistaminea* at different moisture content and dehydration time. Values are mean ± standard error(n = 3). Different letters indicate significant differences between the dehydration time (P <0.05).

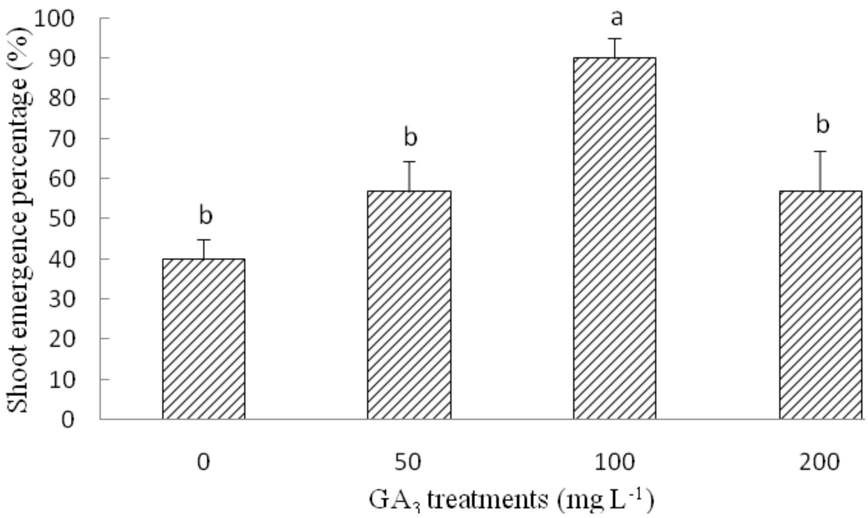

Fig. 3. Effect of gibberellic acid (GA_3) treatment with shoot growth of *Y. longistaminea* under a 12/12 h photoperiod and 28/20 °C. Values are mean ± standard error (n = 3). Different letters indicate significant differences between the different GA_3 solutions ($P < 0.05$).

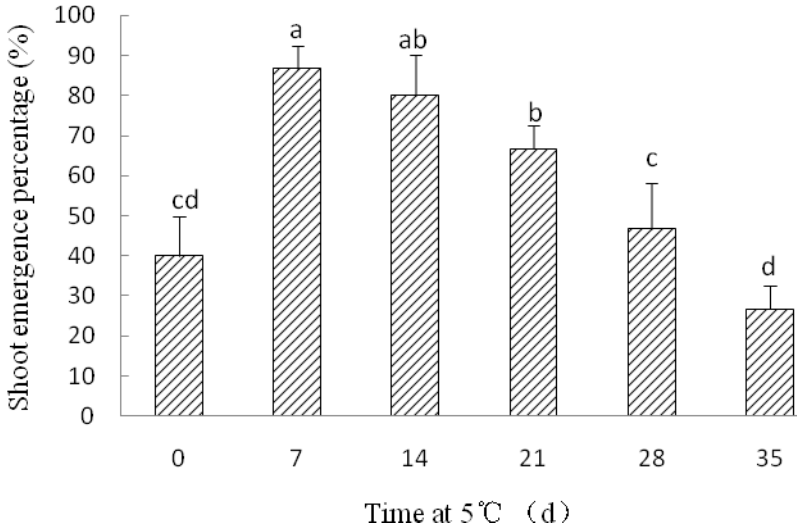

Fig. 4. Effect of shoot growth in germinated seeds of *Y. longistaminea* following 0-35 days of cold treatment at 5°C under a 12/12 h photoperiod and 28/20 °C. Values are mean ± standard error (n = 3). Different letters indicate significant differences between the different cold treatment(P <0.05).