



# Geographic variations in seed germination of *Dalbergia odorifera* T. Chen in response to temperature



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## ABSTRACT

Temperature plays an essential role in regulating seed germination and seedling production. As adaptation of germination requirements can occur in response to slight differences of the local climate, studying the intra-species variations of seed germination is of great importance for its large-scale conservation, introduction and seedling production. In the present study, germination of *Dalbergia odorifera* from four geographic sites (namely Ledong in Hainan province, Pingxiang in Guangxi Zhuang Autonomous region, Zhaoqing in Guangdong province and Longhai in Fujian province) were investigated under five constant temperatures (15 °C, 20 °C, 25 °C, 30 °C and 35 °C). The results showed that there existed statistically significant differences ( $P < 0.001$ ) among these localities, temperatures and their interactions in terms of final germination percentage (FGP) and mean time to germination (MTG). Seeds from all these localities demonstrated the lowest FGP value (27.3%, 0.0%, 8.0% and 10.0% in average) under the temperature of 15 °C, while 25 °C was the optimal germination temperature for seeds collected from Ledong and Pingxiang, and 30 °C for seeds collected from Zhaoqing and Longhai. It could be deduced that intra-specific variations in the optimal germination temperature might occur over a small scale in southern China.

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## 1. Introduction

*Dalbergia odorifera* T. Chen (Leguminosae) is a semi-deciduous tree species endemic to Hainan province, southern China. The wood makes high-grade furniture and extracts from the heartwood are renowned for medicinal properties known as *Jiangxiang* in Traditional Chinese Medicine (Cheng et al., 1998; Choi et al., 2009; Sugiyama et al., 2002; Wang et al., 2000; Yukihiro et al., 1985). Roots and seeds also have special applications in pharmaceutical industry (Yu et al., 2007; Zheng et al., 2011). It has been introduced to subtropical areas of Guangdong, Guangxi and Fujian provinces of China since 1950s (Huang, 1995; Yao et al., 2012). After several decades of domestication, *D. odorifera* have exhibited satisfactory growth performance in most forest sites, and formed valuable heartwood (Li, 2012; Zhu et al., 1997).

Germination traits for tree species especially valuable tree species have important implications for conservation, introduction and plantation establishment. However, seed germination requirements or patterns within a species often vary in response to small differences in environmental conditions (Abe and Matsunaga,

2011; Bevington, 1986; Honěk and Martinková, 1996; Humara et al., 2000; Shimono and Kudo, 2003), and it was considered to be a result of an integration of genetic and environmental factors (Keller and Kollmann, 1999; Meyer et al., 1989). Temperature is one of the most important environmental factors in regulating seed germination (Eberle et al., 2014; Kumar et al., 2011; Verma et al., 2010). Seeds collected from different provenances or localities may differ greatly in the optimal germination temperature (Abe and Matsunaga, 2011; Humara et al., 2000). Several studies have clearly demonstrated the intra-species variations of optimal germination temperature among different collection sites, populations and provenances (Abe and Matsunaga, 2011; Humara et al., 2000; Meyer et al., 1989; Venier et al., 2015). However, there has been no such information for *D. odorifera*.

Due to the economic value and ecological significance in nitrogen fixation (Lu et al., 2012, 2013). *D. odorifera* plantations have been expanded rapidly in southern China in recent years. Propagation from seed is still the main approach for seedling production currently, the four major planting areas of *D. odorifera* in southern China viz. Hainan, Guangdong, Guangxi and Fujian differ considerably in the maximum and minimum temperatures. Therefore, understanding the optimal germination temperature of *D. odorifera* seeds for each region is critical for successful afforestation programs. In the present study, variation in seed germination char-

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acteristics of *D. odorifera* seeds from different localities in response to temperature was examined, and the optimal temperatures for germination were determined for each locality. Results from this study will provide useful information for seedling production and plantation establishment of *D. odorifera* in southern China.

## 2. Materials and methods

### 2.1. Seed collection and pretreatment

Mature fruits of *D. odorifera* were collected in December, 2013 from four locations (namely Ledong in Hainan province, Pingxiang in Guangxi Zhuang Autonomous region, Zhaoqing in Guangdong province and Longhai in Fujian province), which representing the main different geographic regions in southern China. Information of environmental conditions for each region is shown in Table 1. Fruits from each location were bulk of ten parent trees. Fruit coats were removed manually, and thereafter air-dried for 48 h. Seed moisture varied from 14.5% to 21.7% with an average of 18.5%. Fifty seeds were sampled randomly from each seed lot for size and mass measurements. The seeds were stored separately in a large desiccator filled with silica gel until the beginning of the experiment. The temperature was maintained at 17–23 °C with a relative humidity of 40–50%. Before germination tests, seeds were surface-sterilized with 3% (v/v) sodium hypochlorite for 5 min, and then washed with distilled water twice. To stimulate germination, all seeds were soaked in sterilized distilled water under room temperature for 12 h.

### 2.2. Germination test

The germination test was carried out in growth chambers during January–February, 2014. Seeds were placed on top of a filter paper and soaked with sterilized distilled water in Petri dishes (15 cm diameter × 2.5 cm deep), then incubated separately under five constant temperatures (15 °C, 20 °C, 25 °C, 30 °C and 35 °C) in dark conditions. Light was not required during the whole germination process according to the field practice (Deng, 2013; He et al., 2008). Each dish consisted of 50 seeds and replicated four times, thus a total of 80 Petri dishes were used in this experiment.

Germinated seeds were counted daily and removed after counting. Sterilized distilled water was added to the Petri dishes to maintain adequate moisture during seed germination. Emergence of a 2 mm-long radicle was used as the criterion for germination. Germination was monitored until no new germinated seeds were observed over 5 consecutive days, and a total of 40 days were conducted in the experiment. Germination percentage (GP), final germination percentage (FGP) and mean time to germination (MTG) were determined for each seed location to estimate the germination capacity and rate under different temperatures using the following formulas (Czabator, 1962; Xiao et al., 2010):

$$GP(\%) = \frac{n_i}{A} \times 100$$

$$FPG(\%) = \frac{N}{A} \times 100$$

$$MTG = \frac{\sum(n_i \times d_i)}{N}$$

where  $N$  is the total number of seeds germinated at the end of test,  $A$  is the total number of seeds tested in each treatment,  $n$  is the number of seeds germinated at day  $i$ , and  $d$  is the incubation period in days at the time of counting.

### 2.3. Statistical analysis

Seed dimension and mass were subjected to a one-way analysis of variance (ANOVA), and Tukey's multiple comparison tests were used to compare the means between each localities. A general linear model (GLM) including locality, temperature and their interactions as fixed factors was employed to demonstrate various factors affecting the FGP and MTG. An arcsine transformation was applied to FGP before ANOVA in order to make the variances more homogeneous. The Spearman rank correlation coefficients were used to analyze the correlations between seed mass and dimension. All statistical analyses were performed using IBM SPSS statistics Version 18 (IBM Corp., 2010), and  $P < 0.05$  was used as the criterion for rejecting the null hypothesis.

## 3. Results

### 3.1. Germination percentage (GP)

*D. odorifera* seeds began to germinate 2–4 days after treatment except for the seeds from Pingxiang under the temperature of 15 °C (Fig. 1b). The first germination was observed on 2nd day at 30 °C for seeds from Longhai, 3rd day at 30 °C for those from Ledong, 4th day at 30 °C for those from Zhaoqing and 5th day at 35 °C for those from Pingxiang. For each locality, GP tended to increase as the incubation period increased, and reached the maximum under a given period, then finally dropped to zero at the end of the test. The incubation period and temperature where GP was the highest differed greatly among seed localities. For example, for seeds collected from Ledong, Pingxiang and Longhai, GP reached the highest (19.3%, 24.7% and 37.8% on average) under the temperature of 25 °C, and the corresponding incubation period was 10th, 16th and 16th day after treatment, respectively (Fig. 1a, b, and d). For seeds collected from Zhaoqing, GP reached the peak (21.1% on average) at 30 °C, and the corresponding incubation period was 11th day after treatment (Fig. 1c). Seeds collected from all localities exhibited the lowest germination percentage under the lowest temperature (15 °C). Moreover, no germination was observed for seeds from Pingxiang under such low temperature.

### 3.2. Final germination percentage (FGP)

FGP was significantly affected by seed locality ( $F=46.238$ ,  $P<0.001$ ), temperature ( $F=128.205$ ,  $P<0.001$ ) and their interactions ( $F=15.680$ ,  $P<0.001$ ). For each locality, FGP increased with temperature to reach a highest value under a given temperature, then decreased as the temperature increased further (Fig. 2), but the germination temperatures where FGP reached the highest values varied from each other. For example, the highest FGP was observed at 25 °C for seeds collected from Ledong, Pingxiang and Longhai, the corresponding FGP was 82.0%, 86.0% and 100%, respectively. While for seeds collected from Zhaoqing, the FGP value was the highest (100%) under the temperature of 30 °C (Fig. 2). Seeds from all localities recorded the lowest FGP under the temperature of 15 °C; only 27.3%, 0.0%, 8.0% and 10.0% of seeds respectively had germinated at the end of test (Fig. 2).

### 3.3. Mean time to germination (MTG)

Under the temperature of 15 °C, no seeds from Pingxiang region had germinated, therefore the MTG at 15 °C was not calculated and compared. MTG was greatly affected by locality ( $F=45.005$ ,  $P<0.001$ ), temperature ( $F=234.564$ ,  $P<0.001$ ) and the interactions of both parameters ( $F=7.078$ ,  $P<0.001$ ) through a two-way ANOVA. The minimum MTG were observed at the temperature of 30 °C for

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