

Variation in seed and seedling traits among natural populations of *Trigonobalanus doichangensis* (A. Camus) Forman (Fagaceae), a rare and endangered plant in southwest China

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Abstract The extent of genetic variation in seed and seedling traits of *Trigonobalanus doichangensis*, a rare and endangered tree in southwest China, was evaluated at the population level. Seeds were collected from four natural populations in the south and southwest Yunnan province, China. Significant differences ($P < 0.05$) between populations were observed in seed length, seed weight and seedling morphological characters. The analyses revealed that none of the seed and seedling traits had significant correlations with the geo-climatic variables of the populations. Among seed and seedling traits, significant correlations ($P < 0.05$) were discovered among seed length, seed weight and height of 30-day-old seedlings. Significant correlation ($r = 1.00$, $P < 0.01$) occurred also between the height and root collar diameter of 4-year-old seedlings. With the exception of seed width and seed germination percentage, 51–96% of total variation in other seed and seedling related traits was attributed to population effect, as determined by the broad sense heritability. In conclusion, these observations on variation in seed and seedling traits should be a reference point, when considering seed collection of this species for ex situ conservation and species restoration.

Keywords Ex situ conservation · Genetic variation · Natural populations · Seed and seedling traits

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Introduction

The small genus *Trigonobalanus* Forman that was described in 1964 has a present-day intercontinental disjunct distribution in the New World and Asia, areas that are considered a remnant of a former widespread distribution in the tertiary period or before (Sun et al. 2007). The genus *Trigonobalanus* morphologically shares the characteristics with both *Fagus* and *Quercus* (Forman 1964; Crepet and Nixon 1989; Nixon and Crepet 1989; Li et al. 2003) and it has been considered an important taxa to understand phylogeny and biogeography of Fagaceae (Zhou 1992, 1999). The genus includes three species and *T. doichangensis* is the only one occurring in China (Hsu et al. 1981). *T. doichangensis* is now restricted to Thailand and south and southwest Yunnan in southwest China (Fu 1992; Sun et al. 2004). The known localities of this species in China are situated in areas bordering southeast Myanmar, south of the Tropic of Cancer. The climate is of the south subtropical type (EBYV 1987) and the soil is mainly laterite (Xiong 1987).

T. doichangensis has been felled for firewood, house building and making agricultural tools. Its habitat has been greatly degraded while clearing for agriculture. As a scientifically important tree with endangered status, the species has been proposed as a second-ranked plant for national protection in China (Anonymous 1999). At present, *T. doichangensis* is facing a high risk of extinction in the wild (Sun et al. 2006). Practical conservation measures, which can ensure conservation of the maximum genetic diversity of the species, are urgently required. Knowledge of the extent and pattern of intra-specific variation in *T. doichangensis* is essential for both its ex situ and in situ conservation.

Sun et al. (2007) explored the genetic diversity of the species, Zhou et al. (2003) and Zhou (2003) conducted seed morphology and seed germination tests. Seed propagation would be preferred for ex situ conservation, since this would be least detrimental to the extant populations and would include the widest range of genetic diversity (Sun et al. 2006). Thus, genetic analysis of the seeds collected is essential for a successful ex situ conservation program. In order to provide valuable information on sampling for seed collection and compare the findings of previous genetic diversity studies based on the DNA marker RAPD (Sun et al. 2007), this study was done (a) to evaluate the genetic diversity among the four extant populations of the species in southwest China, by analyzing morphological differences in seed and seedling traits, and (b) to examine inter-character correlations and associations between seed characters and geo-climatic variables of the populations.

Materials and methods

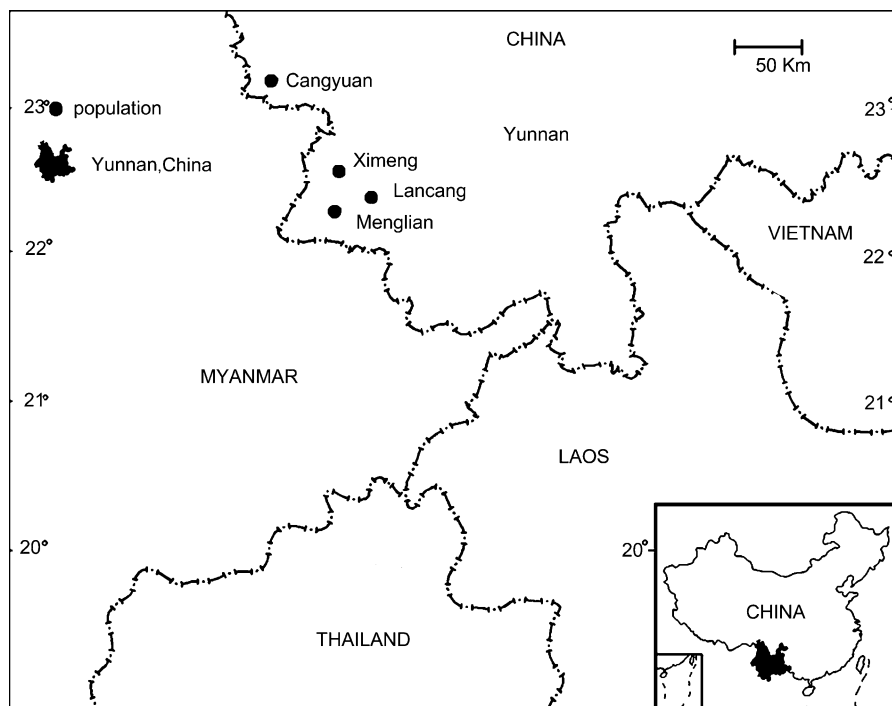
Seed collection

Seeds (botanically called nuts) were collected from the four sites representing the natural populations of the species in China. The geographic locations and climatic conditions of these sites are shown in Table 1 and Fig. 1 and further described in Sun et al. (2004).

Seeds were collected between April and June 2002. The sampled trees per site ranged from 10 to 20 depending on the seed bearing specimens at each site. To ensure inclusion of maximum genetic variation, the selected trees were 50–100 m apart from each other. The seeds collected from each site were brought back to the laboratory at Kunming Botanic Garden (KBG) within 5 days. Then the seeds from individual trees were equally sampled by number, and bulked by population for the experiments. Seed processing, measurement and germination experiments were conducted afterwards.

Table 1 Geographic locations and climatic conditions of seed collection sites

Sites	Latitude (°N)	Longitude (°E)	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperature (°C)
Menglian	22.30	99.53	1,020	1,363.3	27.4
Lancang	22.43	99.80	1,450	1,626.5	27.3
Cangyuan	23.32	99.98	1,730	1,741.1	24.9
Ximeng	22.60	99.63	1,040	2,739.0	19.6

**Fig. 1** Seed collection sites of *Trigonobalanus doichangensis*

Assessment of seed traits

To evaluate the variability in seed characters, seed length, seed width, and 1,000-seed weight were quantified. Seed length and seed width were measured individually using a digital micro caliper. Four replicates of 50 seeds each per site were randomly selected for measurement. For seed weight, four replicates of 100 seeds each per site were weighed and then the 1,000-seed weight was calculated.

Germination tests and seedling traits

Seed germination and seedling growth of *T. doichangensis* originating from the four sites was investigated at KBG (Latitude of 25°01'N and longitude of 102°41'E, at an elevation of 1,990 m). Four replicates of 100 seeds each per population were randomly sampled and

sown in plastic pots in a completely randomized design, under natural conditions (18–25°C). A potting medium of laterite and humus (1:1 by volume) mixture was used. Germination was monitored every day until the seedlings were transplanted. Seedling height was determined 30 days after germination. Height and root collar diameter of 4-year-old seedlings were also measured.

Statistical analysis

Statistical analyses were performed using SPSS 11.0. The data were subjected to one-way analysis of variance (ANOVA). Germination percentage followed a non-normal distribution and was thus subjected to arcsine transformation before statistical analysis. Significant differences between means were tested by Fisher's least significant difference (LSD). Relationships between parameters were examined using the Pearson's product-moment test.

To compare the magnitude of variation due to population and environmental and other non-genetic effects, population coefficient of variation (GCV) and environmental and other non-genetic coefficient of variation (ECV) were computed for the seed and seedling characters using population variance (V_g), environmental variance (V_e) and the overall mean as shown below:

$$\text{GCV} = (V_g)^{1/2} / \text{Mean}$$

$$\text{ECV} = (V_e)^{1/2} / \text{Mean}.$$

To determine to what extent the population variation contributed to the total variation, broad sense heritability (H^2) was calculated as follows:

$$H^2 = V_g / (V_g + V_e).$$

Results

Seed morphology

Seed length and 1,000-seed weight, but not seed width, differed significantly ($P < 0.05$) among populations. The average seed length, seed width and 1,000-seed weight of populations are shown in Fig. 2. The mean seed length varied from 5.29 ± 0.11 (Menglian) to 5.71 ± 0.09 mm (Lancang) among the populations (total mean = 5.46 ± 0.19 mm). The seed weight of populations differed significantly ($P < 0.05$) from each other. The mean 1,000-seed weight varied from 10.78 ± 0.80 to 16.14 ± 0.53 g. Seeds collected from Lancang were the heaviest while those collected from Menglian were the lightest.

Of the seed morphological characters, none showed significant correlation with geo-climatic variables (Table 2). And only the correlation between seed length and seed weight was significant ($r = 0.97$, $P < 0.05$) (Table 3). Tests of population and environmental and other non-genetic effects indicated that 85.7 and 95.2% of the total variation was attributed to population variation for seed length and seed weight, respectively, and environmental and other non-genetic effects played a major role in seed width (Table 4).

Seed germination

Germination percentage of *T. doichangensis* seeds was generally low (total mean = 6.78 ± 0.72) and did not differ significantly among populations (Fig. 2d).

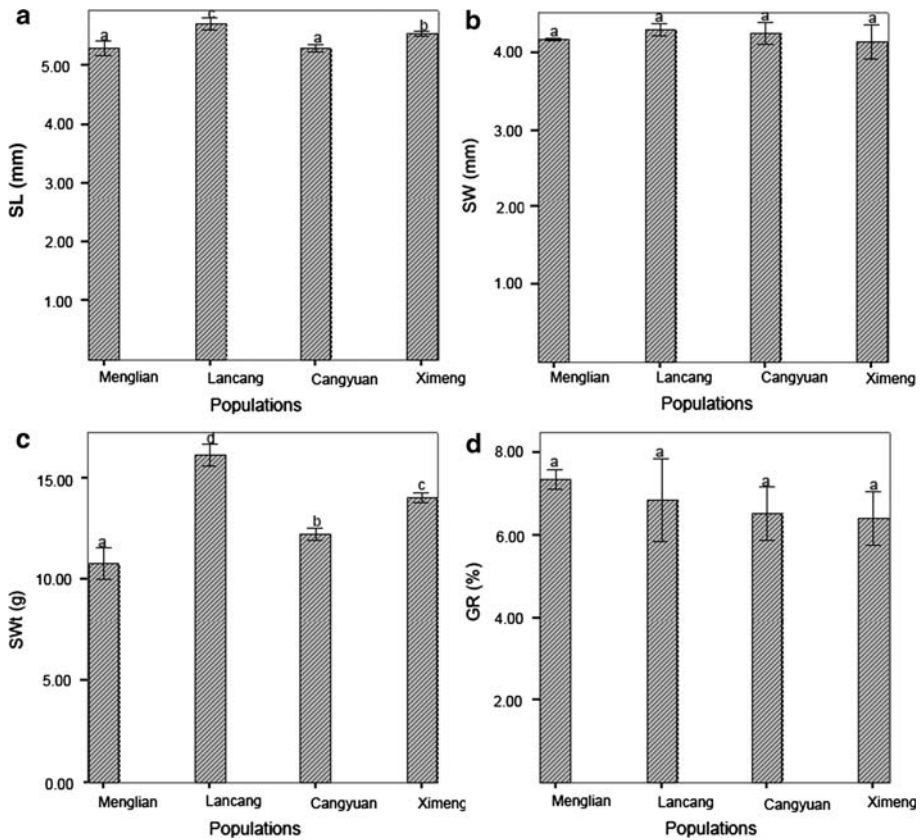


Fig. 2 Mean seed length (a), seed width (b), 1,000-seed weight (c) and seed germination percentage (d) of the four *Trigonobalanus doichangensis* populations (vertical bar represents $\pm$ standard deviation). Bars marked by the same letter are not significantly different at the 0.05 level

Table 2 Correlations between geo-climatic variables of collection sites and seed characters

Characters	Geo-climatic data		
	Altitude	Rainfall	Temperature
SL	-0.01	0.29	-0.09
SW	0.83	-0.47	0.56
Swt	0.20	0.33	-0.13
SH1	0.16	0.12	0.08
SH2	0.66	-0.11	0.26
RD	0.66	-0.08	0.23
GR	-0.36	-0.79	0.78

Values are Pearson correlation coefficient, r

SL seed length; SW seed width; SWt 1,000-seed weight; GR germination percentage; SH1 height of the 30-day-old seedlings; SH2 height of the 4-year-old seedlings; RD root collar diameter of 4-year-old seedlings

Table 3 Intercharacter correlations of seed and seedling parameters of four *Trigonobalanus doichangensis* populations

Parameters	SL	SW	Seed and seedling parameters				
			Swt	SH1	SH2	RD	GR
SL	1	0.40	0.97*	0.98*	0.74	0.73	−0.26
SW		1	0.52	0.57	0.90	0.90	−0.07
Swt			1	0.97*	0.84	0.84	−0.43
SH1				1	0.85	0.84	−0.20
SH2					1	1.00**	−0.30
RD						1	−0.33
GR							1

Values are Pearson correlation coefficient. *SL* seed length; *SW* seed width; *Swt* 1,000-seed weight; *GR* germination percentage; *SH1* height of the 30-day-old seedlings; *SH2* height of the 4-year-old seedlings; *RD* root collar diameter of 4-year-old seedlings

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

Table 4 Population and environmental coefficient of variation, and broad sense heritability for seed and seedling-related characters of *Trigonobalanus doichangensis*

Characters	Overall mean	Coefficient of variation (%)		Heritability (%)
		Provenance	Environment	
SL (mm)	5.46	3.6	1.5	85.7
SW (mm)	4.22	0.6	3.1	3.7
Swt (g)	13.30	17.3	3.9	95.2
SH1 (cm)	6.04	11.8	10.5	55.7
SH2 (m)	3.05	3.3	3.1	51.9
RD (cm)	4.29	12.0	8.1	68.6
GR (%)	6.78	1.8	5.3	10.3

SL seed length; *SW* seed width; *Swt* 1,000-seed weight; *GR* germination percentage; *SH1* height of the 30-day-old seedlings; *SH2* height of the 4-year-old seedlings; *RD* root collar diameter of 4-year-old seedlings

Although not statistically significant, seed germination percentage had a negative correlation ($r = -0.79$) with rainfall and positive correlation ($r = 0.78$) with temperature of seed origin (Table 2). The seed germination percentage had a very weak correlation with all seed morphological traits (Table 3) and it was mainly affected by the environmental and other non-genetic factors, as shown by the relatively low percentage contribution of population variation to the total variation (Table 4).

Seedling growth

The height of 30-day-old seedlings displayed significant differences ($P < 0.05$) among populations. The highest and lowest values were observed for populations of Lancang and Menglian, respectively (Fig. 3a). The height (total mean = 3.05 ± 0.13 m) and root collar diameter (total mean = 4.29 ± 0.58 cm) of 4-year-old seedlings also showed significant differences ($P < 0.05$) among populations (Fig. 3b, c).

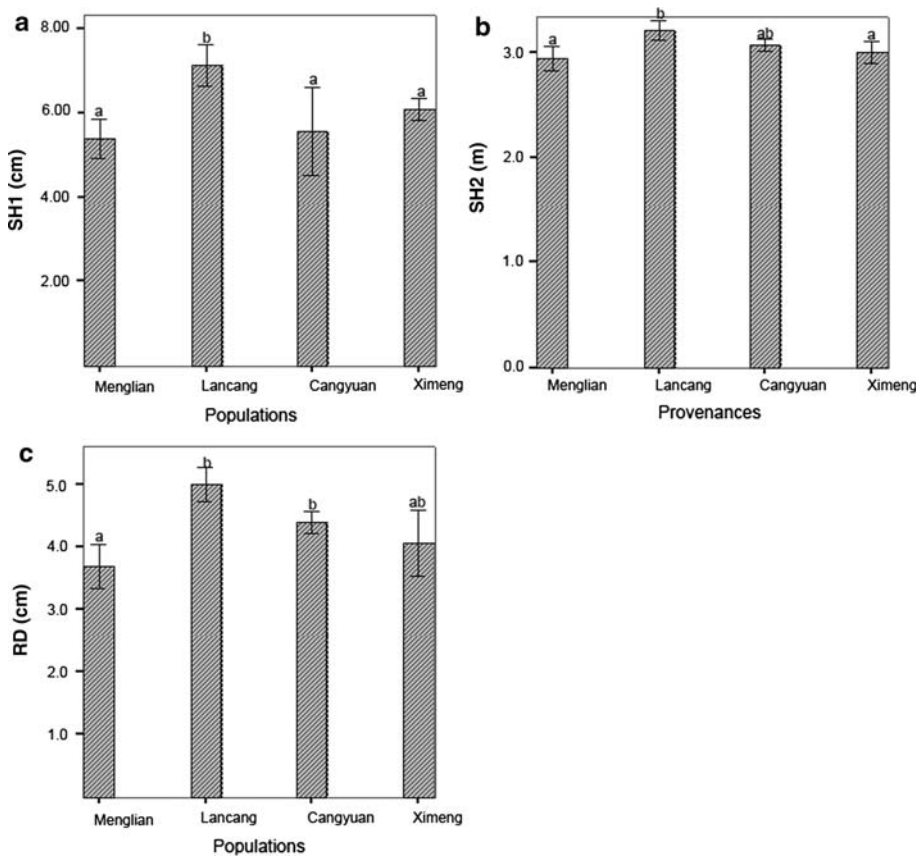


Fig. 3 Mean height (SH1) of the 30-day-old seedlings (a), mean height (SH2) (b) and root collar diameter (RD) (c) of the 4-year-old seedlings of *Trigonobalanus doichangensis* (vertical bar represents $\pm$ standard deviation). Bars marked by the same letter are not significantly different at the 0.05 level

The height of 30-day-old seedlings and height and root collar diameter of 4-year-old seedlings had no significant correlations with geo-climatic variables (Table 2). Among seed parameters, seed length and seed weight showed significant positive correlations ($P < 0.05$) with the height of 30-day-old seedlings. Height and root collar diameter of 4-year-old seedlings were 100% correlated with each other, and both the traits had no significant correlations with all other characters (Table 3). More than 50% of the total variation in seedling height and root collar diameter was attributed to population effect (Table 4).

Discussion

In the present study, the variation in seed morphological traits was observed among populations (Fig. 2). And these traits had weak correlations with geo-climatic variables (Table 2). Some studies dealing with different plant species reported that seed weight increases with altitude (Ayana and Bekele 2000; Holm 1994; Mariko et al. 1993; Boulli

et al. 2001; Oyama 1993; Loha et al. 2006). This does not occur in *T. doichangensis*, and similar results have been reported on *Betula pendula*, *Ranunculus acris* and *Carex flacca* (Holm 1994; Totland and Birks 1996; Pluess et al. 2005). Seed weight has been characterized as one of the co-evolving complex of characters including seed dispersal, seed dormancy, plant biomass, niche specialization and competition ability (Venable and Brown 1988; Rees 1997). Change in seed weight can affect several other traits and phylogenetic constraints may operate against changes in a single trait like seed weight (Hodgson and Mackey 1986; Pigliucci 2003). This can be evidenced by the strong correlation between length and weight of *T. doichangensis* seeds (Table 3). Most of the total variation in seed length and seed weight but not for seed width of *T. doichangensis* was attributed to population effect (Table 4). Other authors have also reported the genetic control of seed morphological traits for several other tree species (Gera et al. 2000; Mkonda et al. 2003; Sivakumar et al. 2002).

Germination percentage of *T. doichangensis* seeds showed a high negative correlation with rainfall, and positive correlation with temperature (Table 2). This might indicate an adaptation of germination and seedling establishment to ecological conditions. As the seed morphological traits of *T. doichangensis* are weakly correlated with percentage seed germination (Table 3), seed morphological traits could not be used to judge the germinability of the species and the traits might be adapted to the seed dispersal, dormancy and other functions. The variation in seed germination percentage of *T. doichangensis* was mainly due to environmental and other non-genetic effects according to the relatively low broad sense heritability (Table 4). Wulff (1995) and Gutterman (2000) reported that maternal factors, such as position of the seed in the fruit/tree and the age of the mother plant markedly influence the germinability of seeds. The point could be confirmed by a parallel study to this that more than 60% of total variation in germinability of *T. doichangensis* was attributed to variation among individual trees within populations (unpublished report). And the variation in the seed germination percentage of *T. doichangensis* might originate from maternal factors, as well as minor environmental conditions.

Significant differences ($P < 0.05$) were found in seedling traits among populations of *T. doichangensis* (Fig. 3). Height of 30-day-old seedlings had, as shown in Table 2, poor correlations with geo-climatic variables, but on the other hand, both height and root collar diameter of 4-year-old seedlings showed relatively high correlations with the altitude. This might suggest that later growth of seedlings is more affected by environment, whereas the emerging seedlings mainly depend on seed reserve for initial growth. This was confirmed by the result that seedling height of 30-day-old seedlings was significantly ($P < 0.05$) correlated with seed length and seed weight. Similar result was reported for *Celtis australis* (Singh et al. 2006). Moreover, Parker et al. (2006) and Rai and Tripathi (1982) reported the positive influence of large seed size and seed reserve on the establishment and early growth of seedlings. Height and root collar diameter of 4-year-old seedlings had 100% correlation, but both those traits had non-significant correlations with traits of seeds and 30-day-old seedlings. Mantovan (2002) reported good correlation between height of 20-day-old seedlings with that of 3 and 7-year-old plants in *Prosopis flexuosa*. Moreover, Loha et al. (2006) reported that for *Cordia africana* Lam., there was a significant strong age–age correlation for mean seedling height after 4 and 8 months of growth. However, early performance of genotypes is sometimes not strongly related to later performance (Mantovan 2002). Therefore, it's necessary that early selection should be done only at a certain age for accuracy in selection (Cundall et al. 2003; Kumar and Singh 2001). As for *T. doichangensis*, further studies would be needed to fix the age at which early selection

can be done. The magnitude of variation due to population effect is over 50% for the seedling traits of *T. doichangensis* (Table 4). It has been reported that there is adequate genetic variability in seedling height growth in several plants (Cundall et al. 2003; Benowicz et al. 2001; Mantovan 2002; Shimizu et al. 2002). However, it is important to consider that broad sense heritability is dependent on experimental conditions and the studied materials, and is therefore unique for the site and experiment.

Most of seed and seedling traits of *T. doichangensis* showed significant differences among populations, confirming the hypothesis that *T. doichangensis* possesses very strong genetic differentiation between its populations (Sun et al. 2007). The environment changes during the evolution of the species, habitat destruction and excessive exploitation may have led to a low gene flow, which in turn resulted in the differences in seed and seedling morphological traits among populations.

Conclusion

Seed and seedling related parameters showed considerable variation among populations of *T. doichangensis*. As the species is facing a high risk of extinction, it is urgent that practical ex situ conservation measures should be undertaken to preserve genetic diversity and maintain multiple specimens. Propagation through seeds is preferred, and collection should be made from large number of individuals in each population, to ensure the widest range of genetic diversity.

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