

Variation in Seed and Seedling Traits among Fifteen Chinese Provenances of *Magnolia officinalis*

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Abstract

Magnolia officinalis seeds were collected from 15 different provenances, ranging from 336 to 1387 m in China. The main objective of this study was to identify germplasm resources of *M. officinalis* by test seed traits, seed germination, seedling traits, and seedling growth rate. There are significant differences among provenances in all studied attributes except relative growth rate and the net assimilation rate. Seed weight exhibited maximum variation among provenances in seed morphometric traits with a significant positive correlation in terms of average annual temperature and average annual rainfall, and negative correlation with latitude of seed origin. Overall average seed germination in nursery was 59.4% varying from 26.6% to 91.2%. Seedling height decreased by geographical latitude gradually from south to north, while stem diameter declines from east to west. That means the geographic variation of the plant at the seedling stage manifests a two-way variation with the latitude and longitude. Seed of Longsheng, Yangxian, Jingning, Wuyishan, Kaixian and Ningqiang provenance have been identified suitable to produce quality seedlings. It can be concluded that the observed patterns of variation will have implications for genetic resources conservation and tree improvement.

Keywords: net assimilation rate, provenances, relative growth rate, seed germination, seed morphology

Introduction

Magnolia officinalis, a deciduous tree propagated from seeds and a traditional Chinese medicinal plant, is distributed throughout the subtropics in China at the range of 300-2,000 m in elevation (Fu, 1992). Individual plants may show some differences in leaf morphology across different fields. It is typically distributed in subtropical broad-leaf forests, flowering from April to May and fruiting in October. The medicinal properties of the plant reside in its bark. The effective ingredients of *M. officinalis* are mainly phenols and alkaloids, of which magnolol and its isomer honokiol absolutely dominate (Shu *et al.*, 2010), is one of the most important constituents of the crude drug prescriptions which are used for the therapy of neurosis, various nervous disorders, including Parkinsonism, and gastrointestinal abnormality (Tong *et al.*, 2002).

Historically, a substantial number of wild populations existed, and were enough to satisfy domestic demands for the cortex. However, in the 1970s, major regions of *M. officinalis* habitat, largely mixed forest, were cut down. As a result, the distribution of wild populations rapidly declined. The species is particularly vulnerable due to its slow initial growth rate and late reproductive maturity. Because of its vulnerable status, *M. officinalis* was listed in the China red data book of plants (Fu, 1992). As one of the more important Chinese medicinal materials, *M. officinalis* has been widely studied. Most studies have focused on effective ingredients, and pharmacology among others

(e.g. Tong *et al.*, 2002). In a traditional opinion, quality of the medicine is mainly influenced by environmental conditions of the producing area, such as climate and soil.

A critical decision in forest resource management is the choice of seed sources for reforestation to ensure a successful crop. Seed zones and seed transfer are essential tools in assisting this decision in order to avoid planting poorly adapted trees (Hamann *et al.*, 2000). Verzino *et al.* (2003) have been suggested to provide guidelines for seed transfer for afforestation and reforestation. Select seed material used in germination is subject of local provenances, because they are expected to be better adapted to local environment conditions facilitating vegetation establishment (Hufford and Mazer, 2003). Selection is expected to favor appropriate responses to local environmental cues that synchronize germination with periods that are optimal for seedling survival. Such a selection may result in site-specific adaptation of germination traits (Bischoff, 2006). Variation in germination behavior that occurs among different populations within the same species has been widely reported (e.g., Marova, 2010). The inter-population variability in germinability can be due to environmental differences or to genetic variation. Indeed, inter-population seed dormancy variability can serve as an adaptation strategy in unpredictable environments (Cruz *et al.*, 2003; Giménez-Benavides, 2005). Cultivation is an ongoing artificially selected process that occurs over time as humans select and cultivate wild plants (Hancock, 2004). The resulting founder effect, coupled with ongoing selection of seeds

from the initial pool of cultivated genotypes, produced a genetic bottleneck in cultivated populations (Miller and Schall, 2006; Zohary, 2004).

Significant provenance variation in seed has been observed in different agroforestry tree species (Meskel and Sinclair, 2000), there should also be clear differences in growth parameters and biomass gain strategies among provenance when they are grown under similar conditions, and such differences could possibly be observed at an early developmental stage. Variability studies are the prerequisite for genetic improvement of any tree species. Selection of the best provenance of desired species for a given site or region is necessary for achieving maximum productivity in plantation forestry. But such information is lacking for *M. officinalis*. So, the main objective was to identify germplasm sources of *M. officinalis* that have a good seed germination capacity and seedling traits suitable for reforestation in the Fuyang city in China. A specific objective was to tentatively explore the adaptive strategy of *M. officinalis* populations in order to provide some orientation for the genetic management of this species. For this purpose, an attempt was made to evaluate the genetic diversity among and within *M. officinalis* provenances based on morphological differences in seed and seedling traits possibly linked to the establishment and end use of this species.

Materials and methods

Seed resources and collection

The present investigation was carried out to study the variation in seed germination in a nursery, and seedling parameters in selected provenances of *M. officinalis*. Fifteen provenance of *M. officinalis* mainly distribute mountains of Zhejiang, Fujian, Guangxi, Chongqing, Shanxi, Gansu, and Sichuan provinces. A total of 150 individuals of *M. officinalis* representing 15 distinct populations were sampled

from its natural habitats (including Jingning, Qingyuan, Suichang, Zhenghe, Pucheng, Wuyishan, Longsheng, Kaixian, Xixiang, Chenggu, Ningqiang, Yanxian, Lueyang, Kangxian, and Baoxing). Seeds were harvested from each source to establish a provenance trial. Selected seed sources ranged from 26°05' N to 33°19' N latitude and 102°35' E to 119°38' E longitude and altitude from 336 to 1387 m (Fig. 1, Tab. 1).

For each site, 3 kg of seeds (approximately 1400 seeds per tree) were collected (October, 2008) from 10 candidate trees from natural stands, which were 50-100 m apart to avoid narrowing down the variation in sample due to relatedness or inbreeding (He, 2009; Zheng *et al.*, 2009). The bulk sample from each site was given an accession number (provenance title) before bringing them to laboratory. Ripen cones of *M. officinalis* were allowed to dry in the sun for 24 h. Thereafter, seeds were soaked in cold water overnight, macerated on a wire mesh and rinsed with water to remove the pulp. Seeds tended sink to the bottom while the soft pulp material tended to float. The seeds were dried in the sun for 24 h. Moisture percentage in seed samples was determined on fresh weight basis by drying the seeds at 103±3°C for 24 h.

Characterization of seed size

Morphological variability in seed characteristics (seed length, width, height, seed length/width ratio and seed weight) was evaluated. The shape of seeds (Vs) can be calculated by measuring length, width, and height of a seed and dividing all values by length, so that length is unity, and then calculating the variance of these three values by dividing the summed squared deviation from the mean by $n=3$ (Bedder *et al.*, 1998). Five replicate samples, each consisting of 50 seeds, were randomly selected from each seed lot and their length, width and height were measured using a micrometer (Besto make). Seed weight of five rep-

Tab. 1. The natural condition of *Magnolia officinalis* in collect seed locus

Provenances	Latitude (N)	Longitude (E)	Altitude (m)	Average annual temperature(°C)	Average annual sun exposure(h)	Average annual rainfall(mm)	Frost-free days(d)
Jingning, Zhejiang	119°38'	27°58'	653	17.6	1774	1542.7	241
Qingyuan, Zhejiang	119°15'	27°45'	1266	17.4	2169	1760	245
Suichang, Zhejiang	119°12'	28°21'	1038	16.8	1755	1510	251
Zhenghe, Fujian	119°10'	27°24'	851	17.5	2800	1600	320
Pucheng, Fujian	118°37'	28°03'	336	17.2	1894	1782.2	255
Wuyishan, Fujian	118°01'	27°47'	1123	17.7	1884	1881	273
Longsheng, Guangxi	110°04'	26°05'	1050	18.1	1408	1932	314
Kaixian, Chongqing	106°17'	29°28'	635	13.5	1695	1153	237
Xixiang, Shanxi	107°25'	32°54'	610	14.4	1624	923.5	253
Chenggu, Shanxi	107°26'	33°03'	1136	14.1	1657	857	245
Ningqiang, Shanxi	106°17'	32°49'	776	12.9	1570	1121	247
Yangxian, Shanxi	107°30'	33°19'	1387	14.7	1544	935.3	201
Lueyang, Shanxi	106°19'	33°16'	1372	13.2	1522	860	203
Kangxian, Gansu	105°41'	33°03'	740	12.6	1434	742	207
Baoxing, Sichuan	102°35'	30°34'	751	16.3	1125	1652	291

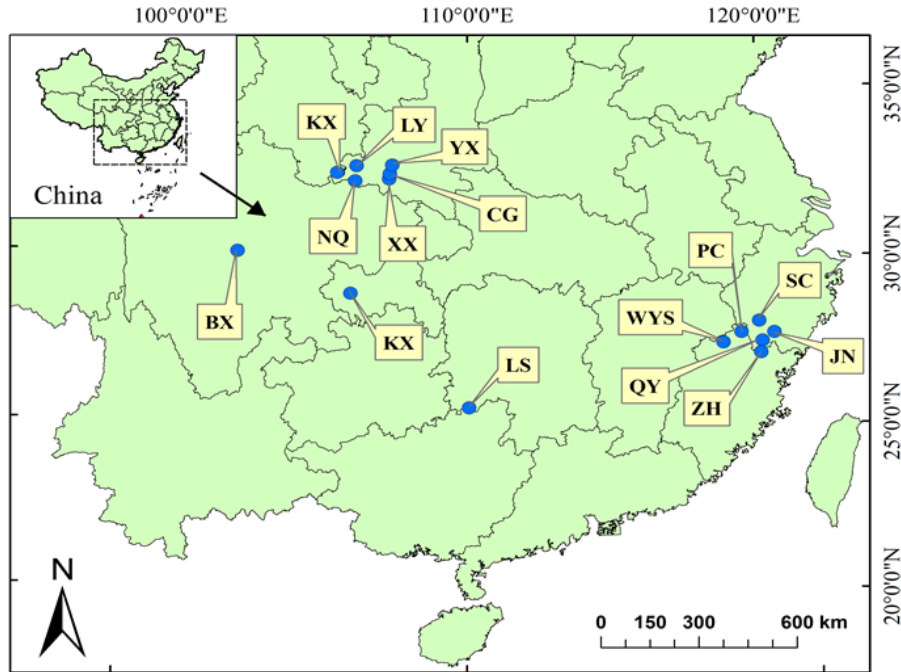


Fig. 1. Geographic distribution of 15 *Magnolia officinalis* provenances in China

Notes: JN-Jingning, QY-Qingyuan, SC-Suichang, ZH-Zhenghe, PC-Pucheng, WYS-Wuyishan, LS-Longsheng, KIX-Kaixian, XX-Xixiang, CG-Chenggu, NQ-Ningqiang, YX-Yangxian, LS-Longsheng, KX-Kangxian, BX-Baoxing. Similarly

licate samples (each of 100 seeds) were also obtained as per ISTA (International Seed Testing Association) regulations using electronic top pan balance. The seeds of each provenance were stored in seed containers of 5 kg capacity consisted of 18 gauge of GI (hot dip galvanized steel) sheets at room temperature and sowing was done in second week on November 2008.

Germination tests and seedling traits

The study was conducted between 2008 and 2009. The research site is located near Fuyang town in the China (long. 29°44' N, lat. 119°25' E, alt. 361 m). The climate is semi-wetness with a mean annual rainfall of 1452 mm. The annual mean temperature is 16.27°C. Seeds were surface sterilized with 0.3% KMnO_4 and soaked in distilled water for 24 h before sowing in 1×1 m well prepared nursery beds (lines 20 cm apart and seeds in lines 5 cm apart, 1-5 to 2.0 cm deep) in a randomized block design with three replicates (200 seeds per replicates). Seed germination was recorded for 40 days or until there was no further germination for a few days. The weeding and irrigation of beds were done manually for one year.

To assess the periodic growth, 10 seedlings of each provenance in each of the three replicates were randomly tagged; plant height and diameter were recorded. Measurements of number of leaves/plant (NL/P), number of lateral root (NLR), leaf area/plant (LA/P), main root length (MRL) were carried out at October 2009. Measurements of shoot height, collar diameter and dry weights of stem, leaf and root, were carried out at June, August and October 2009, respectively. Namely, there were three har-

vests per provenance. Plants were divided into stem, leaf and root portions (5 seedlings of each provenance in each of the three replicates were randomly selected). All plant parts were oven-dried at 85°C to constant weight for determining the stem, leaf and root dry weights. The root to shoot ratio was calculated from the harvest data. The relative growth rate (RGR) and the net assimilation rate NAR were calculated as $RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$ $NAR = [(W_2 - W_1) (\ln L_2 - \ln L_1)] / t (L_2 - L_1)$, where W_1 , L_1 and W_2 , L_2 are seedling dry weights and leaf area/plant (LA/P) at times t_1 and t_2 (Hoffmann and Poorter, 2002; Khurana and Singh, 2004a) where $t_2 - t_1$ was 60 days. Heritability (H^2) was calculated in accordance with Zhang *et al.* (2009).

Statistical analysis

An analysis of variance was performed using the general linear model (GLM) procedure of the statistical software SPSS version (10) for windows. Inc. Chicago, USA, and a regression analysis were done using the JMP 3.2 statistical package. Tukey's HSD test (Steel and Torrie, 1980) was performed to determine possible differences between means ($p \leq 0.05$). Design of experiment was arranged based on randomized complete block designs (RCBD)

Results

Morphological variation in seed characters

There were significant differences ($p \leq 0.01$) among provenances in seed length, width, height, seed length/width ratio, seed weight and V_s , and coefficient of variation were 5.85, 5.74, 6.58, 7.2, 17.54 and 11.16%, respectively.

On average, seed length ranged from 8.84 ± 0.87 (Yangxian) to 10.78 ± 0.72 mm (Baoxing) among provenances (mean = 9.58 mm). Mean seed width was maximum in Qingyuan locations followed by Suichang. Chenggu was recorded lowest mean seed width as compared to the other provenances, seed width ranged from 7.25 ± 0.70 to 8.68 ± 0.83 mm. Seed weight was smaller in north-west population (including Kaixian, Xixiang, Chenggu, Ningqiang, Yangxian, Lueyang, Kangxian, and Baoxing) than in south-east population (including Jingning, Qingyuan, Suichang, Zhenghe, Pucheng, Wuyishan and Longsheng). Yangxian and Wuyishan were recorded lowest V_s values as compared to the other provenances, V_s values ranged from 0.042 to 0.062. Average seed moisture was recorded highest ($12.37 \pm 0.95\%$) from Lueyang population, followed by Suichang ($12.36 \pm 0.90\%$) and Yangxian is minimum ($9.42 \pm 0.09\%$). There was 1.22, 1.20, 1.26, 1.24, 1.76 and

1.48-fold variation between highest and lowest values of seed length, width, height, seed length/ width ratio, seed weight and V_s values, respectively (Tab. 2).

Seed germination

There were significant differences ($p \leq 0.01$) among provenances in seed germination percentage. Seed germination percentage was maximum for Ningqiang locations followed by Chenggu and was lowest in Kaixian, overall seed germination in nursery averaged 59.4% varying from 26.6% to 91.2%. There were high germination potential from Jingning, Wuyishan and Longsheng locations. On average, seedlots from Qingyuan, Zhenghe, Pucheng, Kaixian, Longsheng and Kangxian had 26.6-49.9 % germination, whereas, the rest had germination percentages of 52.2-91.2 % (Fig. 2).

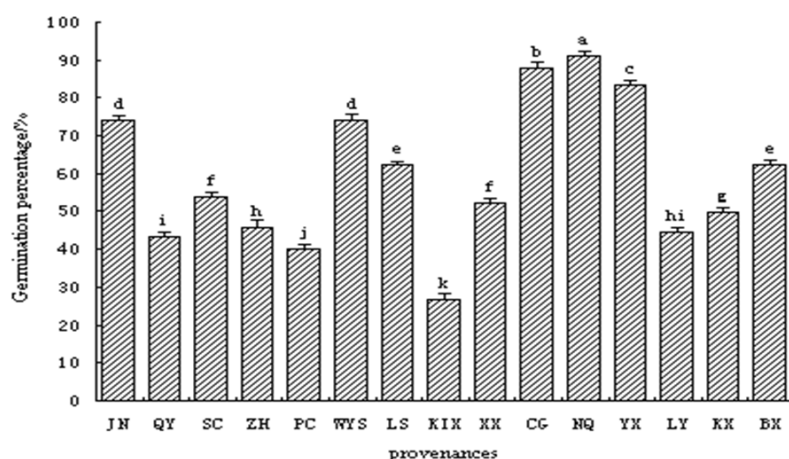


Fig. 2. The seeds germination percentage of *M.officinalis* from different provenances (Different letters indicate significant differences between provenances at $p \leq 0.05$)

Tab. 2. Variation of Seeds morphology of *Magnolia officinalis* from different provenances ($\bar{X} \pm SD$)

Provenances	Seed length (mm)	Seed width (mm)	Seed height (mm)	Seed length/ width ratio	100-seed weight (g)	V_s
Jingning	$9.73 \pm 0.63bc$	$8.11 \pm 0.82cd$	$4.66 \pm 0.49abc$	$1.21 \pm 0.15cde$	$17.57 \pm 0.43c$	0.047
Qingyuan	$9.53 \pm 0.63cd$	$8.68 \pm 0.83a$	$4.25 \pm 0.32hi$	$1.11 \pm 0.13f$	$16.10 \pm 0.34d$	0.059
Suichang	$9.16 \pm 0.54e$	$8.56 \pm 1.06ab$	$4.35 \pm 0.35fgh$	$1.09 \pm 0.19f$	$16.80 \pm 0.64cd$	0.055
Zhenghe	$9.28 \pm 0.86de$	$8.26 \pm 0.90bc$	$4.42 \pm 0.30efg$	$1.14 \pm 0.18ef$	$16.94 \pm 0.63cd$	0.051
Pucheng	$9.95 \pm 1.13b$	$8.00 \pm 1.11cde$	$4.76 \pm 0.40a$	$1.28 \pm 0.30abcd$	$17.40 \pm 1.06c$	0.046
Wuyishan	$9.29 \pm 0.68de$	$7.85 \pm 0.97def$	$4.73 \pm 0.41ab$	$1.20 \pm 0.19de$	$17.75 \pm 0.87bc$	0.042
Longsheng	$10.70 \pm 0.70a$	$8.33 \pm 0.83abc$	$4.60 \pm 0.32abcd$	$1.30 \pm 0.17ab$	$20.37 \pm 0.37a$	0.055
Kaixian	$9.96 \pm 0.92b$	$8.36 \pm 1.03abc$	$4.59 \pm 0.60bcd$	$1.21 \pm 0.16cde$	$16.95 \pm 0.81cd$	0.051
Xixiang	$9.22 \pm 0.65e$	$8.01 \pm 0.78cde$	$4.30 \pm 0.35ghi$	$1.16 \pm 0.17ef$	$16.21 \pm 0.33d$	0.052
Chenggu	$9.19 \pm 0.69e$	$7.25 \pm 0.70g$	$3.90 \pm 0.31j$	$1.28 \pm 0.17abc$	$12.12 \pm 0.50f$	0.057
Ningqiang	$9.41 \pm 0.66de$	$7.54 \pm 0.92fg$	$4.16 \pm 0.25i$	$1.27 \pm 0.19bcd$	$14.47 \pm 0.14e$	0.053
Yangxian	$8.84 \pm 0.87f$	$7.74 \pm 0.92ef$	$4.55 \pm 0.51cde$	$1.15 \pm 0.15ef$	$11.55 \pm 0.23f$	0.042
Longsheng	$9.46 \pm 0.89cde$	$7.37 \pm 1.18g$	$3.77 \pm 0.62j$	$1.33 \pm 0.29ab$	$11.57 \pm 0.64f$	0.062
Kangxian	$9.17 \pm 0.66e$	$7.25 \pm 0.70g$	$3.90 \pm 0.31j$	$1.28 \pm 0.17abcd$	$12.11 \pm 0.46f$	0.056
Baoxing	$10.78 \pm 0.72a$	$8.13 \pm 1.09cd$	$4.49 \pm 0.48def$	$1.35 \pm 0.21a$	$18.62 \pm 0.66b$	0.057
Mean	9.58	7.62	4.36	1.22	15.77	0.052
CV (%)	5.85	5.74	7.2	6.58	17.54	11.16

Notes: Within each provenance, values marked by the same letter are not significantly ($p \leq 0.05$) different

Correlation analysis for seed traits

Seed traits were inter-correlated (Tab. 3). The 100-seed weight showed a higher positive correlation with seed width than with seed length ($r = 0.74$ and 0.70 , respectively). The seed length/width ratio was positively correlated with seed length ($r = 0.56$, $p \leq 0.05$). There were significant negative correlation ($r = -0.56$, $p \leq 0.05$) between seed length/width ratio and seed width. The 100-seed weight was positively correlated with seed height ($r = 0.74$, $p \leq 0.01$).

Tab. 3. Mean correlation coefficients (r) between seed variables of *Magnolia officinalis* provenances

Variables	Seed length	Seed width	Seed height	Seed length/width ratio	100-seed weight
Seed length	1				
Seed width	0.37	1			
Seed height	0.38	0.60*	1		
Seed length/width ratio	0.56*	-0.56*	-0.22	1	
100-seed weight	0.70**	0.74**	0.74**	-0.061	1

Notes: *, ** stand for the significances at the 0.05 and 0.01 levels

The correlation coefficient between geography, climate and seed trait

Correlation analysis between seeds characters and geographical climatic factors showed that: seed length was significantly positively correlated with average annual rainfall or frost-free days. Seed width had a positive and significant correlation with longitude, frost-free days of provenance. Seed width and seed weight while it showed positive and significant correlation with average annual temperature and average annual rainfall. There were significant inverse correlation, seed width and seed weight of *M. officinalis* from different provenances with latitude of seed source. There was significant positive correlation between seed height and average annual rainfall or average annual temperature (Tab. 4).

Genetic variation in seedling traits

There were significant differences ($p \leq 0.001$, $p \leq 0.001$, $p \leq 0.05$, $p \leq 0.001$ and $p \leq 0.001$) existed among provenances in shoot height, collar diameter, number of leaves/plant, main root length and number of lateral root (Fig. 3A, B,

C, D, and E). Provenance Longsheng indicated the greatest shoot height, followed by provenance Yangxian, while provenance Kangxian ranked lowest and other provenances remained intermediate. Collar diameter was considerably higher for Yangxian compared to other provenances. Seedling height and collar diameter had high heritability among provenances ($H^2 = 0.93$ and 0.73 , respectively). The effect of sampling date was significant ($p \leq 0.001$) on shoot height and collar diameter (Fig. 5A, B). At October seedling, Leavers number was highest to Longsheng and least to Kaixian. The provenance Wuyishan showed a higher main root length. The provenance Chenggu and Yangxian showed a higher number of lateral roots.

Root, stem and leaf dry weights

Seedling root dry weight showed significant variation among the provenances. Provenance Ningqiang indicated the greatest root dry weight, followed by provenance Wuyishan, while provenance Kangxian ranked lowest. Root dry weight of Ningqiang is 3 times more than that of Kangxian. There were significant differences ($p \leq 0.001$, $p \leq 0.001$) existed among provenances in stem dry weight and leaf dry weight. (Fig. 3F, G, H). Provenance Longsheng indicated the greatest stem dry weight and leaf dry weight, while provenance Kangxian ranked lowest in stem dry weight. The effect of sampling date was significant ($p \leq 0.001$) on root, stem and leaf dry weights (Fig. 5C, D, F).

Root/shoot ratio and total dry weight

Differences in root to shoot ratio and total dry weight among provenances were significant ($F = 4.079$, $p \leq 0.001$; $F = 5.615$, $p \leq 0.001$). The Provenance Baoxing showed a higher root/shoot ratio compared to the provenance and the lowest one was found in Longsheng, total dry weight was highest to Wuyishan and least to Kangxian (Fig. 4). The effect of sampling date was significant ($p \leq 0.05$) on seedling height, ground diameter, root dry weight, stem dry weight and leaf dry weight (Fig. 5).

The relative growth rate and the net assimilation rate

No significant differences ($F = 1.953$, $F = 1.773$) existed among provenances in the relative growth rate (*RGR*) and the net assimilation rate (*NAR*), although the north-west population indicated, on average slightly higher values to south-east population. Provenance Jingning indicated the

Tab. 4. The correlation coefficient between geographic, climate and seed trait of *Magnolia officinalis*

Item	Seed length	Seed width	Seed height	Seed length/width ratio	100-seed weight
Longitude	-0.16	0.54*	0.44	-0.60*	0.4
Latitude	-0.45	-0.78**	-0.68**	0.3	-0.84**
Altitude	-0.33	-0.12	-0.36	-0.18	-0.42
Average annual temperature	0.35	0.68**	0.68**	-0.3	0.75**
Average annual sun exposure	-0.35	0.37	0.17	-0.62*	0.13
Average annual rainfall	0.55*	0.70**	0.71**	-0.13	0.85**
Frost-free days	0.53*	0.50*	0.45	0.01	0.79**

Notes: *, ** stand for the significances at the 0.05 and 0.01 levels

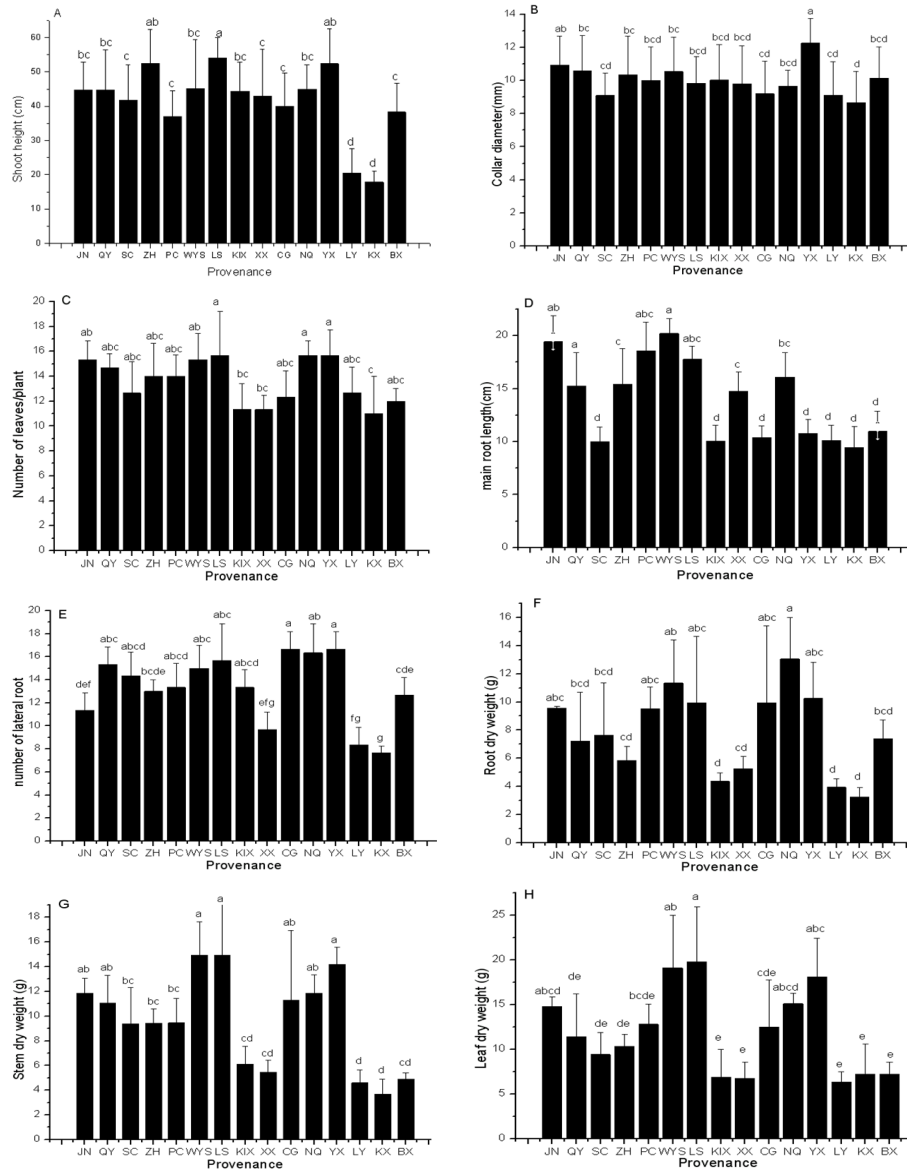


Fig. 3. Seedling growth characteristics in fifteen *M.officinalis* provenances in the nursery at October seedling. (A) height, (B) Collar diameter, (C) number of leaves, (D) main root length, (E) number of lateral root, (F) root dry weight, (G) stem dry weight, and (H) Leaf dry weight. Bars indicate standard error of the mean. Different letters indicate significant differences between provenances at $p \leq 0.05$

greatest *RGR*, followed by provenance Kangxian, while provenance Zhenghe ranked lowest and other provenances remained intermediate, *NAR* was similar for *RGR* in result. (Tab. 5)

The correlation coefficient between geography, climate and seedling growth traits

Correlation analyses revealed that shoot height had a positive correlation with longitude of seed origin ($r = 0.34$), albeit not statistically significant. Shoot height had a significant positive correlation with the average annual temperature and frost-free days ($r = 0.55, p \leq 0.05$; $r = 0.56, P \leq 0.05$, respectively), and negative correlation with latitude of the seed origin ($r = 0.51, p \leq 0.05$) (Tab. 6). Stem

dry weight was significantly positively correlated with average annual temperature ($r = 0.53, p \leq 0.05$) (Tab. 6).

Discussion

Phenotypic variation is determined by genotype and environment interactions and is assumed to be the expression of genotypic variation when environmental conditions are controlled (Li *et al.*, 2009, Westoby *et al.*, 2002). This study of provenance variation in *M. officinalis* has found variation in seed size, shape and weight, in germination percentages and in growth characteristics (seedling height, ground diameter, number of leaves/plant, number of lateral root, leaf area/plant, main root length). Thompson,

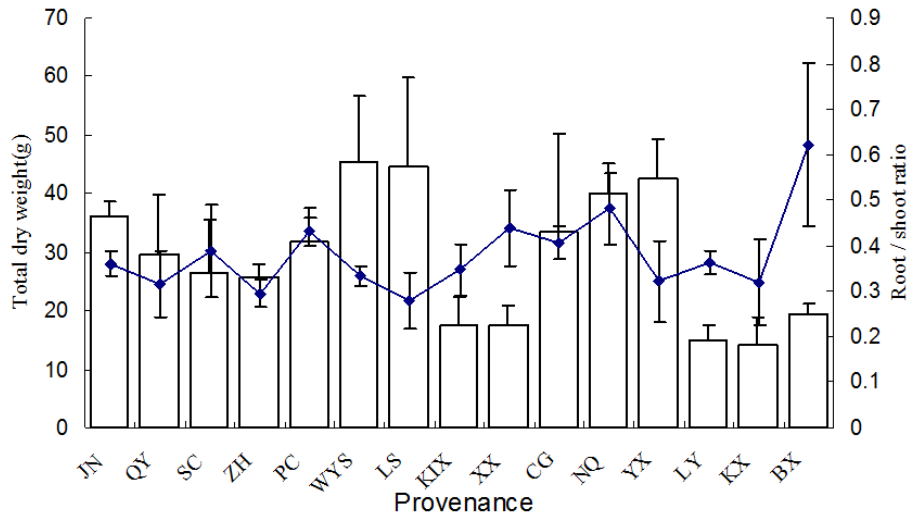


Fig. 4. Root to shoot ratio and total dry weight of at October seedlings in fifteen *M.officinalis* provenances. Bars indicate standard error of the mean. Different letters indicate significant differences between provenances at $p < 0.05$

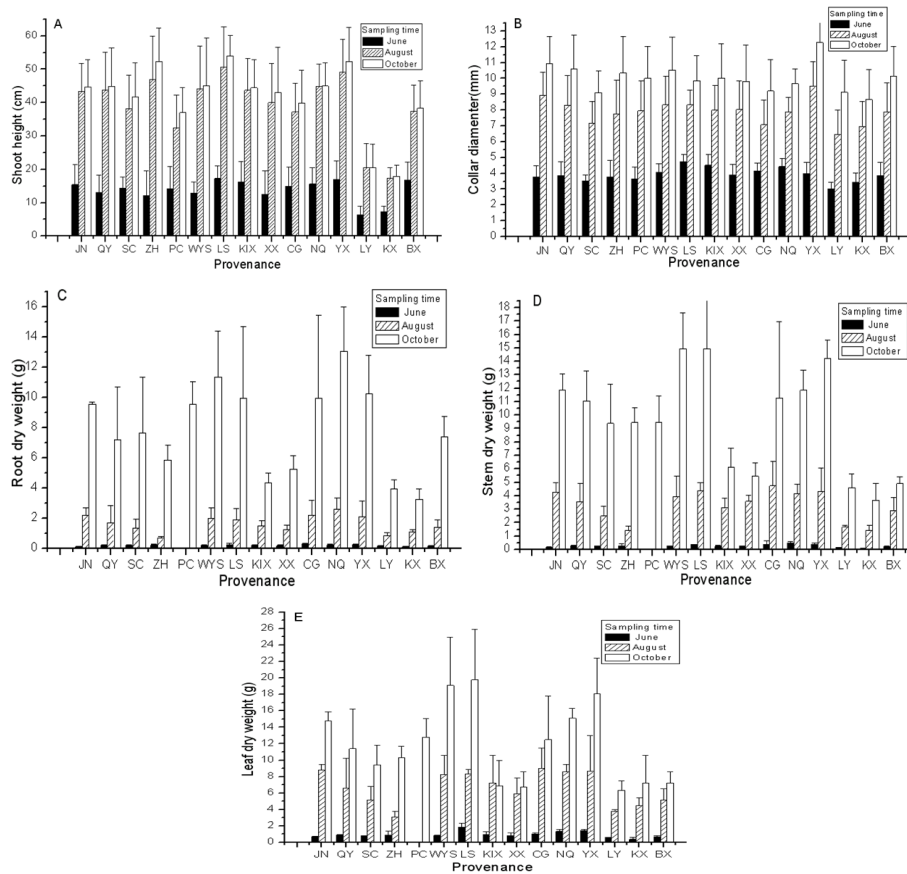


Fig. 5. Effect of sampling time on seedling growth characteristics in the nursery . (A) shoot height, (B) Collar diameter, (C) root dry weight, (D) stem dry weight, (E) leaf dry weight. Bars indicate standard error of the mean

Band and Hodgson (1993) showed that among 97 species of the british flora, seeds which are persistent in the soil tended to be small and compactly shaped, while seeds that are transient in the soil were larger, flattened or elongate. Our result indicates that Yangxian seed are persistent in the soil. It is possible that the variation in morphological

characters among provenances is due to resource availability at the time of fruiting and so to seed size. This in turn could be due to different environments at the geographic origins of each seed and seasonal variables (Murali, 1997). Loha *et al.* (2009) also observed significant variation in seed size traits of *Cordia Africana*.

Tab. 5. The relative growth rate and the net assimilation rate of *Magnolia officinalis* seedlings from different provenances ($\bar{x} \pm SD$)

Provenances	RGR (mg·g ⁻¹ ·d ⁻¹)	NAR (g·m ⁻² ·d ⁻¹)
Jingning	0.0454±0.0029	3.3407±0.3388
Qingyuan	0.0352±0.0080	2.6665±0.6028
Suichang	0.0331±0.0052	2.3646±0.5821
Zhenghe	0.0237±0.0099	1.6594±0.8182
Pucheng	0.0391±0.0056	3.0296±0.7183
Wuyishan	0.0297±0.0022	1.8152±0.1521
Longsheng	0.0358±0.0122	2.5561±1.1497
Kaixian	0.0365±0.0063	2.756±0.5021
Xixiang	0.0374±0.0010	3.3177±0.7607
Chenggu	0.0337±0.0029	2.5812±0.2662
Ningqiang	0.0320±0.0085	2.2958±0.7263
Yangxian	0.0342±0.0053	2.4315±0.1735
Longsheng	0.0419±0.0083	2.9216±0.7212
Kangxian	0.0365±0.0021	2.6083±0.384
F	1.953	1.7730

In the present study, there were significant differences among provenances in seed germination percentage, varying from 26.6% to 91.2% (Fig. 2). A marked intraspecific variability in the germination ability have also been reported in pine species (Lopez-Upton, 2005). Causes of such variability might be generally attributed either to (a) genetic characters of source population/plant (Bewley and Black, 1994), or (b) impact of mother plant environment (Gutterman, 1992). There was a strong intercharacter correlation, particularly between seed weight and seed width (Tab. 3). Correlated quantitative traits are of a major interest in an improvement program, as the improvement of one character may cause simultaneous changes in the other character. Similar results have been reported for *Faidherbia albida* (Dangasuk et al., 1997). In addition, morphometric traits especially seed weight, showed a strong correlation with latitude ($r = -0.84, p \leq 0.01$) and mean annual temperature ($r = 0.75, p \leq 0.05$) of seed origin (Tab. 4). This indicates that seed size is not only heritable but also strongly affected by environmental variations. While it is a well-known fact that latitude and temperature has an inverse relationship, which is consistent with the present finding. Correlation analysis between seed characters and geographical climatic factors showed that the main

variation was of geographical locations. The annual mean temperature, annual precipitation and frost-free period were the leading ecological factors restricting the seed variation.

Large differences in seedling height and collar diameter were found among provenances, and as much as 0.93 of the total variation was due to provenance effect. This indicates that there is adequate genetic variability for seedling height growth in the present material. Similar results have been reported for broadleaved species (Cundall et al., 2003). This also indicates that seedling height could be employed as a proxy indicator for early selection of provenances for further testing. Base on seedling height values and seed germination percentage for fifteen provenances tested, Longsheng, Yangxian, Jingning, Wuyishan, Kaixian and Ningqiang provenance have been identified suitable to produce quality seedlings of *M. officinalis*.

In the present study, there were significant differences among provenances in leaf number, main root length, number of lateral root and root/shoot ratio. A similar result was reported for *Faidherbia albida* (Dangasuk et al., 1997). This also suggests that the root/shoot ratio in seedlings alone indicates adaptation to water stress. Rehfeldt and Wycoff (1981) reported that the *Pinus* seedlings raised from different provenance often display different pattern of root, stem and leaf growth as observed in this study also. These variations in growth characteristics of different *Dalbergia sissoo* provenance may be attributed to adaptation because the seedlings from all the sources were raised under identical conditions (Singh and Pokhriyal, 2000). Leaf number and leaf dry weight/plant varied significantly among provenance of *M. officinalis*. Similar observations have also been documented to leaf biomass of *Acacia mangium* and *Celtis australis* (Salazar, 1989; Singh, 2006).

Relative growth rates (*RGR*) values differ widely among plant species (e.g. between *Briza media* L. and *Festuca ovina* L.), and these differences can be related, to environmental conditions and to inherent species characteristics (Poorter and Remkes, 1990). In our study, provenances showed no statistically significant variation in the *RGR*. The variation in *RGR* in woody species is generally assumed to be related to the variation in leaf area ratio (*LAR*) and to be greater in deciduous species compared to evergreens (Antunez et al., 2001). Physiological mechanisms (Walter et

Tab. 6. The correlation coefficient between geographic, climate and seedling growth traits of *Magnolia officinalis*

Item	Longitude	Latitude	Altitude	Average annual temperature	Average annual sun exposure	Average annual rainfall	Frost-free days
Height	0.34	-0.51*	0.06	0.55*	0.35	0.49	0.56*
Collar diameter	0.24	-0.22	0.19	0.39	0.2	0.28	0.02
Root dry weight	0.22	-0.19	0.09	0.33	-0.05	0.38	0.22
Stem dry weight	0.45	-0.42	0.35	0.53*	0.22	0.47	0.27
Leaf dry weight	0.31	-0.35	0.27	0.46	0.05	0.44	0.23
Total dry weight	0.35	-0.35	0.26	0.47	0.08	0.45	0.25

Notes: *, ** stand for the significances at the 0.05 and 0.01 levels

al., 1993), leaf morphology (Poorter and Remkes, 1990) can be responsible for variation in the *RGR*. Our results suggest some variation in the *RGR* associated with variation in *NAR*.

Correlation was found between seedling height and latitude of seed origin ($r = -0.51$, $p \leq 0.05$). Such correlation is expected from a population of trees which is known to have naturally regenerated without human interference since primary colonization. The stands from which seeds were collected for the present study were remaining relict natural forests. The results are in disagreement with those obtained in early vigor (e.g. Kleinschmit *et al.*, 1996). Seedling height decreased gradually from south to north geographically, while shoot diameter declines from east to west. That means the geographic variation of the plant at the seedling stage manifests a two-way variation with the latitude and longitude, but the variation is more significant with the former. Shoot height had a significant positive correlation with the average annual temperature.

The practical importance of the study is that heavier seedlings raised through suitable provenances may provide fair success in survival and growth of seedlings during out-planting as quality seedlings will only be able to withstand the adverse climatic conditions. However, further research should look into responses of genotypes \times environment interaction in *M. officinalis* at different test sites over a wider range of environmental conditions within.

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