

Variation in needle and cone traits in natural populations of *Pinus yunnanensis*

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Abstract *Pinus yunnanensis* Franch. is a major component of coniferous forests in southwestern China. Little is known about its intraspecific variation. Morphological variations in needle and cone traits of *P. yunnanensis* were analyzed to quantify variability among and within natural populations. Seven traits were measured on 10 needles collected from 30 trees in each of the 18 sampled populations of *P. yunnanensis*. Four cone traits were measured in 221 individual trees from nine populations. The results showed that there were significant differences ($p < 0.01$) both among populations and within populations in each needle and cone trait. The proportion of phenotypic variation of nearly all needle and cone traits was over 50 % within populations, which showed trees within populations accounted for a majority of the total variation. The needle traits showed higher variability within population than cone traits. Variability in the

needle traits was correlated with geo-climatic parameters (longitude, latitude, altitude, temperature, and precipitation). Needle length and the ratio of needle length to fascicle sheath length showed clinal variation in response to latitudinal and altitudinal gradients. A hierarchical classification of all populations based on needle traits led to the formation of four major groups. The findings provide important genetic information for the evaluation of variation. Moreover, it will assist in management of genetic diversity of *P. yunnanensis*.

Keywords Genetic diversity · Conservation · Yunnan pine · Cone morphology · Needle morphology

Introduction

Pinus yunnanensis Franch. is extensively used in reforestation efforts in Southwest China. Its range extends from 23° to 30°N and 96° to 108°E and it is a major component of coniferous forests in this region (Wu 1986; Chinese Academy of Sciences 1978; Jin and Peng 2004). It grows at altitudes ranging from 250 to 3500 m and in a variety of geological areas (Jin and Peng 2004). *P. yunnanensis* is shade-intolerant, deep-rooted, drought-resistant and tolerant of rocky soils with low fertility. It is a pioneer tree species that may be used for afforestation on barren hills (Chinese Academy of Sciences 1978). Its timber is used extensively for a variety of purposes. It plays a crucial role in regional economic development and ecological restoration (Wu 1986; Jin and Peng 2004). In southwest China, it occupies approximately 52 % of the forested area and produces 32 % of the timber volume (Jin and Peng 2004). Unfortunately, human overexploitation and natural disasters, such as fire and drought, have seriously damaged the *P. yunnanensis* resource.

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Conservation and sustainable use of genetic resources depend upon knowledge of the extent and pattern of intra-specific variation (Loha et al. 2006). Characterization of natural forest resources is the necessary first step toward a better understanding of their genetics and implementation of in situ and ex situ conservation activities (Gil et al. 2002; Turna et al. 2006; Turna and Güney 2009). Sevik et al. (2010) emphasized the importance of characterizing similarities or differences among populations as an important stage of breeding. *P. yunnanensis* inhabits an extremely variable geographical range that encompasses great variation in geo-climatic conditions (Li 1984), possibly indicating the existence of ecotypes adapted to different environments (Wang et al. 2003, 2004; Ji et al. 2011). It has been demonstrated that the morphology and anatomy of leaves reflect adaptation to the habitat from which plants originate (Pham et al. 2010; Ali et al. 2011). Thus, variability in needle and cone morphology can be an important indicator of ecological specialization (Donahue and Upton 1996; Boulli et al. 2001; Dangasuk and Panetsos 2004; Wahid et al. 2006).

Genetic improvement of *P. yunnanensis* started in the 1980s. It was focused on identifying phenotypically superior trees from diverse regions and deploying them in seed orchards (Li et al. 2010). The genetic variation of *P. yunnanensis* has been characterized primarily in a few, small provenance tests that sampled a limited part of the species' range (Li 1984; Chen and Wu 1987). There are few descriptions of the intra-specific variability of *P. yunnanensis*, but they have shown that morphological traits varied significantly among populations or provenances (Wang et al. 2003, 2004).

The objectives of this study were to: ascertain variability among and within populations of *P. yunnanensis* based on a range-wide sample of populations, evaluate population differentiation based on the phenotypic variation in needle and cone traits, determine the proportion of variation that exists among and within populations, and explore correlations between phenotypic traits and geographic or climatic factors.

Materials and methods

Sampling

Fresh, mature needle samples were harvested from 18 widely separated populations covering almost all the *P. yunnanensis* range (Fig. 1; Table 1). Sampled trees (30 to 34 per population) were located at least 100 m apart in each population. All sampled trees were more than 25 years old (Dangasuk and Panetsos 2004). Needle traits were analyzed from 30 mother trees in each population. Cones were sampled from

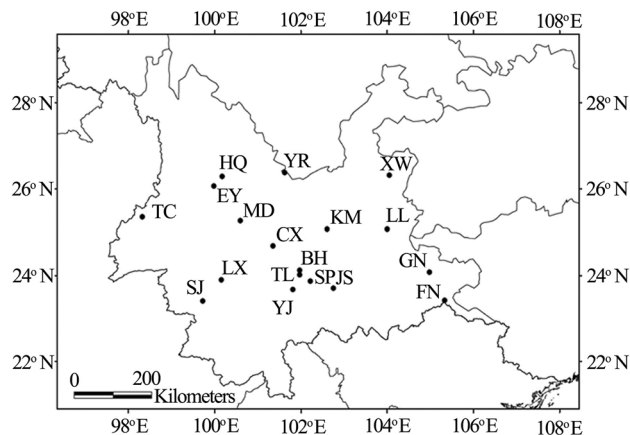


Fig. 1 Distribution of *P. yunnanensis* populations sampled in this study. All sampled sites are in Yunnan province, China

221 individual trees in nine populations. Sample size for cone traits varied from 12 to 34 mother trees in each population (Table 1). Two-year-old needles were sampled randomly from three to five vigorous branches from each sampled tree. The needles and cones were collected from all sides of the canopy, from the upper and middle portion of the crown, and were immediately placed in individually labeled bags. The methods for sampling and trait measurement were derived from previously published studies of congeneric species, such as Scots pine (Bobowicz and Radziejewska 1989; Urbaniak et al. 2003; Boratyńska et al. 2008), slash and longleaf pines (De Vall 1940), and peat-bog pine (*P. uliginosa*) (Boratyńska et al. 2008).

Measurements

Ten fascicles were selected randomly from each sampled tree for the measurement of fascicle sheath length (FSL) and fascicle width (FW), and ten needles (one from each fascicle) were measured for needle length (NL) and needle width (NW) (Panetsos 1975; Urbaniak et al. 2003; Boratyńska et al. 2008). NL and FSL were measured with a metric ruler with an accuracy of 0.1 cm; NW and fascicle width (FW) were measured using digital vernier calipers with an accuracy of 0.1 mm. The ratio of length to width of needle (NL/NW), the ratio of needle length to fascicle sheath length (NL/FSL) and the ratio of fascicle width to needle width (FW/NW) were calculated separately. The mature cones (two to six per sampled tree) were harvested, weighed, and measured for length and width (Table 1). Cone length (CL) was measured in a straight line from the base to the top, cone curvature excluded, and cone diameter (across the broadest portion) (Donahue and Upton 1996) with digital vernier calipers, and then the ratio of cone length to cone diameter (CL/CD) was calculated. Cone weight (CW) was quantified with digital electronic balance.

Table 1 Location and geographic characteristics of eighteen sampled populations of *Pinus yunnanensis*

Population name	Trees sampled (n)	Total cones (n)	Longitude (°)	Latitude (°)	Altitude (m)	Tmean (°C)	Tmax (°C)	Tmin (°C)	Prec (mm)
BH	34	93	101.97	24.12	1834	19	28	5	1008
CX	–	–	101.35	24.68	1997	16	25	2	1015
EY	12	40	99.98	26.06	2290	13	22	–1	1051
FN	–	–	105.33	23.42	1240	17	27	5	1362
GN	–	–	104.98	24.08	1287	17	27	3	1104
HQ	29	136	100.17	26.29	2227	14	23	0	1013
JS	30	144	102.75	23.70	1609	18	27	5	1074
KM	16	69	102.60	25.07	2192	14	23	0	1031
LL	–	–	104.00	25.07	1890	15	25	1	1042
LX	29	135	100.15	23.90	1875	16	25	3	1210
MD	32	158	100.59	25.27	2099	16	26	3	968
SJ	26	114	99.72	23.40	1222	21	31	7	1360
SP	–	–	102.22	23.87	1504	18	27	5	1096
TC	13	46	98.32	25.36	2024	14	22	0	1541
TL	–	–	101.97	24.02	1639	18	27	5	1062
XW	–	–	104.05	26.32	2222	12	23	–1	969
YJ	–	–	101.81	23.68	1654	18	28	5	1188
YR	–	–	101.62	26.37	2132	14	25	0	1013
Total	221	935							

Tmean annual mean temperature, *Tmax* maximum temperature of the warmest month, *Tmin* minimum temperature of the coldest month, *Prec* annual precipitation

Data analysis

The components of variance were determined from within populations and among populations to determine the percentage of variability due to these sources. Data from the measurement of needle and cone traits were subjected to analysis of variance (ANOVA, PROC MIXED) using a nested procedure (needles or cones in trees and trees in populations) (version 9.3, SAS Institute Inc., Cary, NC, USA). For all traits, comparison of means was conducted with Tukey's multiple comparison test at 95 % confidence intervals to determine the differences among populations. The variability within populations was described using the coefficient of variation (CV) (López et al. 2013). The CVs were obtained from population means and standard deviations. The morphological divergence between natural population (Raeymaekers et al. 2007) or phenotypic differentiation (Leinonen et al. 2006) was assessed by calculating the P_{ST} index; analogous to Q_{ST} (Spitze 1993), P_{ST} index was calculated using the formula $P_{ST} = \sigma_{GB}^2 / (\sigma_{GB}^2 + 2\sigma_{GW}^2)$, where σ_{GB}^2 and σ_{GW}^2 are the among-population and within population variance components for a phenotypic trait, respectively (Raeymaekers et al. 2007).

The climatic data—that is the annual mean temperature (*Tmean*), maximum temperature of the warmest month (*Tmax*), minimum temperature of the coldest month (*Tmin*),

and annual precipitation (*prec*)—were extracted from Worldclim using each populations' longitude and latitude (Global Climate Data, <http://www.worldclim.org/>; Hijmans et al. 2005). Then mean values of particular features were used to characterize each population (Boratyńska et al. 2008). To investigate the associations among traits, a Spearman's non-parametric correlation coefficient matrix was constructed between the traits studied and geo-climatic factors, including longitude, latitude, altitude, *Tmean*, *Tmax*, *Tmin*, and *prec*. This correlation coefficient is adequate for samples of small size and non-normal distribution (Steel et al. 1960; Wahid et al. 2006). A principal component analysis (PCA) was performed to display the relationships among populations based on a three-dimensional diagram using a PRINCOMP Procedure (version 9.3, SAS Institute Inc., Cary, NC, USA). SAS for Windows (version 9.3, SAS Institute Inc., Cary, NC, USA) and SPSS software (SPSS version 21, IBM Corporation, North Castle Drive, Armonk, NY, USA) were used in statistical calculations.

Results

High and significant levels of variation were found both among and within populations in all traits measured ($p < 0.01$). Mean needle length ranged from 16.7 cm

(population EY) to 22.1 cm (population FN), and mean needle width ranged from 0.4 to 0.7 mm. Maximum fascicle width (1.5 mm) was observed in the KM and HQ populations, and minimum (1.1 mm) was observed in populations CX, GN, TL, YJ, and YR (Table 2). Fascicle-sheath length ranged from 1.2 to 1.8 cm with an overall average of 1.5 cm. Correspondingly, the ratio of needle length to width, needle length to fascicle sheath length, and fascicle width to needle width varied widely. The FN population presented the highest mean values for three of seven needle traits, i.e., needle length (22.1 ± 3.2 cm), fascicle sheath length (1.8 ± 0.3 cm), and the ratio of needle length to needle width (484.9 ± 87.9). No single population was consistently the lowest for any trait.

Differences in cone traits among populations were comparable to those for needle traits. Cones varied from 57.3 to 68.4 mm in length and from 30.6 to 38.8 mm in diameter (Table 3). Cones of HQ and BH were the largest, while those of population EY were the smallest. Cone weight was the trait with greatest variability. Cones of HQ were nearly twice as heavy as those of EY. Overall, values differed significantly among pair-wise populations (Tukey's multiple comparisons tests significant at $p = 0.05$ for all traits).

The descriptive statistics showed that the coefficient of variation of needle traits within populations did not exceed 33 % or fall below 9 % (Fig. 2). The highest coefficient of variation was recorded for needle width in the XW population (32.13 %) followed by ratio of needle length to needle width in population GN (29.45 %). The trait with lowest intra-population variability was fascicle width (9.02 % in population SP, 9.38 % in population JS, and 9.75 % in population LL) and ratio of fascicle width to needle width (9.80 %). Cone weight presented the highest within population variability; the mean CV for this trait was 37.60 %. Mean CVs of the other cone traits were relatively low. Population TC showed the lowest coefficient of variance in all four cone traits.

The total variance for each trait could be divided into individual differences within populations, among population differences, and error. The results showed individual tree within population (intra-population) variability accounted for most of the total variation with the exception of the ratio of fascicle width to needle width (FW/NW) (Table 4). The morphological divergence between natural populations was further assessed by calculating the P_{ST} index. Values of P_{ST} varied considerably, from 0.18 for needle length to 0.69 for ratio of fascicle width to needle

Table 2 Descriptive statistics (mean \pm standard deviation) for each needle trait among populations of *Pinus yunnanensis*

Population	NL ^{A,B}	NW	FW	FSL	NL/NW	NL/FSL	FW/NW
BH	21.1 \pm 3.6bc	0.7 \pm 0.1a	1.2 \pm 0.2fg	1.8 \pm 0.3ab	307.3 \pm 73.4hi	12.3 \pm 3.2ef	1.7 \pm 0.3j
CX	19.7 \pm 2.6ef	0.5 \pm 0.1hi	1.1 \pm 0.2i	1.6 \pm 0.3cde	415.0 \pm 82.5c	12.5 \pm 2.3ef	2.2 \pm 0.4fg
EY	16.7 \pm 2.1i	0.5 \pm 0.1hij	1.3 \pm 0.2c	1.6 \pm 0.3def	347.6 \pm 56.2ef	11.2 \pm 3.1gh	2.8 \pm 0.3ab
FN	22.1 \pm 3.2a	0.5 \pm 0.1ijk	1.2 \pm 0.2ef	1.8 \pm 0.3a	484.9 \pm 87.9a	12.6 \pm 2.3def	2.7 \pm 0.3cd
GN	21.7 \pm 3.0ab	0.6 \pm 0.2def	1.1 \pm 0.3h	1.6 \pm 0.3def	410.9 \pm 121.0c	14.3 \pm 3.4c	2.1 \pm 0.4h
HQ	18.7 \pm 2.1g	0.6 \pm 0.1ef	1.5 \pm 0.2a	1.5 \pm 0.3ef	347.6 \pm 57.2ef	12.7 \pm 3.3def	2.7 \pm 0.3bc
JS	20.0 \pm 3.1de	0.6 \pm 0.1c	1.3 \pm 0.1d	1.2 \pm 0.2h	316.6 \pm 56.8gh	16.8 \pm 4.2b	2.0 \pm 0.2h
KM	19.7 \pm 3.6ef	0.7 \pm 0.1ab	1.5 \pm 0.2a	1.6 \pm 0.3cde	293.3 \pm 52.3i	12.7 \pm 3.4def	2.3 \pm 0.3ef
LL	17.6 \pm 2.4h	0.6 \pm 0.1def	1.3 \pm 0.1de	1.4 \pm 0.3g	319.0 \pm 58.0gh	12.9 \pm 2.9def	2.3 \pm 0.3ef
LX	20.7 \pm 2.6cd	0.5 \pm 0.1jk	1.3 \pm 0.2de	1.5 \pm 0.3f	459.0 \pm 79.3b	14.2 \pm 3.2c	2.8 \pm 0.3ab
MD	18.9 \pm 2.4fg	0.6 \pm 0.1ef	1.4 \pm 0.2b	1.8 \pm 0.3a	350.0 \pm 52.7def	11.0 \pm 2.4h	2.6 \pm 0.3d
SJ	21.7 \pm 3.0ab	0.5 \pm 0.1gh	1.4 \pm 0.2b	1.2 \pm 0.2h	431.9 \pm 72.1c	19.8 \pm 5.5a	2.8 \pm 0.3abc
SP	21.5 \pm 3.2abc	0.7 \pm 0.1bc	1.3 \pm 0.1de	1.4 \pm 0.3g	332.3 \pm 58.5efg	16.7 \pm 3.9b	2.0 \pm 0.3hi
TC	19.8 \pm 3.2e	0.4 \pm 0.1k	1.2 \pm 0.2de	1.6 \pm 0.3def	458.0 \pm 99.3b	13.1 \pm 2.8de	2.8 \pm 0.3a
TL	21.0 \pm 3.3bc	0.6 \pm 0.2c	1.1 \pm 0.2h	1.6 \pm 0.3cde	353.8 \pm 91.2de	13.5 \pm 3.5cd	1.9 \pm 0.4i
XW	16.8 \pm 2.2hi	0.6 \pm 0.2d	1.3 \pm 0.2cd	1.6 \pm 0.3cd	307.8 \pm 76.8hi	10.6 \pm 2.2h	2.4 \pm 0.6e
YJ	18.6 \pm 4.0g	0.6 \pm 0.1de	1.1 \pm 0.2i	1.2 \pm 0.3h	331.8 \pm 78.4fg	15.9 \pm 4.2b	1.9 \pm 0.4i
YR	19.3 \pm 2.6efg	0.5 \pm 0.1fg	1.1 \pm 0.1gh	1.7 \pm 0.3bc	369.4 \pm 75.1d	12.0 \pm 3.1fg	2.2 \pm 0.3g
Average	19.7 \pm 2.9	0.6 \pm 0.1	1.3 \pm 0.2	1.5 \pm 0.3	368.7 \pm 73.8	13.6 \pm 3.3	2.3 \pm 0.3

^A Each needle was analysed separately on the basis of 7 characters: 4 measured and 3 calculated

^B Mean \pm standard deviation sharing a common letter were not significantly different at $\alpha = 0.05$ by Tukey's test

NL Needle length, NW needle width, FW fascicle width, FSL fascicle sheath length, NL/NW ratio of length to width of needle, NL/FSL ratio of needle length to fascicle sheath length, FW/NW ratio of fascicle width to needle width

Table 3 Descriptive statistics (mean ± standard deviation) for each cone trait among populations of *Pinus yunnanensis*

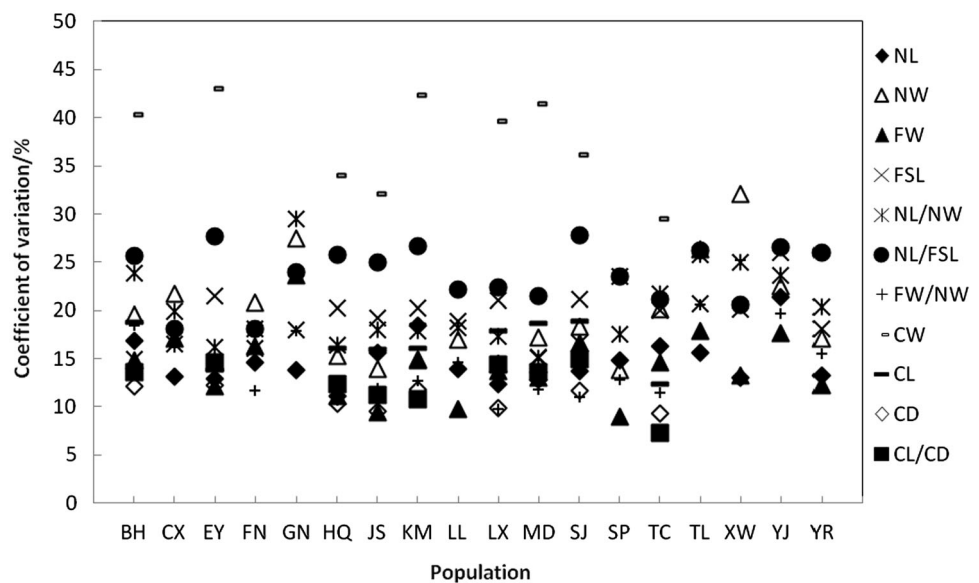
Population	CW ^{A,B}	CL	CD	CL/CD
BH	38.8 ± 15.7ab	68.4 ± 12.9a	38.8 ± 4.7a	1.8 ± 0.2bc
EY	22.2 ± 9.6d	57.3 ± 7.9d	31.4 ± 3.8de	1.8 ± 0.3ab
HQ	43.3 ± 14.7a	64.7 ± 10.4ab	34.3 ± 3.6bc	1.9 ± 0.2a
JS	29.3 ± 9.4c	60.0 ± 9.6bcd	34.7 ± 3.3b	1.7 ± 0.2c
KM	34.1 ± 14.4bc	62.8 ± 10.1bc	35.1 ± 4.2b	1.8 ± 0.2abc
LX	38.7 ± 15.4ab	64.0 ± 11.4abc	33.8 ± 3.4bc	1.9 ± 0.3a
MD	34.0 ± 14.0bc	59.2 ± 11.1cd	32.8 ± 4.5cd	1.8 ± 0.3abc
SJ	27.8 ± 10.0cd	57.6 ± 10.9d	30.6 ± 3.6e	1.9 ± 0.3a
TC	39.3 ± 11.6ab	63.2 ± 7.8bc	34.5 ± 3.2bc	1.8 ± 0.1abc
Average	34.2 ± 12.8	61.9 ± 10.2	34.0 ± 3.8	1.8 ± 0.2

^A Each cone was analyzed separately on the basis of 3 characters: 3 measured and 1 calculated

^B Mean ± standard deviation sharing a common letter were not significantly different at $\alpha = 0.05$ by Tukey's test

CW Cone weight, CL cone length, CD cone diameter, CL/CD ratio of length to diameter of cone

Fig. 2 Coefficients of variation (%) of seven needle traits in 18 populations, and four cone traits in nine populations. Populations are described in Table 1, trait abbreviations are explained in Table 4



width, with an average value of 0.34 among all populations for needle traits, and from 0.03 for ratio of length to width of cone to 0.20 for cone diameter. Over all the traits, the morphological divergence was 0.25 among populations. The cone traits exhibited lower morphological divergence (i.e., P_{ST} index) when compared to needle traits.

We observed significant correlations between the observed needle and cone traits and geo-climatic parameters (Table 5). Needle length (NL) and the ratio of needle length to fascicle-sheath length (NL/FSL) exhibited significant and negative correlations ($p < 0.01$) with latitude and altitude. Thus, shorter needles were associated with higher altitudes and latitudes. The same two traits were positively correlated with temperature ($p < 0.01$) and precipitation factors ($p < 0.05$), which implied that there

were longer needles and higher ratio of needle length to fascicle sheath length in populations that grow in lower altitudes and latitudes and warmer climates with more rainfall.

Needle width (NW) displayed a positive correlation with longitude ($p < 0.05$). From east to west, the needles were narrower. However, the linkage between NW and latitude as well as the correlation between NW and altitude was weak ($r = -0.103$ and -0.119 , respectively) and insignificant. Fascicle width, although different among populations, was not significantly correlated with any geo-climatic parameters we analyzed.

Correlations between cone traits and geo-climatic parameters were not significant except for the correlation between the ratio of length to diameter of cone (CL/CD)

Table 4 Variance component and percentage for each trait in eighteen populations of *Pinus yunnanensis*

Traits	Variance component percentage of total			P_{ST}
	Among populations	Within populations	Error	
Needle length (NL)	22.48	49.86	27.66	0.18
Needle width (NW)	30.60	39.31	30.09	0.28
Fascicle width (FW)	33.96	41.46	24.58	0.29
Fascicle sheath length (FSL)	27.40	42.51	30.10	0.24
Ratio of length to width of needle (NL/NW)	36.97	28.87	34.15	0.39
Ratio of needle length to fascicle sheath length (NL/FSL)	31.93	33.37	34.71	0.32
Ratio of fascicle width to needle width (FW/NW)	53.51	11.90	34.59	0.69
Cone weight (CW)	15.07	58.69	26.24	0.11
Cone length (CL)	7.02	64.35	28.63	0.05
Cone diameter (CD)	23.85	47.33	28.82	0.20
Ratio of length to width of cone (CL/CD)	3.93	63.30	32.76	0.03

Table 5 Spearman correlation between needle traits and geographic parameters

Traits	Longitude	Latitude	Altitude	Tmean	Tmax	Tmin	Precipitation
Needle length (NL)	0.180	-0.687**	-0.822**	0.695**	0.616**	0.716**	0.534*
Needle width (NW)	0.536*	-0.103	-0.119	0.336	0.368	0.251	-0.413
Fascicle width (FW)	-0.209	0.237	0.391	-0.340	-0.367	-0.290	-0.218
Fascicle sheath length (FSL)	0.273	0.403	0.269	-0.275	-0.169	-0.250	-0.488*
Ratio of length to width of needle (NL/NW)	-0.368	-0.228	-0.319	0.070	0.025	0.155	0.577*
Ratio of needle length to fascicle sheath length (NL/FSL)	-0.017	-0.734**	-0.711**	0.640**	0.522*	0.639**	0.708**
Ratio of fascicle width to needle width (FW/NW)	-0.518*	0.252	0.340	-0.531*	-0.582*	-0.441	0.258
Cone weight (CW)	-0.017	0.383	0.133	-0.136	-0.227	-0.171	-0.117
Cone length (CL)	0.217	0.117	-0.067	0.102	0.034	0.060	-0.200
Cone diameter (CD)	0.633	-0.017	-0.083	0.043	0.008	0.026	-0.250
Ratio of length to diameter of cone (CL/CD)	-0.711*	0.151	0.176	-0.180	-0.207	-0.189	0.360

Bold values are statistically significant

* $p < 0.05$; ** $p < 0.01$

and longitude ($\alpha = 0.05$) (Table 5). Cone shape was shorter and thinner with increasing longitude ($r = -0.711$, $p < 0.05$). The results indicated that the observed differences among populations in cone traits could not be associated clearly with any geo-climatic patterns.

Because cones were available from a limited group of populations, principal component analysis (PCA) was based on needle traits only (Fig. 3). The eigenvalues indicated that three components provided a good summary of the data, accounting for 87 % of the total variance. The first component (PC1) explained 35.15 % of the total variance. PC1 had high positive loadings on ratio of length to width of needle (NL/NW) and ratio of fascicle width to needle width (FW/NW), and high negative loadings on needle width (NW). The second component explained 30.27 % of the total variance. PC2 had high positive loadings on needle length (NL) and ratio of needle length

to fascicle sheath length (NL/FSL). There was also a moderate positive loading on ratio of needle length to needle width (NL/NW). PC2 strongly separated population SJ from the others. Populations BH, CX, FN, GN, TL, and YR were separated from the others by PC3, which showed strong loading on fascicle width (FW) but strong negative loading on fascicle sheath length (FSL). The results of principal component analysis indicated that geographic proximity did not predict similarity based on needle traits.

Discussion

Significant differences in needle morphology were found both among and within populations. Similar patterns of variability in those traits were found in other species as well. Calamassi et al. (1988) showed that significant

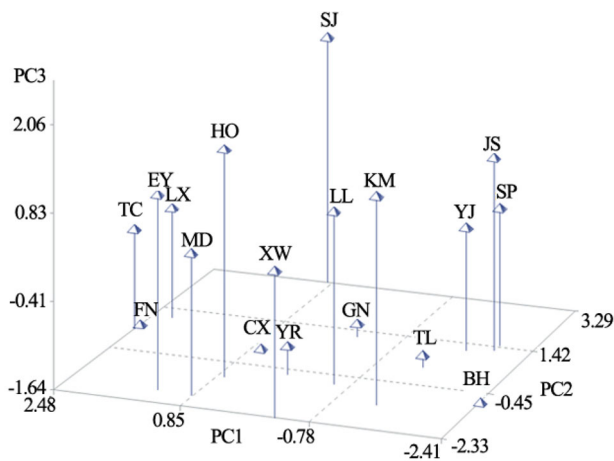


Fig. 3 Three-dimensional view of relationships among 18 populations of *Pinus densata* based on seven needle traits as separated by the first three principal components. The population abbreviations are the same as Table 1

differences existed for all morphological and anatomical needle traits considered among 14 populations from different regions of the natural range of *P. brutia*. We also found high levels of variability in cone morphology among and within populations of *P. yunnanensis*. Similar findings were reported in conifer species such as *P. canariensis* (Gil et al. 2002), *P. halepensis* (Melzack et al. 1981), *P. pinaster* (Wahid et al. 2006), *P. radiata* (Forde 1964), *P. strobus* (Beaulieu and Simon 1995), *P. sylvestris* (Turma and Güney 2009), *P. tecunumanii* (Piedra 1984), and *P. wallichiana* (Singh and Thapliyal 2012).

Morphological traits of conifer species are known to vary adaptively with the geographic and climatic variables (Ji et al. 2011). Patterns of variation within tree species depend on several factors, including geographic distribution, breeding system, and historical events (Wahid et al. 2006). Long generation times, an outcrossing mating system (allogamy), wind pollination, high fecundity, and winged seed dispersal are associated with high levels of genetic and phenotypic variation (Hamrick et al. 1979). The level of genetic diversity detected in limited samples natural populations of *P. yunnanensis* was relatively high based on the analysis of allozymes (Yu et al. 2000).

Observed patterns of phenotypic variation may be the result of local adaptations to a variable environment as was observed for jack pine (*P. banksiana*) (Maley and Parker 1993). Although cone traits of *P. yunnanensis* showed high variability among populations coupled with sizeable variation within population, needle traits had higher variation than cones traits among populations. This may be because foliage size is easily influenced by local environmental conditions (Dangasuk and Panetsos 2004).

Conversely, reproductive traits are believed to be the most conserved plant traits and least affected by the

environment (Vasudeva et al. 2004). Nevertheless, cone size and weight can be influenced by tree age, general health of the trees, and the macro- and micro- habitat of the parent trees (Dangasuk and Panetsos 2004). Long-term adaptation to diverse and changing environmental conditions (including fires, recurrent volcanic events, etc.) and the lack of competition with other pines may maintain high variability in cone morphology (Gil et al. 2002), which may explain the high levels of variability we observed for cone morphology in *P. yunnanensis*.

In the present study, within-population variation accounted for most of the total phenotypic variation for nine of eleven traits studied; the mean proportion of phenotypic variation among populations of *P. yunnanensis* for 11 needle and cone traits was about 38 % (data not shown). A similar conclusion was reached by Ji et al. (2011), who reported the mean proportion of phenotypic variation among populations of *P. tabulaeformis* in 12 measured traits was about 28 %. Piedra (1984) used analysis of variance (ANOVA) to demonstrate that almost two-thirds of the variation for the needle, cone, and seed characters was attributable to between-tree differences, based on 108 trees of *P. tecunumanii* from five geographic regions of Guatemala. Maley and Parker (1993) indicated that most of the variation was expressed between trees and within trees, with relatively little (1.6 to 18.9 % depending on the trait) expressed between sampled populations based on phenotypic variation of cone and needle morphology in jack pine (*P. banksiana*).

Rawat and Bakshi (2011) emphasized that genetic variation within and between populations was a substantial determinant of the adaptive abilities of populations. The high levels of variation in phenotypic traits we observed may reflect adaptation to competition or to a range of environments (Gil et al. 2002). An alternative for Q_{ST} , which is reported widely in tree species (Leinonen et al. 2008; Alberto et al. 2013), is the mean phenotypic differentiation (P_{ST}) (Spitze 1993). Genetic estimates of population differentiation in quantitative traits (Q_{ST}) varied widely among tree species. A review by Alberto et al. (2013) showed that among 16 European conifers species with small or fragmented range, values of Q_{ST} ranged from 0.028 to 0.616, with a mean of 0.192. For 11 European conifer species with a continuous range, Q_{ST} varied from 0.075 to 0.830, with a mean of 0.463. The mean P_{ST} among populations of *P. yunnanensis* for 11 needle and cone traits was 0.25, which probably indicates the adaptive differentiation of populations in phenotypic traits, but the values of P_{ST} were moderate compared to published values for other species, probably indicating that the populations we sampled do not represent unusual patterns of variability.

In *P. yunnanensis*, needle length (NL) and the ratio of needle length to fascicle sheath length (NL/FSL) showed

patterns of variation along latitudinal and altitudinal gradients. In warmer environments (low latitude and altitude), needles were longer, and the ratio of needle length to fascicle sheath length was higher. Other needle traits, such as needle width, fascicle width, and fascicle sheath length, however, showed only loose correlation with geo-climatic parameters. Therefore, particular needle traits, especially needle length and fascicle sheath length, were more sensitive to geographic and climatic gradients than the other measured traits. Similar findings were described in *P. brutia* by Dangasuk and Panetsos (2004), who reported that needle length and width, fascicle sheath length, number of teeth per cm, and the number of resin canals were the most useful traits for identifying altitudinal and longitudinal adaptation variations within and among populations. The relationship between needle length and width and geographic parameters like longitude and altitude for *P. yunnanensis* was in agreement with the findings of Wahid et al. (2006), who found needle length and width correlated negatively with altitude and positively with longitude in maritime pine (*P. pinaster*).

In spite of the overall absence of significant correlation between fascicle width and geo-climatic parameters (Table 5), we observed an association between thick fascicles and dry, cold environments. Populations HQ, EY, XW, and MD all came from high altitudes (Table 1) where precipitation was low and Tmax was generally low. These populations had relatively high fascicle width and short needle length. The population with highest fascicle width was KM, which was also high-altitude. Population EY had the shortest needles and was also from the highest elevation.

Although there was large variation in cone traits of *P. yunnanensis* among populations, no significant correlation was detected between cone traits and geo-climatic parameters except for the negative correlation between the ratio of length to diameter of cone (CL/CD) and longitude. Cone traits were significantly different ($p < 0.01$) among populations, but interpopulation differences were not associated with a climatic gradient. Similar results were observed in *P. brutia* (Dangasuk and Panetsos 2004) and Aleppo pine (*P. halepensis* Mill.) (Melzack et al. 1981). Singh and Thapliyal (2012) pointed out cone characteristics correlated negatively with latitude and positively with altitude in blue pine (*P. wallichiana*), but no particular trend was found between cone characteristics and geographic variables like latitude and altitude. Cone weight, length, and diameter were negatively correlated with precipitation in present study. This result was in agreement with findings in other species of the genus such as *P. roxburghii* (Roy et al. 2004). Cones may be larger in low-precipitation environments (Gil et al. 2002).

It was difficult to discern environmental patterns that explained the clustering of populations in the principal components space (Fig. 3). The most likely reason for this

observation was that population-level trait measures concealed intra-collection-site environmental variation, which, as previously mentioned, can strongly affect needle morphology (Dangasuk and Panetsos 2004). Some traits with high P_{ST} and high loading in PC1, such as NL/NW and NW, showed relatively high intra-population variability (Table 4; Fig. 2). This may indicate that these traits are developmentally plastic in response to environment. As described above, the correlation matrix between needle traits and environmental characteristics (Table 5) presents several strong and highly significant correlations: for example, between short needles and high altitude locations, which are reasonable and worth further investigation.

A lack of geographic correspondence between populations with similar phenotypes was also observed in *P. canariensis* (Gil et al. 2002), *P. radiata* (Forde 1964), and *P. tecunumanii* (Piedra 1984). Beaulieu and Simon (1995) showed that no general geographical trend was detected in the observed variation in *P. strobus* collected from ten natural populations in the Canadian province of Quebec.

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