应用与环境生物学报 2007,13(2):145~151
Chin J Appl Environ Biol = *ISSN 1006-687X* 2007-04-25

Identification of *Tsuga* Germplasm by Morphological Characters and RAPD Markers*

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Abstract Germplasm collection is important to preserve and maximize genetic diversity for germplasm conservation. *Tsuga dumosa* (D. Don) Eichler in Engler & Prantl. and *T. chinensis* var. *forrestii* (Downie) Silba germplasm was collected from three localities in China: Mt. Yulong, Wenfeng Temple and Mt. Dishiergu, Yunnan Province. Accessions were identified based on morphological characters and RAPD markers. The shapes of the apices and margins of needles were examined, and the length and width of needles, cones and seeds from accessions of mature plants were used to compare the morphological differences and to identify the germplasm. Molecular markers generated by randomly amplified polymorphic DNA (RAPD) were also used to characterize the taxa. Although the clustering based on RAPD markers was inconsistent with the morphological characters of the needles, based on the overall morphological characters and on RAPD markers, the accessions from Mt. Yulong and Wenfeng Temple were identified as *T. chinensis* var. *forrestii*, and those from Mt. Dishiergu identified as *T. dumosa*. Taxonomic identification of the accessions was made based on morphology and by RAPD markers concurred. The results indicate that the shapes of the apices and margins of needles particularly from young plants could not be used as a possible key to identify *T. dumosa* and *T. chinensis* var. *forrestii*. Fig 6, Tab 3, Ref 24

Keywords Identification of germplasm; *Tsuga dumosa*; *Tsuga chinensis* var. *forrestii*; morphological characters; RAPD
CLC Q949.665.03 (274)

The genus *Tsuga* (Pinaceae) comprises 10 to 11 species, of which four Asian species, *T. dumosa*, *T. chinensis* (Franchet) Pritzel, *T. oblongisquamata* (W. C. Cheng & L. K. Fu) L. K. Fu & Nan Li and *T. longibracteata* W. C. Cheng, are distributed in China [1]. Since the hemlock woolly adelgid (*Adelges tsugae* Annand) has become a serious pest of *T. canadensis* (L.) Carr. and *T. caroliniana* Engelm. [2], the populations of native *Tsuga* in eastern North America have decreased. Introduction of foreign germplasm is needed to broaden the genetic diversity available for breeding insect and disease resistance.

T. dumosa occurs along the Himalayas between 2 600 and 3 200 m, and from northwestern Yunnan to southwestern Sichuan between 1 700 m and 3 500 m [1,3~5]. *T. chinensis* var. *forrestii* is a high-mountain species occurring between 2 000 and 3 500 m and it grows in the area where *T. dumosa* and *T. chinensis* var. *chinensis* are sympatric at Mt. Yulong, Yunnan and the southwest of Sichuan. *Tsuga* is usually identified based on the shape and size of cone and seed scales of mature plants.

Although needle morphology of mature plants that produce cones has not been used as a major key to identify *Tsuga* [1] due to a considerable variability of the morphological features of the needles [6], the shapes of the needle apices and leaf margins could be a valuable tool to identify young seedlings before they reach maturity. The apices of *T. dumosa* needles can be acute, obtuse or intermediate, and the needles are rarely emarginate (notched) or denticulate at the apical half of the needles. For *T. chinensis* var. *forrestii*, needles are emarginate, obtuse, or entire or rarely serrulate [4,5].

Randomly amplified polymorphic DNA (RAPD) [7] has been applied to identify species and cultivars [8~12], and to characterize germplasm of conifers [13~15]. The objective of this study was to identify *Tsuga* germplasm from Yunnan, China based on the molecular markers obtained by RAPD, and compare the results with the morphological characters of young seedlings and mature plants.

1 Material & Methods

1.1 Accession of germplasm

Needles of *Tsuga* were collected from 30 young plants and 9 mature plants in the areas of Mt. Yulong (Y), Wenfeng Temple (W) and Mt. Dishiergu (D), Yunnan, China (Table 1) to examine germplasm according to the locations and elevations, development stages (mature and young plants) and morphological variations within *T. dumosa* and *T. chinensis* var. *forrestii*. To examine the variations due to elevation for the Mt. Dishiergu population, samples, as indicated in Table 2 were collected from young and adult trees between 2 800 m and 3 290 m of the mountain top. Herbarium specimens from 7 mature plants were deposited in the US National Arboretum Herbarium. Samples of specimens of *T.*

Received: 2006-12-08 Accepted: 2006-12-26
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dumosa (accession No. 91.114) and *T. chinensis* var. *forrestii* (accession No. 91.267) from the Quarryhill Botanical Gardens (Glen Ellen, CA, USA) were included to confirm the identification of each taxon.

Table 1 Accession information of *Tsuga* germplasm

DNA No.	Description	Needle apex		Needle margin	
		Acute	Emarginate	Prickly	Entire
Location: Mt. Yulong, Lijiang County, Yunnan					
Y1*	RFZX-1 (N 27.08.788; E100.15.181)	0	5	5	0
Y2	Young plants around Y4	0	5	0	5
Y3	Young plants around Y4	0	5	0	5
Y4*	RFZX-2, (50 m from Y 1)	5	0	0	5
Y5	RFZX-4, across the stream where Y1 stands	0	5	1	4
Location: Wenfeng Temple					
W6*	RFZX-6 (N 26.48.544; E 100.09.484)	0	5	5	0
W7*	RFZX-7 (200 m from W 6)	0	5	5	0
W8*	RFZX-8 (100 m west of RFZX-7)	0	5	5	0
W9	Young plant around W 8	0	5	5	0
W10	Young plant around W 8	0	5	0	5
W11	Young plant around W 8	0	5	0	5
W12	Young plant around W 6	4	1	3	2
Location: Dishiergu Mountain.					
D13*	Young plant, 3 m from the oldest tree (N 27.14.625; E 99.25.720) 30 cm tall	0	5	0	5
D14	4 m from D13, 15 to 20 cm tall	0	5	3	2
D15	4 m from D 13, 8-10 cm tall	5	0	0	5
D16	7 m from D 13	5	0	2	3
D17*	RSZ-2	5	0	5	0
D17	Young plant, 10 m from D 17, 30 cm tall	5	0	1	4
D19	Young plant near D 17, 30 cm tall	5	0	0	5
D20*	Many seeds were formed, 3200 m	5	0	1	4
D21	Young plant, 10 m from D 20	5	0	0	5
D22	Young plant, 10 m from D 20	5	0	0	5
D23	100 m lower from D 20	5	0	0	5
D24*	RSZ-3, 280 m	5	0	0	5
D25	Young plant around D 24	5	0	5	0
D26	Young plant around D 24	5	0	5	0
D27	Young plant around D 24	5	0	4	1
D28	Young plant around D 24	5	0	4	1
D29	Young plant (3290 m)	5	0	0	5
D30	Young plant (3250 m)	5	0	3	5
D31	Young plant (3200 m)	5	0	0	5
D32	Young plant (3150 m)	5	0	4	5
D33	Young plant (3100 m)	5	0	2	5
D34	Young plant (3050 m)	5	0	0	5
D35	Young plant (3000 m)	5	0	5	1
D36	Young plant (2950 m)	5	0	2	5
D37	Young plant (2900 m)	5	0	5	0
D38	Young plant (2950 m)	5	0	0	5
D39	Young plant (2800 m)	5	0	5	0
T. dumosa (accession No. 91.114)		5	0	5[z]	0
T. chinensis var. *forrestii* (accession No. 91.267)		0	5	0	5[z]

Asterisks represent mature or standard trees for measuring distance of young plants. Height of accessions of young plants (D24 ~ D39) was about 20 to 30 cm. [z]Based on the information from Fu, *et al.*, 1999

Table 2 Morphological data and identification of *Tsuga* accessions from Yunnan, China

Specimen Id[z]	DNA Id[z]	Collection site	Leaf (cm)		Cone (cm)		Scale (cm)		Seed wing (cm)		Identification
			Length	Width	Length	Width	Length	Width	Length	Width	
RFZX-1	Y1	Mt. Yulong	1.51	0.17	3.34	1.32	1.21	1.04	1.02	0.38	*T. chinensis* var. *forrestii*
RFZX-2	Y2	Mt. Yulong	1.63	0.17	3.21	1.34	1.43	0.93	1.20	0.39	*T. chinensis* var. *forrestii*
RFZX-4	Y5	Mt. Yulong	1.34	0.17	1.94	1.06	1.06	0.83	0.94	0.35	*T. chinensis* var. *forrestii*
RFZX-6	W6	Wenfeng Temple	1.64	0.17	2.89	1.13	1.32	1.03	1.02	0.43	*T. chinensis* var. *forrestii*
RFZX-7	W7	Wenfeng Temple	1.44	0.16	1.63	0.94	0.97	0.80	0.88	0.34	*T. chinensis* var. *forrestii*
RSZ-2	D17	Mt. Dishiergu	1.39	0.17	1.49	0.83	0.88	0.64	0.84	0.31	*T. dumosa*
RSZ-3	D24	Mt. Dishiergu	1.18	0.17	1.49	0.80	1.03	0.65	0.82	0.32	*T. dumosa*
Significance (HSD at 5%)			0.14	ns	0.37	0.18	0.2	0.13	0.18	0.09	

Herbarium specimens are deposited in the US National Arboretum Herbarium. No accession numbers are assigned

1.2 Morphological characters

The length and width of 30 needles, the length and width of 5 cones, and the length of 5 scales and 10 seed wings sampled from the middle part of a cone were measured for each accession, respectively. Analysis of variance was conducted and means were compared by Tukey's honestly significant difference (HSD) [16]. The needle apices and margins from a minimum of five needles of each accession were examined under a dissecting microscope at 70×. The apices were recorded either as acute or emarginate (notched) (Table 1, Fig. 1). The presence or absence of prickle-like structures at the margin of the apical half of the needles were also recorded either as 'prickly' when present, which are often referred to as prickle-shaped teeth, or 'entire' when absent. For *T. dumosa* and *T. chinensis* var. *forrestii* provided from the Quarryhill Botanical Gardens, information on needle margins was based on the description by Fu *et al.* (1999) [1]. Data on the morphological characters of the needles were analyzed using the Mantel-Haenszel analysis for categorical data [17]. Location and age of the trees, i. e., mature plants and young plants, from which the needles were collected were tested as main effects.

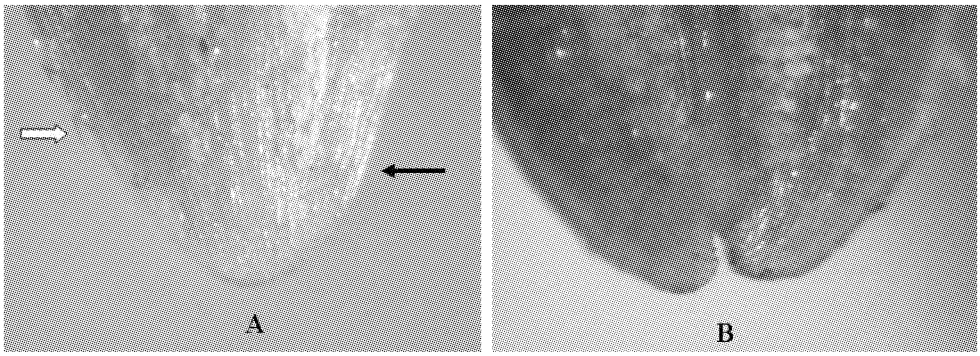

Fig. 1 Morphological difference of needles of *T. dumosa* (left) and *T. chinensis* var. *forrestii* (right)
The apex of needles is either acute (A) or emarginate (B), and the margin of the needles near the apex is either prickly (⇒) or entire (←)

1.3 DNA extraction, amplification and molecular markers

DNA was extracted from 70 mg (dried) or 100 mg (fresh) needles using a CTAB buffer with a Lysis Matrix (Q-Bio Gene, Calsbad, CA), following the protocol of Doyle and Doyle [18]. The DNA was quantified by fluorometry (Hoefer Pharmacia Biotech Inc., San Francisco, CA, USA). After screening 43 random 10-mer primers using 7 mature samples (RFZX-1,-2,-6,-7,-8, RSZ-2,-3), 8 primers (OP-A07, OP-A11, OP-A-15, OPA-17, OPA-19, OP-B05, OP-B12 and OP-C04; Operon Technologies, Alemada, CA, USA) were selected for analyses. Amplification for RAPD was performed using 10 ng of template DNA, 5 pmoles of a primer and Ready-To-Go PCR Beads (PCR bead 27-9555-01, Pharmacia Biotech Inc., Piscataway, NJ) for a total volume of 25 L, using PTC-100 Programmable Thermal Cycler (MJ Research, Watertown, MA, USA). The PCR profile consisted of one cycle of 3 min at 94 ℃, 36 cycles of 5 s at 94 ℃, 30 s at 37 ℃, 1 min at 72 ℃ and then the final extension at 72 ℃ for 3 min. Amplifications were repeated twice. Amplified products were separated on 1.2% agarose gel (0.5× TBE buffer) for 3 h at 107 volts, stained with ethidium bromide and visualized on a UV trans-illuminator.

Accessions were divided arbitrarily into two groups so that each group could be loaded into a 30-well gel for electrophoresis. The first group consisted of the accessions from Mt. Yulong, Wenfeng Temple and some accessions from Mt. Dishiergu, and the second group included 16 accessions from Mt. Dishiergu to examine the variation between young and adult individuals (Table 1). Based on the preliminary results, a final analysis was performed with representative accessions (Y1, Y4, W6, W8, W12, D17, D24, D28, D31 and D39) including *T. dumosa* (accession No. 91.114) and *T. chinensis* var. *forrestii* (accession No. 91.267).

Amplification products ranging in size from 400 bp to 1 800 bp were selected for scoring manually. Only reproducible bands appearing on all gels following adjustment of the contrast scales were selected for scoring. Reproducibility of bands was determined as described [11]. Polymorphic bands were scored and the corresponding matrix was used to construct dendrograms by the unweighted pair-group method using arithmetic average (UPGMA) and Molecular Evolutionary Genetics Analysis (MEGA) program, and p-distance was obtained [19].

2 Results

2.1 Identification of germplasm by morphological characters

The needles of the accessions collected from Mt. Yulong (Y) and Wenfeng Temple (W) were longer than those from Mt. Dishiergu (D) (Table 2). The length and width of cone scales and length of seed wings of Y1, Y2 and W8 were greater than those of other accessions. The needle length of Y2 (1.63 cm), cone scale size of Y1 (3.34 cm) and seed wing length of Y2 (1.20 cm) were longest, which were significantly different from those of D17 or D24 collected from Mt. Dishiergu (Table 2). The cone length of Y1 (3.34 cm) and Y2 (3.21 cm) from Mt. Yulong was significantly greater than that of W6 (2.89 cm) from the Wenfeng

Temple (Table 1, Fig. 2). All accessions from Mt. Yulong (Y), with an exception of Y4, and Wenfeng Temple (W) had needles with emarginate apices (Fig. 1).

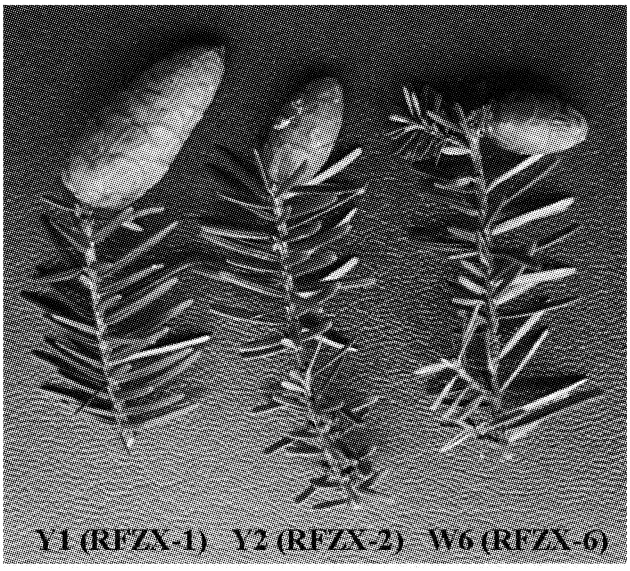

Fig. 2 Difference in size of the cones of Y1 (RFZX-1) and Y2 (RFZX-2) from Mt. Yulong, and W6 (RFZX-6) from the Wenfeng Temple

The accessions collected from Mt. Dishiergu (D) had shorter needles, small cones, scales and seeds, and acute apex on needles, except D13 and D14 having emarginate needles. The cones of 4 *T. chinensis* var. *forrestii* accessions except RFZX-7 (W7) were significantly larger than those of *T. dumosa* accessions. Variations within a taxon were found in the needle length, and in cone and scale size of both *T. dumosa* and *T. chinensis* var. *forrestii*. With these observed morphological characters based on the previous studies [1,4,5], the accessions collected from Mt. Yulong (Y) and from the Wenfeng Temple (W) were identified as *T. chinensis* var. *forrestii*, and those from Mt. Dishiergu as *T. dumosa*.

However, some accessions, such as Y4 (RFZX-2) and W12 (a young plant growing near W6) (Table 1) showed an acute needle apex, which was different from the majority of the accessions within the same locality. Overall, the margin near needle apex, either prickly or entire, was variable among the accessions within the same locality and was not affected by the maturity of plants (Table 1). Overall data analysis indicated that a significant association occurred among location, age of the tree and the frequency of each needle type (MH value = 152.733 1, $P < 0.000\ 1$) (Table 3). In Mt. Yulong, while no acute apex of needles was observed from young plants, acute apex was recorded from mature plants. Needles with prickly margins were observed only in mature plants. Needles with emarginate apex and entire margins did not differ between mature trees and young plants. In the Wenfeng Temple, needles with acute apex and prickly margins were obtained from one (W12) young plant. The plants from both Mt. Yulong and the Wenfeng Temple had higher percentage of emarginate needles regardless of their maturity, while only one young plant (D14) from Mt. Dishiergu had emarginate needles. The young plants from Mt. Dishiergu were found with a significantly higher percentage of entire than prickly needles, whereas young plants from Mt. Yulong had entire needles, while those from the Wenfeng Temple had either entire or prickly needles.

Table 3 Percentage of each needle type collected from mature trees and young plants at three locations

Location	Maturity	Morphological characters of the needles			
		Needle apex		Needle margin	
		Acute	Emarginate	Prickly	Entire
Mt. Yulong	Mature	1.24[z]	2.49	2.24	1.49
	Young	0	2.49	2.49	0
Wenfeng Temple	Mature	0	3.73	0	3.73
	Young	1.00	3.98	2.99	1.99
Mt. Dishiergu	Mature	2.49	0	1.24	1.24
	Young	28.61	2.49	21.39	12.69

[z]Needles of mature plants collected from Mt. Yulong had acute needle apex, which account for of 1.24 %. The total, when all respective percentages are added, will be 100%

2.2 Characterization using RAPD markers

A total of 95 polymorphic bands were scored for 23 samples from Mt. Yulong (Y), Wenfeng Temple (W) and Mt. Dishiergu (D) (data not presented), and a dendrogram was constructed (Fig. 3). All accessions from Mt. Yulong were clustered together, and all but W6 and W12 accessions from the Wenfeng Temple were clustered together; W6 and W12 at the Wenfeng Temple were clustered with the accessions from Mt. Yulong. The accessions of Mt. Dishiergu formed one cluster separated from the other clusters of Mt. Yulong and the Wenfeng Temple. The Mt. Dishiergu clade seemed to have separate clusters based on altitude. The accessions from lower altitudes (D35 through D39) were clustered separately from those from higher altitudes (D30 through D34). One exception was D29 that was collected from the highest altitude near the oldest plants (Table 1, Fig. 3). All needles had an acute apex, except D14 that had an emarginated leaf apex, and D14 was distantly clustered from all accessions (Fig. 4) with an acute apex (Fig. 2). No correlation between these sub-groupings of accessions and morphological characters, particularly the margin of needles, was found (Table 1).

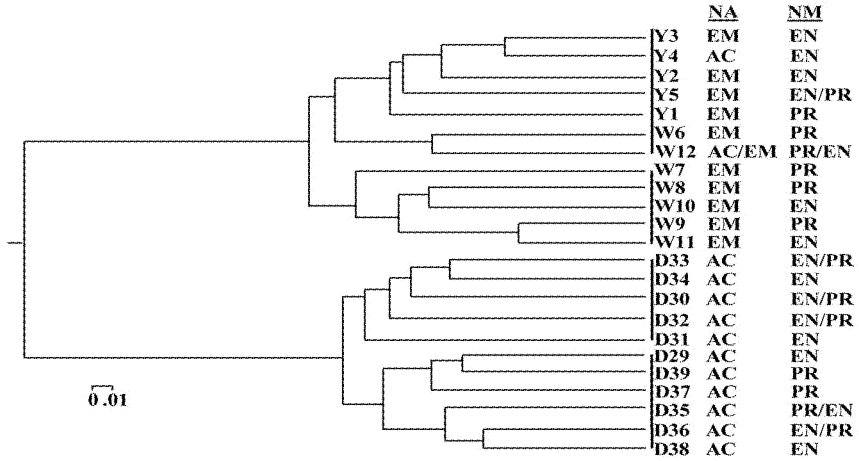

Fig. 3　Cluster analysis by UPGMA obtained by RAPD markers in *Tsuga* germplasm collected from Mt. Yulong (accessions Y), Wenfeng Temple (accessions W) and Mt. Dishiergu (accessions D)

NA: Apex of needles is either acute (AC) or emarginate (EN). NM: Margin of the needles at apex is either prickly (PR) or entire (EN). P-distance scale bar is presented in the figure. The same below

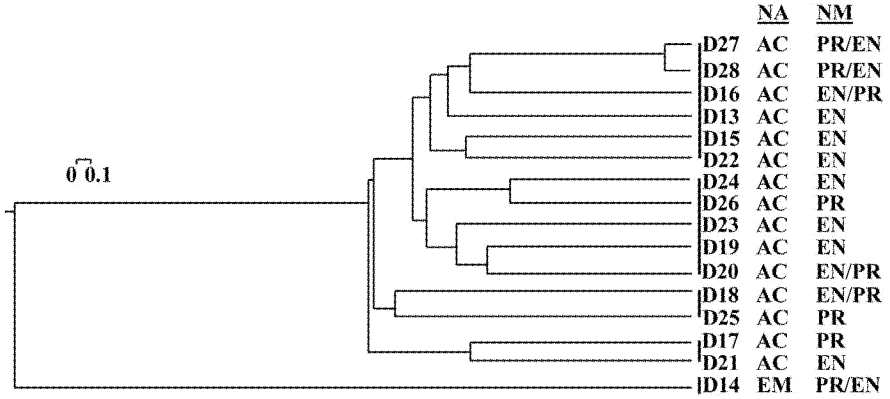

Fig. 4　Cluster analysis by UPGMA obtained by RAPD markers in *Tsuga* germplasm collected from Mt. Dishiergu (accessions D)

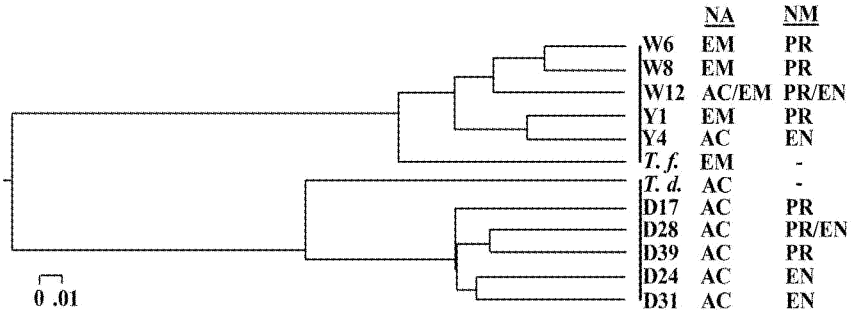

Fig. 5　Cluster analysis by UPGMA obtained by RAPD markers in *Tsuga* germplasm collected from Mt. Yulong (accessions Y), Wenfeng Temple (accessions W) and Mt. Dishiergu (accessions D), and *T. chinensis* var. *forrestii* (*T. f.*) and *T. dumosa* (*T. d.*)

RAPD analysis was further performed with selected accessions (Y1, Y4, W6, W8, W12, D17, D24, D28, D31 and D39) including *T. dumosa* and *T. chinensis* var. *forrestii* established at the Quarryhill Botanical Garden. During field collection, accession W6 could not be easily identified in the field. A total of 69 polymorphic bands were scored (8, 9, 10, 9, 8, 8, 9 and 8 bands with OP-A07, OP-A11, OP-A15, OP-A17, OP-A19, OP-B05, OP-B12 and OP-C04, respectively). Accessions Y1 and Y4 from Mt. Yulong, and W6, W8 and W12 from the Wenfeng Temple were clustered together with *T. chinensis* var. *forrestii*, while D17, D24, D28, D31 and D39 from Mt. Dishiergu clustered with *T. dumosa* (Fig. 5). Although the accessions from Mt. Yulong and the Wenfeng Temple were clustered with *T. dumosa*, this grouping based on RAPD markers was not congruent with the findings based on the morphological characters of needles. RAPD markers from OP-A11 primer generated from *T. chinensis* var. *forrestii* (*T. f.*) and *T. dumosa* (*T. d.*), and the accessions collected from Mt. Yulong (Y1, Y4), Wenfeng Temple (W6, W8, W12) and Mt. Dishiergu (D17, D24, D28, D31, D39) are shown in Fig. 6. Two *T. dumosa* specific bands [760 bp (Td-1) and 470 bp (Td-2)] and two [710 bp (Tf-1) and 410 bp (Tf-3)] or three *T. chinensis* var. *forrestii* specific bands [710 bp, 600 bp (Tf-2),

and 410 bp] or sequence-characterized amplified region (SCAR) markers [20] could be used to identify young plants of these two species. The accessions from the Wenfeng Temple appeared not to have any *T. dumosa* bands.

3 Discussion

Based on the morphological characters and RAPD analyses, the accessions collected from Mt. Yulong (Y) and the Wenfeng Temple were confirmed as *T. chinensis* var. *forrestii* and those from Mt. Dishiergu (D) as *T. dumosa*. The size of cones and scales from mature plants at the Wenfeng Temple (W) showed intermediate morphological characters between *T. dumosa* and *T. chinensis* var. *forrestii*. Judging from the characters of cone size and scale shape, W6 could be assigned to either species. But RAPD data clearly showed that it was *T. chinensis* var. *forrestii*. We observed no correlation among the morphological characters of the accessions collected from Mt. Yulong, Wenfeng Temple and Mt. Dishiergu. Further, the juvenility or maturity of trees did not influence the margin shape. The margins of young needles of *T. dumosa* are denticulate although the mature ones are entire [4]. Therefore, we conclude that needle morphological characters are not influenced by the maturity of the plants, and the morphological characters are not a species-specific. Therefore, needle morphology could not be used to assign specific designation to *Tsuga* germplasm collected in Yunnan Province.

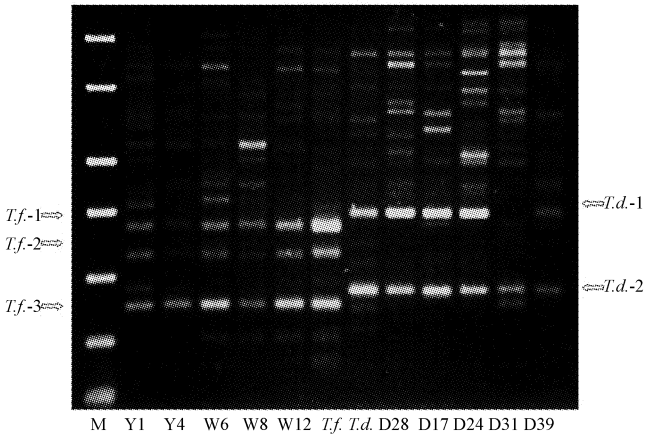

Fig. 6 RAPD amplification products using OPA 11 primer generated from *T. chinensis* var. *forrestii* (T. f.) and *T. dumosa* (T. d.), and accessions collected from Mt. Yulong (Y1, Y4), Wenfeng Temple (W6, W8, W12) and Mt. Dishiergu (D17, D24, D28, D31, D39)

M: Molecular marker (Sigma P 9577, St. Louis, MO). Possible marker bands specific for each species (species specific) are noted: Two *T. dumosa* specific bands [760 bp (Td-1) and 470 bp (Td-2)] and two [710 bp (Tf-1) and 410 bp (Tf-3)] or three *T. chinensis* var. *forrestii* specific bands [710 bp. 600 bp (Tf-2) and 410 bp]

Clustering of Y1 ~ Y4 from Mt. Yulong and of W6, W8 and W12 from the Wenfeng Temple in the final analysis in the same group suggests that the accessions from Mt. Yulong and the Wenfeng Temple are *T. chinensis* var. *forrestii*. If the accessions of Y1 and Y4 that are clustered closely are considered genetically different from the accessions of W6 and W12 in the same region, W6 can be considered different from Y1 or Y4 (Fig. 5), which have an acute apex and entire margins. Based on the RAPD profiles, the possibility of a hybrid nature of W6 and W12 is excluded, since there are no bands that are specific to *T. dumosa* (Fig. 6). Gene flow between two populations of *T. chinensis* var. *forrestii*, not necessarily between *T. chinensis* var. *forrestii* and *T. dumosa*, would be possible even though both species are sympatric in the Lijiang area where Mt. Yulong and the Wenfeng Temple are located [4, 5, 21]. Hybridization has been documented between *T. heterophylla* and *T. mertensiana* based on phytochemical and morphological interpretations [22], between *T. canadensis* and *T. caroliniana* based on DNA polymorphisms [23], and between eastern North American (*T. canadensis*) and Asian (*T. chinensis*) hemlock [24].

4 Conclusion

Based on RAPD markers, *Tsuga* accessions collected from Mt. Yulong and the Wenfeng Temple are *T. chinensis* var. *forrestii*, and the accessions from Mt. Dishiergu are *T. dumosa*. Two *T. dumosa* specific and three *T. chinensis* var. *forrestii* specific bands produced by using OPA-11 primer could be used to identify young plants of the two species. Identification of germplasm should not solely rely on morphological characters of needles from young plants that do not bear cones.

Acknowledgements We thank Bill McNamara, Peter Clements, Kim Hummer, Jianhua Li, Alan Whittemore and Nick Gawel for reviewing the manuscript; the statistical analysis of morphological characters by Jo-Ann Bentz is greatly appreciated and the support from the Committee Member Institutes of the North America-China Plant Exploration Consortium is greatly appreciated.

References

1 Fu L, Li N, Mill RR. Pinaceae. In: Wu W, Raven PH. Flora of China. Vol. 4. Science Press (Beijing, China) and Missouri Bot. Garden (St. Louis, USA), 1999. 11 ~ 41

2 Souto D, Luther T, Chianese B. Past and current status of HWA in Eastern and Carolina hemlock stands. In: Proc. of the First Hemlock Wooly Adelgid Review. USDA, Forest Service Publication FHTET 96-10, 1996. 9 ~ 15

3 Farjon A. Pinaceae: drawings and description of the genera. Koeltz Scientific Books. Federal Republic of Germany, 1990

4 Krüssmann G. Manual of Cultivated Conifers. 2nd rev. ed. Timber Press, Portland, OR, 1985. 361

5 Zhen W. Sylvia Sinica. Vol. 1. Beijing: China Timber Press, 1982. 203 ~ 210

6 Lepage BA. A new species of *Tsuga* (Pinaceae) from the middle Eocene of Axel Heiberg Island, Canada, and an assessment of the evolution and biogeoraphical history of the genus. *Bot J Linn Soc*, 2003, **141**: 257 ~ 296

7 Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*, 1990, **22**: 6531 ~ 6535

8 Asemota HN, Ramser J, Lop-Peralta C, Weising K, Kahl G. Genetic variation and cultivar identification of Jamaican yam germplasm by random amplified polymorphic DNA analysis. *Euphytica*, 1996, **92**: 341 ~ 351

9 Joung YH, Roh MS. Mapping characterization of *Pinus sylvestris* var. *sylvestriformis based on chloroplast DNA microsatellite markers*. *For Gen*, 2005, **12**: 89 ~ 98

10 Joung YH, Roh MS, Bentz SE. Characterization of *Acer griseum* and its putative interspecific hybrids. *Acta Hort*, 2001, **546**: 217 ~ 220

11 Lee A-K, Suh J-K, Roh MS, Slovin JP. Analysis of genetic relationships of *Ardisia* spp. using RAPD markers. *J Hort Sci & Biotech*, 2002, **78**: 24 ~ 28

12 Roh MS, Kurita S, Zhao XY, Suh JK. Identification and classification of the genus *Lycoris* using molecular markers. *J Kor Soc Hort Sci*, 2002, **43**:120 ~ 132

13 Hicks M, Adams D, O'Keefe S, McDonald E, Hodgetts R. The development of RAPD and microsatellite markers in lodgepole pine (*Pinus contorta* var. *latifolia*). *Genome*, 1998, **40**: 1 ~ 9

14 Leibenguth F, Shoghi F. Analysis of random amplified polymorphic DNA markers in three conifer species. *Silv Gen*, 1998, **47**: 120 ~ 126

15 Scheepers D, Eloy MC, Briquet M. Use of RAPD patterns for clone verification and in studying provenance relationships in Norway spruce (*Picea abies*). *Theor Appl Genet*, 1997, **94**: 480 ~ 485

16 Steel RGD, Torrie JH. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc. New York, NY, 1960

17 Stokes ME, Davis CS, Koch GG. Categorical data analysis using the SAS system. 2nd ed. Cary, NC: SAS Institute Inc., 2000

18 Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. *Focus*, 1990, **12**: 13 ~ 15

19 Kumar S, Tamura K, Nei M. MEGA: Molecular Evolutionary Genetics Analysis. 1st ed. The Pennsylvania State University, University Park, PA, USA, 1993

20 Roh MS, Lee A-K, Suh JK, Slovin J. Confirmation of cross-pollination of *Ardisia crenata* by sequence-characterized amplified region (SCAR) markers. *Sci Hort*, 2006, **109**: 361 ~ 367

21 Wang CW. The forest in China; with a Survey of Grassland and Desert Vegetation. Publ. Maria Moors Cabot. Found Bot. Res. 5 (Harvard Univ.), Cambridge, Mass., 1961

22 Taylor RJ. The relationship and origin of *Tsuga heterophylla* and *Tsuga mertensiana* based on phytochemical and morphological interpretations. *Amer J Bot*, 1972, **59**: 149 ~ 157

23 Wang C, Perlin MH, Van Stockum Jr, RR, Hamilton CH, Wagner DB. Chloroplast DNA polymorphisms in *Tsuga canadensis* and *Tsuga caroliniana*. *Can J For Res*, 1997, **27**: 1329 ~ 1335

24 Bentz SE, Riedel L, Pooler MR, Townsend AM. Hybridization and self-compatibility in controlled pollinations of eastern North American and Asian hemlock (*Tsuga*) species. *J Arboric*, 2002, **28**: 200 ~ 205