Electrophoretic Characterization of *Taxus* Cultivars

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Summary. Starch gel electrophoresis was used to fingerprint 55 *Taxus* plants, listed as 21 species and/or cultivars. Plants were analyzed for six enzymes, representing eight putative loci. Within many of the cultivars, different fingerprints were observed, indicating nomenclatural errors in *Taxus*.

Correct identification of *Taxus* species and cultivars can be difficult and misleading if based solely on morphological characteristics (Dirr, 1983). Misidentification of *Taxus* plants may stem from harvesting bulk cutting wood for propagation and extensive pruning and shaping, which greatly influence plant appearance.

Difficulties in the identification of *Taxus* have been compounded by nomenclature errors that can be traced back to 1918 (Chadwick and Keen, 1976), when Quarantine 37 was appropriated (U.S. Secretary of State, 1919). This law, which gave the Secretary of Agriculture the power to regulate imports and exports, prohibited importation of nursery stock into the United States (U.S. Secretary of State, 1913). The resulting lack of supply provoked some nursery operators to assign marketable plant names to poor-quality stock from unknown origins.

"The confusion of names resulting from such practices presented taxonomic difficulties of the genus which still exist today" (Chadwick and Keen, 1976). Dirr (1983) and Wyman (1990) agree that nomenclature problems still exist among the *Taxus* varieties, and Dirr (1983) specifies the problem "is particularly serious in the *T. cuspidata* and *T. × media* types."

Isozyme analysis is one method of testing suited to differentiating among many cultivars and species (Peirce and Brewbaker, 1973). Each protein has a specific net charge, seen as different rates of migration through a medium, resolved by staining. The sum of the results of different stains creates a "fingerprint" unique to each species and cultivar. This fingerprint can be used for identification purposes, as well as measuring genetic diversity within a species. Using isozyme analysis, nomenclature errors have been detected in some of the most-reliable sources of some species. For example, labeling errors of raspberry cultivars at the National Clonal Germplasm Repository were detected by Cousineau and Donnelly (1989). Recently, isozyme analysis was used to distinguish red maple cultivars (Tobolski and Kemery, 1992), apple rootstocks (Samimy and Cummins, 1992), and North American potato cultivars (Douches and Ludlam, 1991).

Our objective was to conduct isozyme analysis and fingerprint 55 plants of *Taxus* grown as 21 species and/or cultivars. The fingerprints would provide initial information for the review of these species and/or cultivars. Fingerprinting *Taxus* species and cultivars can help to avoid confusion within nursery production systems, and, ultimately, to protect the consumer from incorrectly named stock.

Materials and methods

Horizontal starch gel electrophoresis was used to analyze needles from 21 *Taxus* phenotypes, including species and cultivars collected from arboretum and nursery sites in Michigan and Ohio. The 55 plants sampled were 8 to 60 years old. When feasible, more than one plant of each species or cultivar was sampled from different sources. Collections were conducted between 24 Apr. and 30 June 1992. All samples were chilled during transportation and kept at $\approx 4^{\circ}\text{C}$ until prepa-

ration. Needles were ground in a chilled mortar and pestle. Six drops of the phosphate grinding buffer used by Soltis et al. (1983) and a spatula-tip-full of polyvinylpyrrolidone (#P-6755, Sigma, St. Louis) saturated with the grinding buffer were added to the mortar. Once a homogeneous consistency was reached, a nylon mesh screen was placed in the mortar and the solution was absorbed through the mesh onto a wick cut from Whatman #3 chromatography paper. The $5 \times 3 \times 1$ -mm wicks were stored in a Corning Elisa plate, double-wrapped with cellophane, sealed in plastic zipper bags, and stored at -80°C until loading onto the starch gel.

Samples were analyzed for six enzymes using procedures described previously by Clayton and Tretiak (1972). Morpholine-citrate system (pH 6.1) was used for malate dehydrogenase (MDH; EC 1.1.1.37), isocitrate dehydrogenase (IDH; EC 1.1.1.41), and 6-phosphogluconic dehydrogenase (6-PGDH; EC 1.1.1.44), while the Tris-citrate lithiumborate buffer system (pH 8.3) of Scandalios (1969) was used for phosphoglucosyltransferase (PGI; EC 5.3.1.9), phosphoglucosyltransferase (PGM; EC 5.4.2.2), and leucine amino peptidase (LAP; EC 3.4.11.1). Enzyme commission numbers given are according to the International Union of Biochemists (Soltis and Soltis, 1989).

The morpholine-citrate system was run at 55 mA for the first hour, and then maintained between 55 and 65 mA for an additional 5 h. The Tris-citrate lithium borate buffer system was run at 75 mA until 275 V were reached. The system was then maintained at 275 V for a total of 6 h.

Results and discussion

Plant identification (ID) numbers and the isozyme banding patterns for four polymorphic loci are presented in Table 1. The four letters following the ID number create a four-letter fingerprint specific to that plant. Arbitrary letter designations were assigned to each putative locus found within each enzyme system.

6-PGDH, PGM, PGI, MDH, IDH, and LAP resolved well (Figs. 1-4). 6-PGDH, MDH, PGI and PGM were produced by multiple loci. On the gel, loci were numbered in order, from the fastest migrating band to the slowest. Putative alleles were assigned

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Table 1. Isozyme banding patterns of *Taxus* species and cultivars examined in this study. A different letter was assigned to each pattern within each enzyme system*. ID numbers were assigned to the 55 individual plants.

Species and cultivar	ID no.	6-PGDH	PGM	MDH ₁	MDH ₂
<i>T. media</i> 'Hicksii'	1-9	D	A	A	A
<i>T. media</i> 'Hicksii'	10	D	A	A	B
<i>T. media</i> 'Hicksii'	11, 12	D	A	---	A
<i>T. media</i> 'Hicksii'	13	D	A	A	B
<i>T. media</i> 'L. C. Bobbink'	14, 15	C	H	A	A
<i>T. media</i> 'L. C. Bobbink'	16	C	G	A	A
<i>T. media</i> 'Dark Green Spreader'	17	D	B	A	A
<i>T. cuspidata</i> 'Capitata'	18, 19	C	B	A	A
<i>T. cuspidata</i> 'Capitata'	20	C	B	A	B
<i>T. cuspidata</i>	21	D	A	A	A
<i>T. cuspidata</i>	22	C	B	A	A
<i>T. cuspidata</i> 'Adams'	23, 24	D	A	A	A
<i>T. media</i> 'Runyan'	25	D	D	A	B
<i>T. media</i> 'Runyan'	26, 27	D	A	A	B
<i>T. media</i> 'Fairview'	28-30	D	D	A	B
<i>T. media</i> 'Brownii'	31	D	G	A	B
<i>T. media</i> 'Brownii'	32	D	D	A	B
<i>T. media</i> 'Brownii'	33, 34	D	G	A	B
<i>T. media</i> 'Densiformis'	35	D	D	A	B
<i>T. media</i> 'Densiformis'	36	D	D	A	A
<i>T. media</i> 'Densiformis'	37	D	D	A	B
<i>T. media</i> 'Wardii'	38-40	D	A	A	B
<i>T. media</i> 'Wardii'	41	C	D	A	B
<i>T. brevifolia</i>	42	B	C	A	B
<i>T. baccata</i> 'Fastigiata Aurea'	43	B	---	A	A
<i>T. baccata</i> 'Nigra'	44	B	I	A	A
<i>T. baccata</i> 'Overeynderi'	45	D	E	A	B
<i>T. baccata</i> 'Repandens'	46	B	I	A	B
<i>T. canadensis</i>	47, 48	C	A	B	A
<i>T. cuspidata</i>	49	C	A	A	A
<i>T. cuspidata</i>	50	A	B	A	A
<i>T. cuspidata</i> 'Nana'	51	C	B	A	A
<i>T. hunnewelliana</i>	52	C	A	B	B
<i>T. hunnewelliana</i>	53	C	A	A	B
<i>T. media</i> 'Citation'	54	C	---	A	A
<i>T. media</i> 'Coleana'	55	D	---	A	A

*Subscripts denote different putative loci. Numbering begins at the loci migrated farthest from the origin.

to loci based on patterns of variability and results from previous plant enzyme investigations (Gottlieb, 1981; Kephart, 1990). MDH, 6-PGDH, PGI, and IDH were interpreted as dimeric enzymes, whereas PGM and LAP were interpreted as monomeric enzymes. Genetic variation was highest in 6-PGDH, PGM, MDH₁, and MDH₂. Data is not presented for the monomorphic loci PGI₁, MDH₂, IDH, or LAP for the 55 plants tested. 6-PGDH₂ and PGI₂ could not be read clearly; therefore, they are not included in the analysis.

In several cases, plants listed by the same name showed different fingerprints. The fingerprints for plants 10 and 13 have pattern B for MDH₂ loci (Table 1). This pattern is clearly

different from the remaining plants named *Taxus media* 'Hicksii'; however, only one fingerprint can be the true *T. media* 'Hicksii'. Similar variations were found within all of the other cultivars, except for *T. cuspidata* 'Adams' and *T. media* 'Fairview'. Genetic variation, as expected within a species, was found among unnamed selections of *T. cuspidata*.

Four enzyme patterns were found for 6-PGDH (Fig. 1). Only one plant, number 50, a *T. cuspidata*, exhibited pattern A (Table 1). Four plants, numbers 42, a *T. brevifolia*; 43, a *T. baccata* 'Fastigiata Aurea'; 44, a *T. baccata* 'Nigra'; and 46, a *T. baccata* 'Repandens' exhibited pattern B. Fifty plants exhibited either C or D patterns for 6-PGDH. The only intracultivar variations were found in *T. media* 'Wardii'—numbers 38, 39, and 40 exhibited pattern D; however, number 41 exhibited pattern C.

Twenty-six plants exhibited pattern A (Table 1) for the PGM enzyme system (Fig. 2). Seven plants exhibited pattern B. Pattern C was evident only for plant 42, a *T. brevifolia*. Nine plants showed pattern D. One plant, 45, a *T. baccata* 'Overeynderi', exhibited pattern E. Plants 44, *T. baccata* 'Nigra', and 46, *T. baccata* 'Repandens', exhibited pattern I. Within each cultivar, many differences were found. Plants labeled *T. media* 'L.C. Bobbink' (14, 15, and 16) had either G or H patterns. Out of three *T. media* 'Runyan' samples, one, plant 25, exhibited a D pattern, while the rest exhibited an A pattern. Within the four *T. media* 'Brownii' samples, plant 32 exhibited

Fig. 1. 6-PGDH isozyme patterns of various *Taxus* species and cultivars. Interpretive drawing on the left and a picture of the gel on the right. The letters correspond to those listed in Table 1. The relative mobility (RM+) is shown on the left axis.

Fig. 2. PGM isozyme patterns of various *Taxus* species and cultivars. Interpretive drawing on the left and a picture of the gel on the right. The letters correspond to those listed in Table 1. The relative mobility (RM+) is shown on the left axis.

Fig. 3. MDH₁, MDH₂, and MDH₃ isozyme patterns of various *Taxus* species and cultivars. Interpretive drawing on the left and a picture of the gel on the right. The letters correspond to those listed in Table 1. The relative mobility (RM+) is shown on the left axis.

Fig. 4. Interpretive drawings of IDH, LAP, and PGI₁ isozyme patterns of *Taxus* species and cultivars. The letters correspond to those listed in Table 1. The relative mobility (RM+) is shown on the left axis.

a D pattern, while numbers 31, 33, and 34 exhibited pattern G. Pattern D was found in *T. media* 'Wardii', number 41, while pattern A was found in *T. media* 'Wardii' numbers 38, 39, and 40.

MDH₁ showed two distinct banding patterns, A and B (Fig. 3). Both *T. canadensis*, 47 and 48, exhibited pattern B (Table 1). Of two *T. hunnewelliana* sampled, one, number 52, showed pattern B, while the other showed pattern A. The remaining samples all showed pattern A at the MDH₁ loci. MDH₂ showed two patterns, A and B. Of 13 *T. media* 'Hicksii' sampled, plants 10 and 13 had B patterns; all other *T. media* 'Hicksii' exhibited pattern A. Differences among the *T. media* 'Densifomis' were also found at the MDH₂ loci. Plants 35 and 37 had pattern B, while plant 36 exhibited pattern A.

Seven plants with different names exhibited the same four-letter fingerprint. These plants include two *T. media* 'Hicksii' (10 and 13), two *T. media* 'Runyan' (26 and 27), and three *T. media* 'Wardii' (38, 39, and 40). These differently named types might carry the same fingerprint due to the chance selection of non-divergent loci or many other factors, including labeling errors in the 1700s or mix-ups in the field. The origins of *T. media*, *T. media* 'Runyan', *T. media* 'Wardii', and *T. media* 'Brownii' can be traced to the Hunnewell Estate in Massachusetts, where head gardener T.D. Hatfield raised the first hybrids (Chadwick and Keen, 1976). Although the original *T. media* 'Hicksii' originated at the Hicks Nursery in Long Island (Chadwick and Keen, 1976), the similarities in these seven plants may be due to similar origins (the Hunnewell Estate), whereas the other plants with those names could have come from another source. Staining for other enzymes could lead to further separation of these cultivars. In addition, *T. cuspidata* (21) and *T. cuspidata* 'Adams' (23 and 24) are similar. *T. media* 'Fairview' (28, 29, and 30), *T. media* 'Brownii' (32), and *T. media* 'Densifomis' (35 and 37) share the same four-letter fingerprint.

Although the samples came from reliable and reputable sources, the diversity of enzyme patterns within cultivars indicates there may be nomenclature errors in the genus *Taxus*.

This study shows that starch gel

electrophoresis can be a useful tool in the characterization of *Taxus* species and cultivars. The intracultivar variation found provides information for further genetic and taxonomic investigation. The proper labeling of cultivars, however, will depend ultimately on the characterization of the original registered plants. Unfortunately, many of the records are no longer available and the existence of the original selections is questionable. Efforts to address these discrepancies would require an expanded collection of species and cultivars from both the nursery industry and known collections at arboreta and botanical gardens.

Compared to other molecular biological techniques of cultivar identification, such as restriction fragment length polymorphisms (RFLPs), two-dimensional page electrophoresis, and randomly amplified polymorphic DNA (RAPD), isozyme electrophoresis has some practical advantages: It is relatively inexpensive and can be used to assay many tissues quite rapidly. Isozyme electrophoresis could be expanded to systematically characterize important or commonly mislabeled species. The characterization of these crops would benefit germplasm repositories, researchers, propagators, and retail consumers. In the future, mislabeling can be avoided if new cultivars include isozymic patterns or some other form of genetic characterization in their registration or patents. Periodic testing and registration documents can be used to confirm the authenticity of the propagation stock.

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