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Using Amplified Fragment Length Polymorphism Markers to Confirm Identity and Correct Labeling of Japanese Barberry (*Berberis thunbergii*) Cultivars in the Market

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***Abstract*. Japanese barberry (*Berberis thunbergii* DC.) is a popular ornamental shrub used in garden and urban landscaping. Currently there are over 60 *B. thunbergii* cultivars in the market. To better distinguish its cultivars, we used the amplified fragment length polymorphism (AFLP) technique to develop DNA marker profiles for 59 cultivars and hybrids. These markers were used to authenticate the trueness-to-name of *B. thunbergii* cultivars in production and in the market, control for intracultivar genetic variants, and develop a molecular key to identify cultivars approved for importation in Canada. Polymorphic markers from seven primer combinations were able to clearly differentiate 57 of 59 cultivars evaluated. Two cultivars, Aurea and Aurea Nana, could not be differentiated because they had identical marker profiles. Among the 274 plants tested, 263 were confirmed to be true-to-name and correctly labeled, whereas 11 plants could not be confirmed true-to-name. Seven of the 20 cultivars evaluated exhibited detectable intracultivar genetic variation. 'Crimson Pygmy' had the highest number of plants exhibiting genetic variability. Overall, nursery producers and retailers do not appear to be mixing or mislabeling cultivars. A molecular key developed from a subset of 25 markers was able to accurately identify and differentiate the 11 *B. thunbergii* cultivars approved for importation in Canada. This key could be used in a cultivar verification program to facilitate international trade of *B. thunbergii* cultivars where wheat rust is a concern.**

Japanese barberry (*Berberis thunbergii* DC.) is a deciduous spiny woody shrub of the barberry family (Berberidaceae). This species is native to Japan and was introduced to the United States in the late nineteenth century (Dirr, 1998). It is currently naturalized in ≈30 states across the eastern and central United States (Silander and Klepeis, 1999). Many cultivars of *B. thunbergii* have been developed for use as ornamental plants and currently there are over 60 cultivars in the market and more continue to be introduced (Dirr, 2009). *Berberis thunbergii* cultivars are hardy, easy to grow, and resistant to deer browsing (Dirr, 1998, 2009; Lubell et al., 2008). The cultivars are characterized by variations in growth habits ranging from dwarf to tall, and their foliage can be red, purple, green, yellow, or variegated (Dirr, 2009). All these characteristics make *B. thunbergii* cultivars attractive as garden and urban landscaping plants.

Plants of the genus *Berberis* are known to be alternate hosts of the fungus *Puccinnia graminis* Pers., which causes black stem rust (BSR) disease in wheat (CFIA, 2012; USDA, 2002). Therefore, the movement of *B. thunbergii* plants across some state and international borders is restricted. For instance, only 11 *B. thunbergii* cultivars classified as highly resistant to BSR are allowed entry into Canada (CFIA, 2012). Identification and verification of approved cultivars is mainly by visual inspection of their morphological characteristics. However, it is often difficult to distinguish cultivars, especially those with similar morphological traits. Furthermore, plants are sold when young and the morphological traits used for their identification are not very distinct at this stage. Additionally, morphological characteristics can be influenced by variation in environmental and growing conditions making it difficult to accurately identify and differentiate *B. thunbergii* cultivars. Modern technology using molecular markers offers a reliable and efficient way to identify and differentiate plant cultivars. Molecular markers are not influenced by variable environmental conditions where plants are grown and can be applied at almost any stage of plant development, thus presenting a great potential for use in a barberry authentication program. Also, because molecular marker profiles can be unique to each cultivar, they can be used to provide protection of plant breeders' intellectual property rights when applying for plant patents for new cultivars.

AFLP is one of the techniques that could be used to develop molecular markers to identify and differentiate *B. thunbergii* cultivars. AFLP is a DNA fingerprinting assay that combines restriction enzyme digestion and polymerase chain reaction (PCR) amplification of DNA fragments (Vos et al., 1995). The technique is highly reproducible, does not require prior knowledge of the target genome, and uses genome-wide distribution of restriction enzyme sites to yield abundant informative molecular markers (Mueller and Wolfenbarger, 1999; Vos et al., 1995). AFLP markers have been successfully used to evaluate genetic relationships in several ornamental plants, including *Dieffenbachia* (Chen et al., 2004), *Prunus* (Hu et al., 2005), *Cornus* (Smith et al., 2007), and *Ficus* (Fang et al., 2007). Also, previous studies have used AFLP markers to identify and differentiate *B. thunbergii* cultivars and interspecific hybrids (Cote and Leduc, 2007; Lubell et al., 2008).

In this study, our objectives were to use AFLP markers to: 1) authenticate the trueness-to-name of *B. thunbergii* plants from different nurseries and control for mislabeled plants in the market; 2) evaluate the occurrence of genetic variants within cultivars; and 3) develop a molecular key that could be used by regulatory personnel to ascertain cultivar identity to facilitate international trade of *B. thunbergii* cultivars where wheat rust is a concern. The presence of genetic variants or subclones in some *B. thunbergii* cultivars in the market has been long suspected by the nursery industry, but their occurrence has not been evaluated genetically.

Materials and Methods

Plant materials. B. thunbergii cultivars maintained in a replicated research collection at the University of Connecticut Plant Science Research Farm, Storrs, CT, were used to generate reference AFLP marker profiles. Plant samples of different barberry cultivars were collected from seven nurseries in Michigan and several retail outlets in Michigan to authenticate their trueness-to-name. The plants collected from Michigan retail outlets originated from 35 different nurseries across

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the United States. In total, 274 samples representing 20 different cultivars based on container labels were collected. Once the plants arrived at collection centers at the University of Connecticut and Michigan Department of Agriculture research locations, they were placed in outdoor container growing facilities to allow regeneration of new growth. Young leaf tissues were collected from plants and stored in a –80 °C freezer until DNA extraction.

DNA extraction. DNA was extracted following the protocol outlined in Lubell et al. (2008). Quality of extracted DNA was assessed by gel electrophoresis and a spectrophotometer (NanoDrop ND-1000; Thermo Scientific, Wilmington, DE). Only non-degraded and high-quality DNA samples (absorbance ratio 260/280 ≥ 1.8; 260/230 ≥ 1.8) were used in AFLP analyses. Extracted DNA samples were stored at –80 °C until needed for the AFLP procedure.

AFLP procedure. The AFLP steps including restriction digestion, adaptor ligation, and pre-selective and selective amplification reactions were carried out as outlined in the AFLP plant mapping protocol (Anonymous, 2007). Selective PCR was done using seven primer combinations (*Eco* RI-AGG + *Mse* I-CAT, *Eco* RI-ACC + *Mse* I-CAC, *Eco* RI-AGG + *Mse* I-CAA, *Eco* RI-AGG + *Mse* I-CTG, *Eco* RI-ACC + *Mse* I-CTC, *Eco* RI-ACA+ *Mse* I-CTG, and *Eco* RI-ACG + *Mse* I-CAC)

Table 1. Species, cultivar name, and general morphological characteristics of 59 *Berberis* cultivars whose amplified fragment length polymorphism profiles were developed.

Species	Cultivar name[z]	Foliage characteristics and plant form
B. koreana × *B. thunbergii*	'Bailsel' Golden Carousel®	Golden yellow, rounded habit with somewhat arching branches
B. koreana × *B. thunbergii*	**'Tara' Emerald Carousel®**	Green, rounded habit with arching branches
B. ×*mentorensis* L. Ames		Green with spiny margins, round open branched habit
B. ×*ottawensis* Schneid.	**'Concorde'**	Deep red–purple, compact habit
B. ×*ottawensis*	'Crimson Velvet'	Smoky maroon, tall habit
B. ×*ottawensis*	'Criruzam' Crimson Ruby™	Deep burgundy, small upright branching habit
B. ×*ottawensis*	**'Royal Cloak'**	Deep red–purple, tall upright with spreading top habit
B. ×*ottawensis*	'Silver Mile'	Red–purple some with whitish variegation, tall spreading habit
B. ×*ottawensis*	'Superba'	Burgundy with serrate margins, tall open habit
B. thunbergii DC.	'Admiration'	Red with yellow–cream ring, compact habit
B. thunbergii	'Anderson' Lustre Green®	Green, rounded compact habit
B. thunbergii	'Angel Wings'	Red–purple with golden ring, spreading growth habit
B. thunbergii	'Antares'	Red, spreading growth habit
B. thunbergii	'Aurea'	Yellow, medium dense habit
B. thunbergii	**'Aurea Nana'**	Yellow, medium dense habit
B. thunbergii	'Bagatelle'	Red–purple glossy, compact habit
B. thunbergii	**'Bailgreen' Jade Carousel®**	Green, upright habit
B. thunbergii	**'Bailone' Ruby Carousel™**	Burgundy–purple, tall spreading habit
B. thunbergii	'Bailtwo' Burgundy Carousel®	Burgundy–purple, spreading growth habit
B. thunbergii	'Bogozam' Bonanza Gold®	Yellow, dwarf habit
B. thunbergii	'Crimson Dwarf'	Red, less dense mounded habit
B. thunbergii	'Crimson Pygmy'	Red, dense mounded habit
B. thunbergii	'Erecta'	Green, medium rounded habit
B. thunbergii	'Fireball'	Red
B. thunbergii	**'Gentry' Royal Burgundy™**	Burgundy, low-mounding habit
B. thunbergii	'Golden Devine'	Bright yellow, dwarf mound-like habit
B. thunbergii	'Golden Ring'	Red–purple with golden ring margins, spreading habit
B. thunbergii	'Golden Rocket'	Yellow, upright columnar habit
B. thunbergii	'Goruzam' Golden Ruby™	Red with golden ring margins, compact mounded habit
B. thunbergii	'Grhozam' Green Hornet™	Green, dwarf compact habit
B. thunbergii	'Green Pygmy'	Green, dwarf mounded habit
B. thunbergii	'Helmond Pillar'	Red–purple, upright columnar habit
B. thunbergii	'Inermis'	Green, rounded dense habit
B. thunbergii	'J.N. Redleaf' Ruby Jewel™	Glossy red, dense rounded habit
B. thunbergii	'J.N. Variegated' Stardust™	Green with white variegation, mounded compact habit
B. thunbergii	'Kelleris'	Green with white variegation on new shoots, compact habit
B. thunbergii	'Kobold'	Deep green, mounded compact habit
B. thunbergii	'Lime Glow'	Green with white variegation, rounded open branching habit
B. thunbergii	'Maria' Sunjoy™ Gold Pillar	Yellow, upright columnar habit
B. thunbergii	'Marshall Upright'	Burgundy, upright habit
B. thunbergii	'Miruzam' Midnight Ruby™	Burgundy–purple, compact habit
B. thunbergii	**'Monlers' Gold Nugget™**	Golden yellow, compact mounded habit
B. thunbergii	**'Monomb' Cherry Bomb™**	Red–purple, open mounded habit
B. thunbergii	**'Monry' Sunsation™**	Yellow, upright dense vase-shaped habit
B. thunbergii	'Orange Rocket'	Rusty orange, some with gold margins, upright habit
B. thunbergii	'Painters Palette'	Green with white variegation, dense rounded habit
B. thunbergii	'PowWow'	Green with white variegation on new shoots, upright habit
B. thunbergii	'Pyruzam' Pygmy Ruby™	Burgundy–purple, compact habit
B. thunbergii	'Red Bird'	Burgundy, medium open branching habit
B. thunbergii	'Red Chief'	Red–purple, upright stems with open branching habit
B. thunbergii	'Red Rocket'	Red–purple, tall dense habit
B. thunbergii	**'Rose Glow'**	Red–purple with red–purple splotches, tall open habit
B. thunbergii	'Rosy Rocket'	Red–purple, upright
B. thunbergii	'Sparkle'	Glossy dark green, rounded with arching horizontal branches
B. thunbergii	'Sparkler'	Green, new shoots have green and red variegation, rounded habit
B. thunbergii	'Stans Variegated'	Green with white variegation, upright dense habit
B. thunbergii	'Talago' Sunjoy™ Gold Beret	Yellow, dwarf habit
B. thunbergii	'Tiny Gold'	Yellow, dwarf habit
B. thunbergii	'24 Karat Gold'	Yellow, small habit

[z]Cultivars in bold are approved for importation in Canada.

(Cote and Leduc, 2007; Lubell et al., 2008). Restriction enzymes were from New England Biolabs (Ipswich, MA), and all fluorescently labeled primers and PCR reagents were from Applied Biosystems (Foster City, CA). The DNA fragments from selective PCR were visualized by capillary electrophoresis on an ABI3730xl analyzer (Applied Biosystems) using GeneScan™ 500 LIZ® size standard. The AFLP procedure was repeated once, including DNA extractions and AFLP reactions, for the standard plants to ensure reproducibility of reference AFLP marker profiles. The AFLP procedure on all other plant samples was done only once with positive and negative controls included in each PCR run. The controls were used to control for reproducibility of amplicons and presence of contaminants in the

Table 2. Number of polymorphic amplified fragment length polymorphism markers generated by each primer pair on 59 *Berberis* cultivars evaluated.

Primer pair[z]	Polymorphic markers	Cultivars differentiated	Cultivars not differentiated	Names of cultivars not differentiated	PDP[y]
P1	24	54	5	'Aurea', 'Aurea Nana' and 'Painters Palette'; 'Erecta' and 'Lime Glow'	0.92
P2	31	51	8	'Aurea' and 'Aurea Nana'; 'Kobold' and 'Bagatelle'; 'Sparkle' and 'Crimson Dwarf', 'Kelleris' and 'Lime Glow'	0.86
P3	36	57	2	'Aurea' and 'Aurea Nana'	0.97
P4	34	49	10	'Aurea' and 'Aurea Nana'; 'Crimson Dwarf' and 'Crimson Pygmy'; 'Gold Nugget' and 'Pow Wow'; 'Bogozam' Bonanza Gold® and 'Grhozam' Green Hornet™; 'Kelleris' and 'Lime Glow'	0.83
P5	53	57	2	'Aurea' and 'Aurea Nana'	0.97
P6	36	51	8	'Aurea' and 'Aurea Nana'; 'Golden Rocket' and 'Rosy Rocket'; 'Pyruzam' Pygmy Ruby™ and 'Tiny Gold'; 'Kelleris' and 'Lime Glow'	0.86
P7	31	44	15	'Aurea' and 'Aurea Nana'; 'Bagatelle', 'Crimson Dwarf', 'Gentry' Royal Burgundy™ and 'Sparkle'; 'Pyruzam' Pygmy Ruby™ and '24 Karat Gold'; 'Red Bird', 'Bogozam' Bonanza Gold® and 'Grhozam' Green Hornet™; 'Fireball' and 'Tiny Gold'; 'Helmond Pillar' and 'Golden Rocket'	0.75
All primers	245	57	2	'Aurea' and 'Aurea Nana'	0.97

[z]P1 = *Eco* RI-AGG + *Mse* I-CAT; P2 = *Eco* RI-ACC + *Mse* I-CAC; P3 = *Eco* RI-AGG + *Mse* I-CAA; P4 = *Eco* RI-AGG + *Mse* I-CTG; P5 = *Eco* RI-ACC + *Mse* I-CTC; P6 = *Eco* RI-ACA + *Mse* I-CTG; P7 = *Eco* RI-ACG + *Mse* I-CAC.
[y]Primer discriminative power (PDP) = number of cultivars differentiated divided by total number of cultivars evaluated.

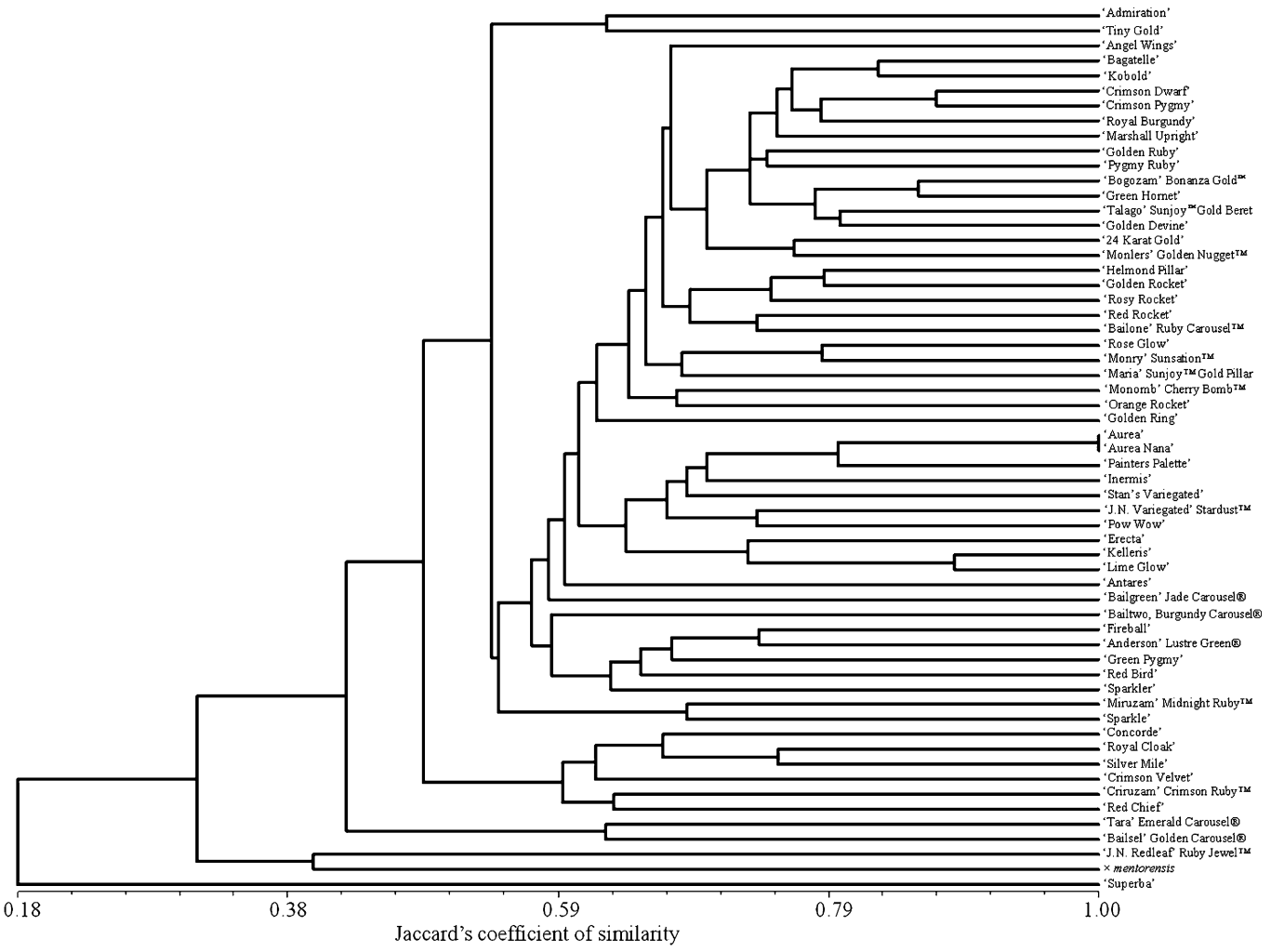

Fig. 1. Unweighted pair group method with arithmetic averaging phenogram of 59 *Berberis* cultivars evaluated using amplified fragment length polymorphism markers.

reagents. When results from any DNA sample were questionable, the AFLP procedure was repeated for that plant sample.

AFLP data analysis. Fragments between 100 and 600 bp long generated by each primer pair were scored using GeneMarker® software Version 1.95 (SoftGenetics, LLC, State College, PA). Peaks were first scored using automatic settings followed by visual inspection and manual adjustment of each peak to ensure accurate scoring. To differentiate between cultivars, only polymorphic markers within the scored range were used. The polymorphic markers were binary scored (1 for peak present and 0 for peak absent) in each standard cultivar to create a primer specific binary data set of AFLP marker profiles. The binary data from all seven primer pairs were combined in a spreadsheet (Microsoft Office Excel; Microsoft, Redmond, WA) and Jaccard's similarity coefficients were calculated for all pairwise cultivar comparisons using the SIMQUAL module in NTSYSpc software Version 2.21L (Exeter Software, Setauket, NY). To determine if the scored data set could differentiate cultivars, a phenogram based on similarity coefficients was generated using unweighted pair group method with arithmetic averaging (UPGMA) in the SAHN module of NTSYSpc software.

To verify a sample as true-to-name, the AFLP profiles of a test sample were scored alongside those of its reference standard for each primer pair to create a binary data set. The binary data sets from all primer pairs were combined and the similarity coefficient of the test plant and standard was calculated. The coefficient was converted to a percentage to reflect the percent similarity between the test sample's AFLP profiles and those of its reference standard. Intracultivar genetic variation was assessed through pairwise comparisons of similarity coefficients among plants of the same cultivar. A molecular identification key was developed using a subset of highly informative polymorphic markers that could identify and differentiate the cultivars approved for importation in Canada. AFLP markers that produced fragment sizes unique to individual cultivars within the approved group were identified and included in the key as identity confirmations.

Results and Discussion

Differentiating B. thunbergii *cultivars using AFLP markers.* A total of 245 polymorphic markers was generated using seven primer pairs on 59 cultivars (Table 1). The number of polymorphic markers ranged from 24 (primer pair P1) to 53 (primer pair P5) with an average of 35 polymorphic markers per primer pair (Table 2). The primer discriminative power, calculated as number of cultivars differentiated divided by total number of cultivars evaluated, ranged from 0.75 to 0.97 (Table 2). Primer pairs P3 and P5 differentiated the most number of cultivars and P7 differentiated the least (Table 2). The similarity coefficient among cultivars, excluding 'Aurea' and 'Aurea Nana', ranged from 0.12 to 0.89 (mean = 0.54). 'Aurea' and 'Aurea Nana' plants could not be differentiated from each other because they produced identical AFLP marker profiles with all primer pairs used in this study (Fig. 1), implying that the plants of these two cultivars that we analyzed were of the same genotype. Cote and Leduc (2007) were not able to differentiate 'Aurea Nana' from another cultivar referred to as 'Golden'. We are not aware of any cultivar named 'Golden', but because some barberry plants are sold under multiple names (Lubell et al., 2008), it is possible that the Golden cultivar in Cote and Leduc's study could have been 'Aurea' or mislabeled 'Aurea Nana'.

Mature (greater than five years old) replicates of 'Aurea' and 'Aurea Nana' plants growing in our research farm exhibit indistinguishable morphological characteristics and habits. Only the cultivar Aurea is described in the literature (Dirr, 1998, 2009), and 'Aurea Nana' is only mentioned as a possible rename of the cultivar Bogozam Bonanza Gold™ (Dirr, 1998). In our study, plants labeled 'Aurea Nana' and those labeled 'Bogozam' Bonanza Gold™ were clearly distinguishable with our AFLP markers (similarity coefficient between the two cultivars was 0.68). However, the same 'Aurea Nana' plants were

Table 3. *Berberis* cultivars submitted for true-to-name verification using amplified fragment length polymorphism markers developed for barberry.

Cultivar name[z]	Samples submitted	Samples confirmed true-to-name: Matched standard 98% or greater	Samples confirmed true-to-name: Matched standard 90% or greater, less than 98%[y]	Samples not true-to-name[x]
'Aurea'	15	10	4	1
'Bagatelle'	6	4	1	1
'Bailone' Ruby Carousel™	3	3		
'Bailsel' Golden Carousel	2	2		
'Bogozam' Bonanza Gold™	23	22		1
'Concorde'	12	9	2	1
'Crimson Pygmy'	62	28	30	4
'Gentry' Royal Burgundy™	23	21		2
'Golden Ring'	1	1		
'Goruzam' Golden Ruby™	7	7		
'Helmond Pillar'	27	23	4	
'Lime Glow'	9	7	1	1
'Maria' Sunjoy™ Gold Pillar	15	15		
'Marshall Upright'	3	3		
'Monlers' Gold Nugget™	4	4		
'Pow Wow'	1	1		
'Rose Glow'	52	52		
'Royal Cloak'	2	2		
'Talago' Sunjoy™ Gold Beret	1	1		
'Tara' Emerald Carousel®	6	5	1	
Total	274	220	43	11

[z]Cultivars in bold are approved for importation in Canada.
[y]These plants showed slight genetic variation from the reference standard and same named plants.
[x]DNA fingerprints of these plants matched those of reference standard less than 90%.

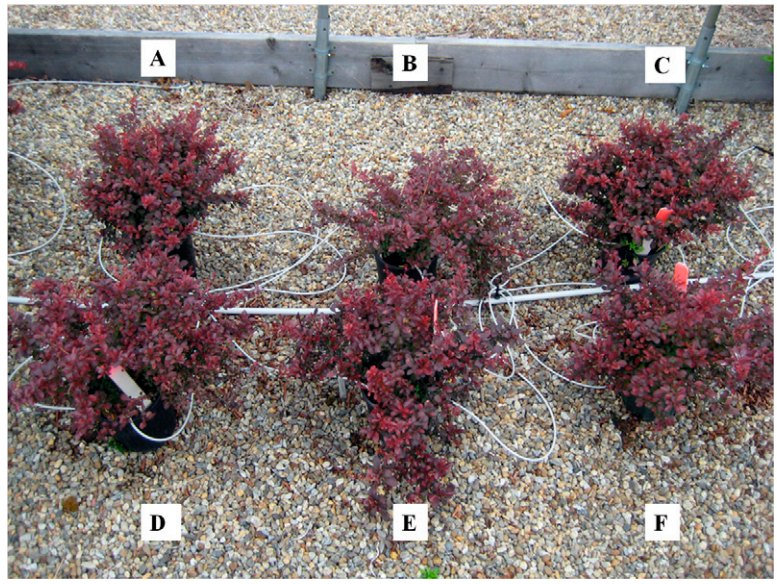

Fig. 2. 'Crimson Pygmy' plants showing a lack of morphological differences between those plants confirmed to be true-to-type (**B** and **F**) and those confirmed not to be true-to-type (**A, C, D,** and **E**) with amplified fragment length polymorphism markers.

indistinguishable from plants labeled 'Aurea', which raises more suspicion about the 'Aurea Nana' genotype. Both 'Aurea Nana' and 'Bogozam' Bonanza Gold™ are described as dwarf forms of golden barberry by the nurseries that introduced them, Spring Meadow Nursery and Lake County Nursery, respectively. However, only 'Bogozam' Bonanza Gold™ is patented (PP 8,215). It would be interesting to determine how plants of these two cultivars obtained directly from their original nurseries compare genetically, but these plants were not included in our sampling. In ornamental cultivars, sometimes a single genotype can be incorrectly sold under different names. For example, the *Hydrangea paniculata* cultivars White Tiara and White Moth are sold as different cultivars, but they have identical DNA fingerprints (Reed and Rinehart, 2009). Likewise, the *Hydrangea macrophylla* cultivars Glory Blue and Charm have identical DNA profiles despite being labeled and sold as different cultivars (Rinehart and Reed, 2006), and neither plant is described to have originated as a sport of the other (Dirr, 1998).

Crimson Pygmy, Monomb Cherry Bomb™, and Crimson Dwarf cultivars, which could not be differentiated in previous studies (Cote and Leduc, 2007; Lubell et al., 2008), were successfully differentiated with our AFLP markers. This could be attributed to the higher number of polymorphic markers (245) used in our study compared with those used in the previous two studies (33 and 148, respectively). Using more AFLP markers generated from several primer combinations ensures significant representation of hypervariable loci and enables differentiation between closely related individuals (Mueller and Wolfenbarger, 1999; Vos et al., 1995).

Verifying the identity and correct labeling of B. thunbergii *cultivars.* The similarity coefficient between two different cultivars as determined from the polymorphic markers used was lowest between 'Superba' and 'Admiration' (0.12) and highest between 'Kelleris' and 'Lime Glow' (0.89). We therefore determined that if the similarity coefficient between a sample and its reference standard was less than 0.90 (90% match) it would be regarded as not true-to-name. Cote and Leduc (2007) determined a sample to be of a different cultivar from its reference standard if it matched 28 markers or less of the 33 polymorphic markers they used (85% match or less) for cultivar verification. Based on our authentication criteria, 263 of the 274 plants evaluated (96%) were confirmed to be true-to-name and correctly labeled, and 11 plants (4%) were determined to be not true-to-name (Table 3). These plants included: four plants labeled 'Crimson Pygmy' (similarity coefficient with standard cultivar ranged from 0.78 to 0.86); two plants labeled 'Gentry' Royal Burgundy™ (similarity coefficients with standard cultivar were 0.85 and 0.88); and one plant each for 'Aurea' (0.89), 'Bogozam' Bonanza Gold® (0.86), 'Lime Glow'(0.82), 'Bagatelle'

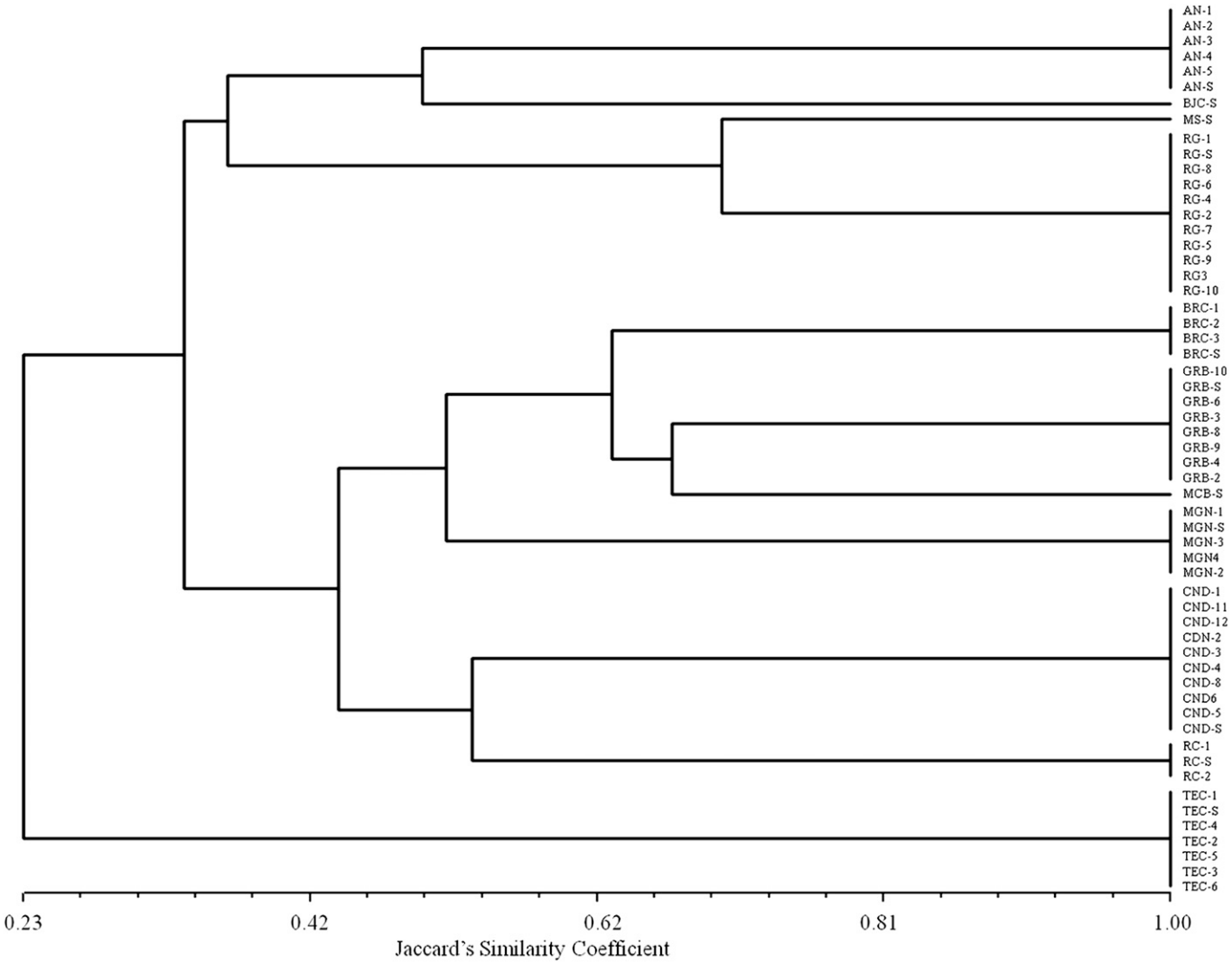

Fig. 3. Unweighted pair group method with arithmetic averaging phenogram of 11 *Berberis* cultivars approved for importation in Canada. The phenogram is based on a subset of 25 select markers out of the 245 polymorphic markers used in analyzing 59 *Berberis* cultivars. AN = 'Aurea Nana'; BJC = 'Bailgreen' Jade Carousel®; BRC = 'Bailone' Ruby Carousel™; CND = 'Concorde'; GRB = 'Gentry' Royal Burgundy™; MGN = 'Monlers' Gold Nugget™; MCB = 'Monomb' Cherry Bomb™; MS = 'Monry' Sunsation™; RC = 'Royal Cloak'; RG = 'Rose Glow'; TEC = 'Tara' Emerald Carousel®. Cultivar code followed by –S designates a cultivar standard, and code followed by a number indicates a test sample.

(0.88), and 'Concorde' (0.80). Although these plants did not meet the criteria, we used for true-to-name confirmations, they did not appear to be mislabeled or misidentified cultivars, but rather subclones, which could be resulting from spontaneous vegetative sports or genetic mutations that can occur in clonally propagated cultivars (De Riek et al., 2001; Kimball et al., 2012). Overall, these results indicate that nursery producers and retailers do not appear to be mixing or mislabeling barberry cultivars.

Intracultivar genetic variation of B. thunbergii *cultivars.* Following duplicate AFLP analyses, including DNA isolation and AFLP reactions, we estimated that up to 2% of the observed variation within a cultivar could be attributed to artifacts of the AFLP procedure (such as peak scoring errors as a result of low signal intensity). We therefore determined that similarity coefficients above 0.98 (98% match or greater) among plants of the same cultivar implied lack of intracultivar genetic variation and similarity coefficients of between 0.90 and 0.98 (90% or greater, less than 98%) suggested presence of slight genetic variation within a cultivar. Based on these criteria, 220 of the 274 plants evaluated had similarity coefficients of 0.98 or greater with their respective cultivar standard and same-named plants (Table 3), which implied genetic homogeneity. Similarity coefficients of 43 plants were between 0.90 and 0.98 (Table 3), which suggested occurrence of intracultivar genetic variation. In comparison with other cultivars evaluated, 'Crimson Pygmy' had the highest number of plants (48%) exhibiting genetic variability. This cultivar is one of the oldest (introduced in 1942) and the most popular of all *B. thunbergii* cultivars (Dirr, 2009); therefore, the relatively higher number of its plants exhibiting intracultivar genetic variability could be attributed to natural mutations that have occurred as a result of its repeated vegetative propagation over a long period of time. The genetically heterogenous plants, however, did not exhibit morphological differences from those whose AFLP profiles matched (98% or greater) the reference profiles (Fig. 2). This could be attributed to either genetic variability occurring in the noncoding regions of the genome and therefore not manifested in the phenotype or variation could be occurring in the coding regions but does not affect the genes involved with morphological development.

Molecular identification of approved cultivars. Currently, only 11 barberry cultivars are approved for import in Canada. To facilitate the identification process of these cultivars, we selected 25 highly informative markers from the 245 polymorphic markers used to differentiate 59 cultivars. The Jaccard's similarity coefficients among the 11 cultivars were calculated from the subset binary data and a phenogram was constructed from the similarity matrix using UPGMA (Fig. 3). The cultivars clustered on the phenogram according to their known breeding origin. 'Tara' Emerald Carousel®, which is a hybrid between *B. koreana* and *B. thunbergii*, clustered separately from other cultivars. 'Concorde' and 'Royal Cloak', which are *B.* × *ottawensis* cultivars, formed a separate cluster from *B. thunbergii* cultivars. Further analysis using principal coordinate analysis grouped cultivars (Fig. 4) relatively similar to the clusters in the phenogram with the first three vectors explaining 65.82% of the variation. To test if the clustering was an artifact of the small number of polymorphic markers used, we randomly picked 12 polymorphic markers from the 245 and Jaccard's similarity coefficients were calculated among the 11 cultivars, and a new phenogram was constructed. Some unrelated cultivars were clustered together in the new phenogram (data not shown), confirming that the clustering attained with the 25 selected polymorphic markers was not an artifact of the small number of markers used and that the selected markers represented polymorphisms that were able to clearly differentiate the approved cultivars.

Using the 25 select polymorphic markers, we developed a molecular identification key for the approved cultivars (Fig. 5; Table 4). The key was able to accurately identify test plants of the same cultivar originating from multiple sources. The small number of markers and primers involved in developing this key ensures a quick, accurate, and cost-effective way to identify the approved cultivars and could be used in addition to morphological characteristics. The key can be expanded to accommodate identification of other cultivars that may be added to the approved list in the future. The large data set of polymorphic AFLP markers we have developed offers additional resources that could be used to identify most *B. thunbergii* cultivars currently in the market.

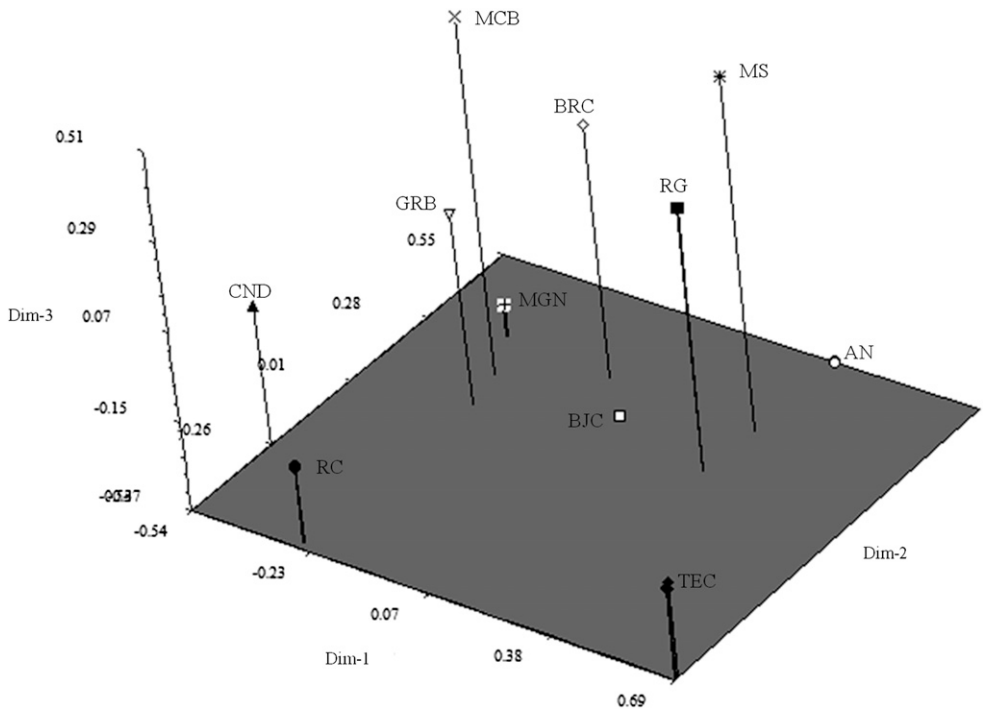

Fig. 4. Principal coordinate analysis plot showing the relationship of 11 Berberis cultivars approved for importation in Canada. The first three eigenvectors accounted for a total of 65.82% of variation among cultivars. AN = 'Aurea Nana'; BJC = 'Bailgreen' Jade Carousel®; BRC = 'Bailone' Ruby Carousel™; CND = 'Concorde'; GRB = 'Gentry' Royal Burgundy™; MGN = 'Monlers' Gold Nugget™; MCB = 'Monomb' Cherry Bomb™; MS = 'Monry' Sunsation™; RC = 'Royal Cloak'; RG = 'Rose Glow'; TEC = 'Tara' Emerald Carousel®.

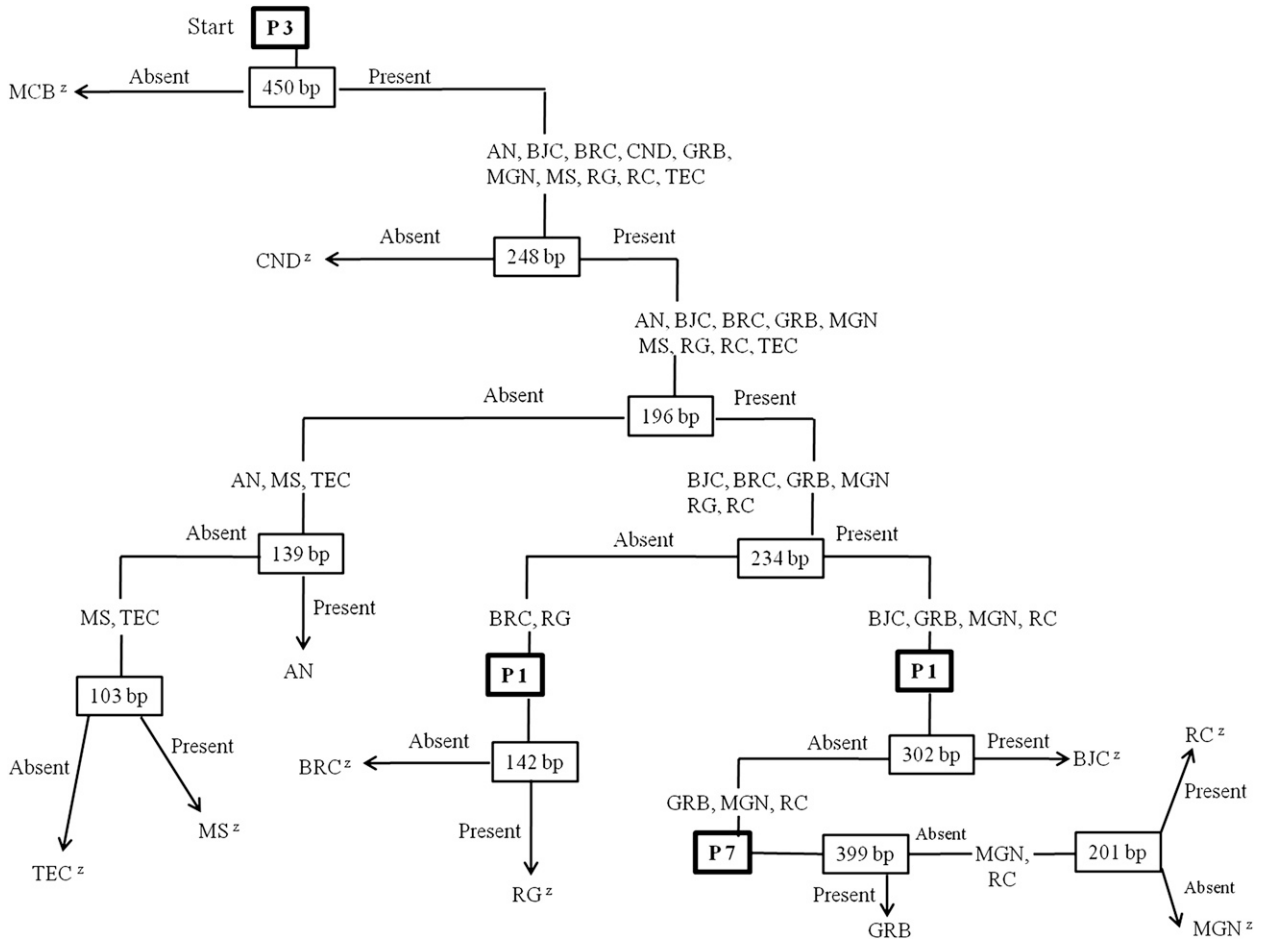

Fig. 5. Identification key for 11 *Berberis* cultivars approved for importation in Canada. Key is derived from 25 select polymorphic markers generated by three primer pairs; P1 = *Eco* RI-AGG + *Mse* I-CAT; P3 = *Eco* RI-AGG + *Mse* I-CAA; P7 = *Eco* RI-ACG + *Mse* I-CAC. AN = 'Aurea Nana'; BJC = 'Bailgreen' Jade Carousel®; BRC = 'Bailone' Ruby Carousel™; CND = 'Concorde'; GRB = 'Gentry' Royal Burgundy™; MGN = 'Monlers' Gold Nugget™; MCB = 'Monomb' Cherry Bomb™; MS = 'Monry' Sunsation™; RC = 'Royal Cloak'; RG = 'Rose Glow'; TEC = 'Tara' Emerald Carousel®. [z]Unique marker(s) used for confirming the cultivar identity are indicated in Table 4.

Table 4. Unique amplified fragment length polymorphism markers used in the identification of 11 *Berberis* cultivars approved for importation in Canada.

Cultivar name	Cultivar code	Primer pair[z]	Unique marker
'Aurea Nana'	AN		None
'Bailgreen' Jade Carousel®	BJC	P7	214 bp
'Bailone' Ruby Carousel ™	BRC	P1	287 bp
		P7	219 bp
'Concorde'	CND	P1	316 bp
'Gentry' Royal Burgundy™	GRB		None
'Monlers' Gold Nugget™	MGN	P3	272 bp
'Monomb' Cherry Bomb™	MCB	P7	128 bp
'Monry' Sunsation™	MS	P7	234 bp
'Royal Cloak'	RC	P3	266 bp
		P7	187, 239, 293 bp
'Rose Glow'	RG	P7	217 and 234 bp[y]
'Tara' Emerald Carousel®	TEC	P3	148 bp
		P7	164, 374 bp

[z]P1 = *Eco* RI-AGG + *Mse* I-CAT; P3 = *Eco* RI-AGG + *Mse* I-CAA; P7 = *Eco* RI-ACG + *Mse* I-CAC.
[y]The two markers have to be present to identify this cultivar.

In conclusion, with exception to 'Aurea' and 'Aurea Nana', we did not find any evidence that barberry cultivars currently sold in the market are mislabeled or misidentified. Genetic variants do exist in some barberry cultivars; however, these genetic variants are hard to detect morphologically. It is unknown if the underlying genetic variability within a cultivar could affect the cultivar's attributes such as BSR resistance or susceptibility and will require further investigation. The molecular key developed in this study can be used by the regulatory personnel to establish barberry cultivar identity and could also be used to develop a system that gives true-to-name guarantees to nursery producers, therefore facilitating international trade of barberry cultivars where wheat rust is a concern.

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