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APPLICATION OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) BASED GENOTYPING FOR VARIETY IDENTIFICATION OF *BERBERIS THUNBERGII* (DC) (JAPANESE BARBERRY) IN A REGULATORY DIAGNOSTIC LABORATORY

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APPLICATION OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) BASED GENOTYPING FOR VARIETY IDENTIFICATION OF *BERBERIS THUNBERGII* DC. (JAPANESE BARBERRY) IN A REGULATORY DIAGNOSTIC LABORATORY

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INTRODUCTION

1. *Puccinia graminis* is a fungal pathogen that causes black stem rust in cereals and grasses. The fungus has a complex life cycle involving different spore forms and a requirement for alternate hosts which enables it to over-winter in climates where it would normally not survive year round. This ability to over winter on alternate host plants increases the incidence of outbreaks in regions involved in cereal production and could lead to development of more virulent strains such as UG99, currently the source of an epidemic of stem rust in Africa and Asia. Common barberry (*Berberis vulgaris* DC.) is a naturalized species and the primary alternate host to black stem rust in North America. An average-sized barberry bush can produce more than 64,000,000,000 spores at one time, and consequently, the species has been the target of various eradication programs since the early 1900's in an attempt to protect cereal crop production. Today, common barberry remains on Federal regulated pest lists and in some states and provinces as well. In the 1990s Canada banned import and domestic movement of all *Berberis* species to try to mitigate the threat of this potentially devastating disease.

2. *Berberis thunbergii* DC. – Japanese Barberry - is a an ornamental species of barberry native to Japan and South-East Asia imported for use in landscaping and gardening. Variety morphology is quite diverse - foliage color, as an example, ranges from pale yellow to green to deep burgundy, solids and variegated and brilliant orange-reds in fall. It is extremely hardy and was a popular choice among gardeners across North America for many years prior to the ban. Most varieties are rust resistant, however, some are not. In 2002, the Government of Canada implemented a Barberry Certification Program in an attempt to mitigate the threat of the fungal pest *Puccinia graminis*, while allowing the importation and sale of rust resistant varieties of *Berberis thunbergii* DC.for use in horticultural trade. Since that time, eleven rust resistant varieties of Japanese Barberry have been authorized for importation and sale in Canada: Aurea Nana, "Monomb" Cherry bomb, Concorde, "Tara" Emerald Carousel, "Monlers" Golden Nugget, "Bailgreen" Jade Carousel, Rose Glow, "Gentry" Royal Burgundy, Royal Cloak, "Bailone" Ruby Carousel, and "Monroy" Sunsation. The Program requires that plants imported from the United States of America, or those moved, sold and/or propagated within Canada to be labeled with the variety name. Thus, success of the program is dependent on accurate identification of the approved varieties. CFIA inspection staff evaluate thousands of nursery plants each year to verify these labels against variety descriptions which were developed using features observable on mature plants. Occasionally, verification is not possible through visual inspection alone, as morphology can conflict with variety descriptions, and in some cases, insufficient morphology is available for positive identification, such as when plants are dormant, bare root, or immature.

3. To assist inspectors in situations such as this, a molecular based test was developed within CFIA by Côté and Leduc (2007) using the DNA fingerprinting technique of Amplified Fragment Length Polymorphism (AFLP). Barberry fingerprints are generated and

33 polymorphic bands within the fingerprint are scored for presence/absence (+/-) in each sample. The genotype obtained after scoring is compared against a database containing reference genotypes of the 11 approved varieties. This database was established during development and validation of the method. A variety is considered to be the same as a reference if >/= 31 of 33 markers are shared. Using this technique, all 11 approved varieties are able to differentiated, allowing for identity verification of plants in situations where morphology alone is insufficient.

4. The method was transferred to the CFIA Genotyping/Botany diagnostic laboratory for routine use in 2005 and has been successfully used to support Canada's Barberry Certification Program for the past several years. Testing numbers have varied since implementation of the testing program – but have generally increased since 2005 (Fig 1). The technique has successfully verified variety identity of 384 samples submitted to the lab for genotyping, and has detected mix-ups and occasional mislabeling. In 2008, the method not only detected an unapproved variety that was being sold throughout Canada, but also was applied to assist in sorting out the source of this unapproved variety at the suspected origin.

DIAGNOSTIC CASE: DETECTION OF AN "UNKNOWN" VARIETY

5. In 2008, the laboratory received samples of starter plants, or "liners" from a nursery in Quebec with a request for cultivar verification. Barberry plants at this stage can be difficult to evaluate because immature plants do not always display the morphological traits required for variety verification. Genotyping using the AFLP method revealed that most specimens were true to type, sharing 33/33 markers with the variety indicated on the label. However, two specimens in the lot shared only 24/33 markers with that of the reference genotype (Figure 2). Interestingly, both specimens showed 33 of 33 markers in common with each other, suggesting they might be the same variety. The genotype observed for these two individuals did not match any approved variety with sufficient homology to be identified as one of the Canadian approved varieties and thus were considered to be "Unknown". Through the course of the 2008 testing season, the "Unknown" genotype was detected in samples received from two other nurseries located in British Columbia. These individuals produced genotypes that did not match any Canadian approved variety, but were consistent with the genotype first detected in samples from the nursery in Quebec. In total 6 plants were detected with this genotype in the spring of 2008.

6. Trace back investigation determined all of the "Unknown" plants had originated from a single exporter located in the United States of America. It was suspected that a mix up with another variety sharing similar morphology at early growth stage could have been the source of the unknown genotype. In cooperation with the nursery of origin, 27 blind samples were submitted to for AFLP fingerprinting and genotype analysis. Twenty specimens were taken from the mother block for the variety in question, and seven specimens were taken from a second block of plants with very similar morphology. This second block was suspected as a potential source of the "Unknown" genotype.

7. The results showed 21 of the 27 specimens analyzed had genotypes true-to-type and matched their variety reference. Twenty of these were from the Mother block and one was from the second block. The remaining six samples taken from this second block matched that of the "Unknown" genotype detected in the Canadian samples (Figure 3). Without a verified reference genotype in the database that matched the unknown genotype, it was not possible to identify the "Unknown" genotype.

CONCLUSION

8. AFLP fingerprinting is a powerful tool for discrimination of plant varieties. This technique has been successfully used within the CFIA in support of Canada's Barberry Certification Program which was established in an attempt to mitigate the threat of black stem rust, while allowing the importation and sale of rust resistant varieties of Japanese Barberry for use in horticultural trade. The method developed by CFIA is able to discriminate all 11 Canadian approved varieties and has been used as a diagnostic tool since 2005.

REFERENCES

Arnold Arboretum of Harvard University Bulletin of Popular information. 1937. Barberries immune or highly resistant to black stem rust of cereals. Series 4. Vol V. n.11-18

Côté, M.-J., and Leduc, L. 2007. Molecular Identification of Japanese Barberry (*Berberis thunbergii*) Cultivars Using Amplified Fragment Length Polymorphism. HortScience 42(3):478-482.

CFIA D-01-04: Plant protection import and domestic movement requirements for barberry (*Berberis, Mahoberberis* and *Mahonia spp*.) under the Canadian Barberry Certification Program

Doyle J.J. and Doyle J.L. 1990. Focus 12 #1

Fig 1: Japanese Barberry Fingerprinting – CFIA Genotyping Botany sample numbers - 2005 to present:

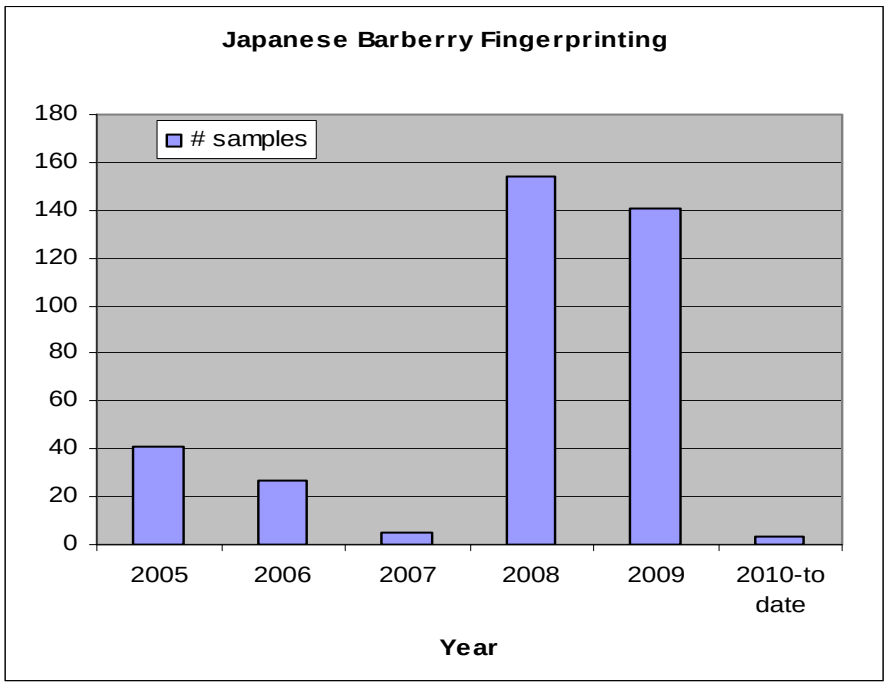

Fig 2: “Unknown” Genotypes compared against Variety Reference:

	Marker number:																															
ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
REF1	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
REF2	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B13	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+
B14	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+
B33	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+
B36	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+
B87	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+
B91	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+

Ref 1 and 2 are reference Genotypes for the variety in question (2 individuals). Red text indicates diagnostic samples displaying the unknown genotype observed in samples submitted from 3 different Nurseries in Canada. Yellow highlighted cells indicate discrepancies between reference genotype and specimens that were taken from the different Canadian nurseries.

Figure 3: Genotype Results of plants from Nursery of Origin:

	Marker number:																															
ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
REF1	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
REF2	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
UNK	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+
B118	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B119	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B120	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B121	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B122	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B123	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B124	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B125	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B126	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B127	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B128	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B129	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B130	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B131	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B132	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B133	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B134	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B135	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B136	+	-	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B137	+	-	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B141	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+
B142	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+
B143	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	-	-	+	-	-	-	+
B144	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+
B145	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+
B146	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+
B147	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	+

Ref 1 and 2 are reference Genotypes for the variety in question (2 individuals). UNK is the unknown genotype observed in Canadian samples from 3 different Nurseries. Yellow highlighted cells indicate discrepancies between the reference genotype and specimens submitted from the Nursery of origin. Samples B118 through B137 were taken from a Mother block, Specimens B141 through B147 were taken from a 2nd block thought to be a potential source of the unknown variety. Red text indicates the samples from block 2 which displayed the "Unknown" genotype, consistent with that observed in the Canadian samples in question.

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