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GENEVA

**WORKING GROUP ON BIOCHEMICAL AND MOLECULAR
TECHNIQUES AND DNA PROFILING IN PARTICULAR**

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Ottawa, Canada, May 11 to 13, 2010

ADDENDUM

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

Document prepared by experts from Canada



Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments

Canadian Food Inspection Agency



Our vision:
To meet an evidence-based regulatory market and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Application of amplified fragment length polymorphism (AFLP) based genotyping for Variety Identification of *Berberis thunbergii* (Japanese Barberry) in a Regulatory Diagnostic Laboratory.

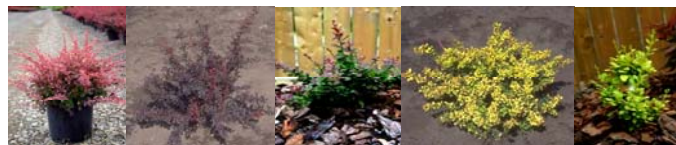
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Background: Japanese Barberry Genotyping

Wild barberry (*Berberis vulgaris*) is a known host of black stem rust of wheat (*Puccinia graminis*) and as a result, plants of the genus *Berberis* were forbidden entry in Canada in the 1990's.

Japanese barberry (*Berberis thunbergii*) is a popular ornamental plant with many varieties appreciated for hardiness and attractive foliage, and generally are considered immune to rust.



Rose glow

Ruby carousel

Emerald carousel

Sunstation

Aurea nana

The Canadian Food Inspection Agency (CFIA) has established a regulatory Program allowing importation of 11 approved Japanese barberry varieties known to be immune to rust.

Why is molecular identification needed?

The success of the program relies on the proper identification of the approved varieties, which may not always be possible by morphology if the plant is missing key features for any reason (i.e. immature, environmental influence, dormancy).

A genotyping method using amplified fragment length polymorphism (AFLP) was developed to be used as a diagnostic test to support delivery of this Program and assists CFIA operations staff with inspection of Japanese barberry imports when:

1. plants display morphology that does not correspond entirely to the variety description,
2. plants are in dormant state and the morphology required for varietal ID is missing



Royal Burgundy



Aurea Nana



???

Japanese Barberry Genotyping

AFLP: A combination of RFLP and randomly primed amplification which generates complex fingerprints comprised of a series of amplified bands (i.e. markers). The technique does not rely on sequence knowledge.

Bands are scored as present (+)/absent(-) for specific bands in the fingerprint.

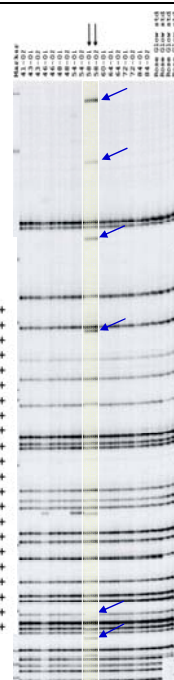
Data is stored in a database for comparison and historical reference.

Sample fingerprints are compared against reference fingerprints for variety identification and/or verification.

Variety verification of Japanese Barberry "Rose Glow"

mb-03-bar-41-02	---+---+---+---+---
mb-03-bar-43-01	---+---+---+---+---
mb-03-bar-43-02	---+---+---+---+---
mb-03-bar-46-01	---+---+---+---+---
mb-03-bar-46-02	---+---+---+---+---
mb-03-bar-48-01	---+---+---+---+---
mb-03-bar-48-02	---+---+---+---+---
mb-03-bar-54-01	---+---+---+---+---
mb-03-bar-54-02	---+---+---+---+---
mb-03-bar-58-01	++-+---+---+---+---
mb-03-bar-58-02	++-+---+---+---+---
mb-03-bar-60-01	---+---+---+---+---
mb-03-bar-64-01	---+---+---+---+---
mb-03-bar-64-02	---+---+---+---+---
mb-03-bar-72-01	---+---+---+---+---
mb-03-bar-72-02	---+---+---+---+---
mb-03-bar-84-01	---+---+---+---+---
mb-03-bar-84-02	---+---+---+---+---
Rose Glow Std	---+---+---+---+---
Rose Glow Std	---+---+---+---+---
Rose Glow Std	---+---+---+---+---
Rose Glow Std	---+---+---+---+---

Image: Validation gel for variety Rose Glow.



Japanese Barberry Genotyping con't

Scoring:

Naturally occurring variation may result in loss (→) or gain (→) of an allele or two.

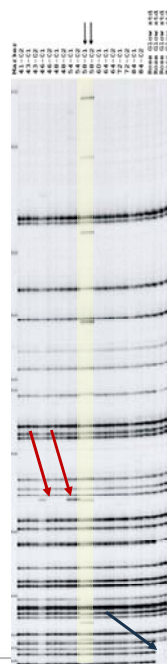
This may/may not impact bands that are scored, but the assay is robust enough to deal with such minor variations:

-> A variety is considered the same as the reference if 31 or more of 33 markers are shared.

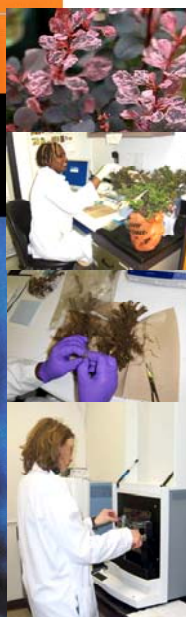
-> A variety is considered not to be the same as the reference if 28 or less of 33 markers are shared.

The 33 markers are generated by 2 different AFLP reactions using 2 primer sets.

(2 additional primer sets are validated for additional discrimination if required for a total of 64 possible alleles)



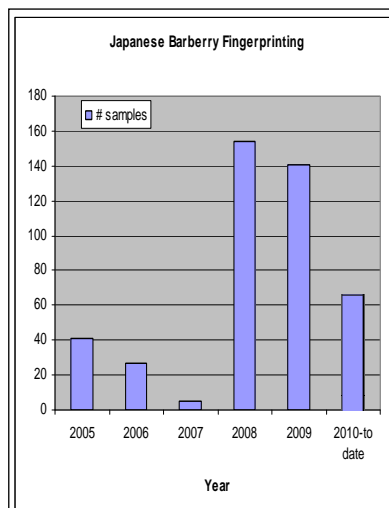
Historical Use at CFIA:



Since it was transferred to the diagnostic lab, 384 specimens sampled from Canadian nurseries across the country have been tested.

The technique has enabled verification of varietal identity, detection of mix-ups and occasional mislabeling.

In 2008, the technique detected an unapproved variety that was being sold throughout Canada and was also able to determine the source of the unapproved variety at the suspected origin.

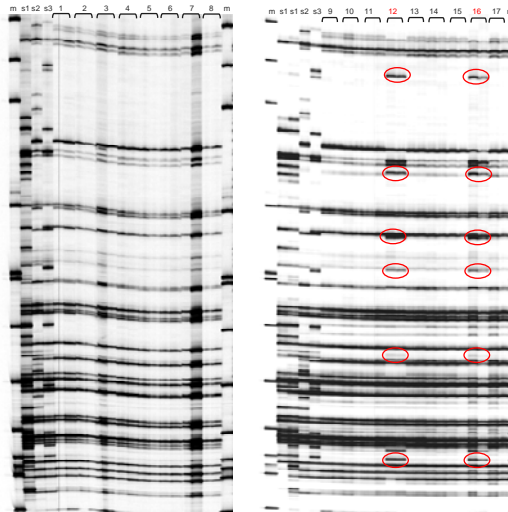


The story of detecting unknown variety "X"

A set of samples selected from liner plants representing a single lot were submitted for confirmation of varietal identity.

Primer set 1 results:
17 markers are scored in each fingerprint with this primer set

Scored bands not corresponding to those expected for this variety are indicated in Red



AFLP Gels for 1st primer set: M: Marker, S1: Std 1, S2: Std 2, S3: Std 3, 1-17: samples

Detection of unknown variety "X", con't

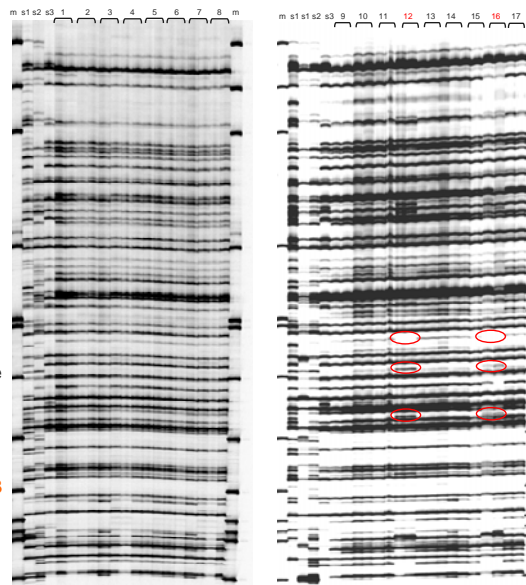
Primer set 2 results:
16 markers are scored in each fingerprint with this primer set

Again, red circles indicate scored bands which did not correspond to the reference

Samples 12 and 16 displayed genotypes that did not match the reference for the variety in question (24/33)

Comparison to the genotypes for the other approved varieties determined they did not match any approved variety.

BUT - both individuals shared 33/33 markers scored with each other!



AFLP Gels for 2nd primer set: M: Marker, S1: Std 1, S2: Std 2, S3: Std 3, 1-17: samples

Detection of unknown variety "X", con't

During the course of the testing season, this genotype was observed in 6 individual plants sampled from 3 different Nurseries across Canada.

ID	Marker number:																																
	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: reference Genotypes of the variety in question (2 Individuals). Red text: genotype observed in diagnostic samples from 3 different Nurseries in Canada. Yellow: highlighting discrepancies between reference genotype and diagnostic samples.

For all 6 samples, the same genotype was observed consistently.

Traceback indicated all 6 plants originated from the same nursery.

Origin of the genotype:

A mix-up with another variety with similar morphology at early growth stage was suspected.

In co-operation with the nursery, an additional 27 additional samples were collected at source and submitted to the lab for AFLP genotyping.

The sample set was comprised of:

- 20 plants from the mother block of the variety in question
- 7 plants from a neighboring block of different variety, which displayed similar morphology and was suspected to be the source of the unknown genotype.



Results:

The genotypes obtained:

ID	Marker number:																																	
	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	+	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	+
B137	+	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	+
B141	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	+
B142	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	+
B143	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	+
B144	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	+
B145	+	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	+
B146	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	+
B147	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	+

The mother block plants – B118 through B137 – all displayed genotypes identical to the reference, as expected; 1 plant, B145, from the neighboring block also displayed this same genotype.

The other plants from the neighboring block displayed genotypes identical to the unknown

Conclusions:

AFLP fingerprinting is a powerful tool for discrimination of plant varieties.

This technique has been successfully used within CFIA in support of Canada's Barberry Certification Program which was established to mitigate the threat of black stem rust while allowing importation and sale of rust resistant varieties for use in the Horticultural trade

The method is able to discriminate all 11 approved varieties and has been used as a regulatory diagnostic tool since 2005.

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