

Genetic Fingerprinting of

(*Hydrastis canadensis* L. [Ranunculaceae])

# Golden





# seal

using AFLP markers

| Suping Zhou and Roger J Sauvé

## ABSTRACT

Goldenseal (*Hydrastis canadensis* L. [Ranunculaceae]) could be considered a worthy ornamental plant for a forest-like garden setting, however, its primary use is medicinal. Research described in this paper demonstrates that AFLP analysis can be used to determine the genetic relationships between accessions of a plant species collected from different regions. The genetic relationships of 9 goldenseal accessions collected in 3 neighboring states, Tennessee, Georgia, and Florida, were determined. A cluster analysis from AFLP data showed that the 2 Tennessee accessions were very closely related to each other with a high bootstrap value of 93%. The 4 Georgia accessions were more diversified with a bootstrap value ranging from 38% to 63%. Among the 3 Florida accessions, genetic relatedness was very low. One Florida accession was closely related to the 2 Tennessee accessions (96%), one with the Georgia accessions (88%), and the other was distant to all accessions. The molecular marker technique developed for determining the genetic relationship and the genetic diversity between accessions could be used by plant breeders for the selection of parental material.

Zhou S, Sauvé RJ. 2006. Genetic fingerprinting of goldenseal (*Hydrastis canadensis* L. [Ranunculaceae]) using AFLP markers. *Native Plants Journal* 7(1):72–77.

## KEY WORDS

genetic diversity, molecular marker, plant DNA profile

## NOMENCLATURE

USDA NRCS (2005)

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Photo by Joseph G Strauch Jr

**G**oldenseal (*Hydrastis canadensis* L. [Ranunculaceae]) is an herbaceous perennial species that is becoming rarer within its range because of excessive harvesting. Goldenseal is classified as a threatened species by the Scientific Committee on the Status of Endangered Wildlife in Canada (COSEWIC). This diminutive herb is found in rich soils of open woodlands in eastern and southern states of the US (Hoffmann 1995). The rhizomes and roots contain large amounts of alkaloids, berberine, and hydrastine, which have medicinal properties. As a botanical supplement, it is one of the top-selling herbs in US health food markets.

Herbalists use goldenseal's rhizomes and roots for their anti-inflammatory effect and antibiotic-like actions. This herb is used to treat a wide variety of ailments such as diarrhea, eczema, eye inflammations, flatulence, gallbladder disease, gastritis, giardia, hemorrhoids, impetigo, indigestion, infections, liver disease, excessive menstrual flow, mouth sores, rhinitis, ringworm, and ulcers. Because goldenseal contains berberine, which can protect against both gram-positive and negative bacteria, it can be more effective for treating gastrointestinal infections and reducing vaginal and uterine inflammations than standard antibiotics (Harding 1936; Hoffmann 1995).

Genetic characterization with molecular (DNA) markers is one of the most reliable methods for providing reference data on genetic diversity of germplasm collections (Zhou and Sauvé 2002). Fluorescent-based amplified fragment length polymorphism (AFLP) in combination with fluorescent detection instrumentation can automatically and accurately size AFLP fragments and provides the data necessary to accurately separate individual plants (Vos and others 1995). Genetic marker-tagged information related to biochemical traits is useful during selection of parental plants for breeding and during evaluation of progenies (Quagliaro and others 2001). Genetic markers are also valuable for determining the phylogenetic relationships among accessions and for true-to-type plant identification (Aggarwal and others 1999). AFLP analysis has also been used in forensic sciences to track clonal sources of illegal plants (Coyle and others 2002). In this study AFLP molecular markers were developed for each goldenseal accession evaluated. These accessions were phenotypically similar but genotypically different. Evidence of genetic variations in individual goldenseal accession and in different colonies determined through F-AFLP analysis is presented.

## MATERIALS AND METHODS

### Plant Material

Goldenseal plants were collected from 3 different neighboring states in the southern part of the US. Four accessions were collected from 2 different locations in Georgia (HGGA1, HGGA2, WCGA1, and WCGA2), 2 accessions from the same county in Tennessee (OCTN1 and OCTN2), and 3 accessions

from 3 different locations in Florida (SHF1, SHF2, and SHF3). All accessions were potted into 4-l (1-gal) pots (#1) in a synthetic medium composed of processed pine bark, Canadian peat moss, and coarse builder's sand (2:1:1; v:v:v) and maintained under 50% shade until needed. For each accession, the genomic DNA was extracted using the methods described by Sauvé and others (2005). Resulting DNA extracts were quantified by fluorometry (DyNA Quant 200 DNA fluorometer, Hoefer Scientific, San Francisco, California) and stored at  $-20^{\circ}\text{C}$  ( $-4^{\circ}\text{F}$ ) until needed.

The AFLP system and thermal cycling parameter used were those described by Zhou and Sauvé (2002), and the selective amplification reactions were performed with ALFP® Analysis System I (Invitrogen Corporation, Carlsbad, California [formerly Gibco BRL Life Technologies]) using the following primers: *EcoR I* 5'-labeled with IR-800 (LI-COR Biosciences, Lincoln, Nebraska) and *Mse I*. The selective nucleotides for *EcoR I* were: E-AAA, E-AAG, E-ACA, E-ACC, E-ACG, E-ACT, E-AGC and E-AGG and for *Mse I*: M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG, and M-CTT. Sixty-four *EcoR I* / *Mse I* primer combinations were evaluated for selective amplifications, thermal cycling, preparation of PCR products, and electrophoresis analyses were performed as previously described (Zhou and Sauvé 2002).

To determine the phylogenetic distance among all accessions, AFLP profiles generated with the primer combinations M-CAC + E-ACA, M-CAT + E-ACA, M-CTG + E-AGG, M-CTT + E-ACG, and M-CTT + E-AGG were combined to generate the database. After filtering out bands that were smaller than 85 bp, the database was binned at 2% tolerance and exported in the TreeCon format.

### Data Collection and Analysis

Image data (16-bits) were automatically collected and recorded during electrophoresis. AFLP fragments were scored, analyzed, and converted into numerical data using the Gene ImagIR™ software (Version 4.02, LI-COR Biosciences, Lincoln, Nebraska). Following band filtration ( $> 85$  bp), all markers were binned with a 2% tolerance and scored as dominant markers. Similarity between different accessions was measured using the similarity index (percentages of shared fragments in the total number of fragments). Genetic distances between plant accessions were estimated according to Nei and Li (1979) and a dendrogram was produced using UPGMA (unweighted pair group method with arithmetic mean) clustering (TreeCon for Windows; Van de Peer and De Wachter 1994).

## RESULTS AND DISCUSSION

### Establishment of F-AFLP Procedures for Goldenseals

The first objective in this experiment was to establish reliable F-AFLP procedures for *H. canadensis*. Previous

TABLE 1

AFLP fragment size distribution and band number (> 80 bp).

Band number Size (bp)	E-AAA	E-AAG	E-ACA	E-ACC	E-ACG	E-ACT	E-AGC	E-AGG
M-CAA	0	3 194–204	5 90–254	2 128–132	7 99–132	0	4 155–292	0
M-CAC	10 119–543	10 82–362	13 125–390	4 89–173	10 83–167	6 84–259	8 88–291	4 269–311
M-CAG	6 96–275	7 90–185	0	3 81–160	17 77–600	7 101–389	0	10 107–197
M-CAT	14 89–230	26 88–661	4 81–264	8 81–639	10 83–327	15 84–284	9 101–241	16 90–455
M-CTA	0	14 85–309	9 90–235	9 90–249	14 81–561	15 81–498	6 83–483	11 97–471
M-CTC	7 88–201	4 116–227	5 85–242	4 127–204	19 81–585	3 120–266	11 91–406	6 81–127
M-CTG	3 139–233	22 91–369	16 91–364	2 113–139	8 97–251	8 94–388	14 100–360	16 92–364
M-CTT	25 93–455	9 133–293	16 99–460	13 94–375	19 93–474	21 93–364	14 98–428	17 97–419

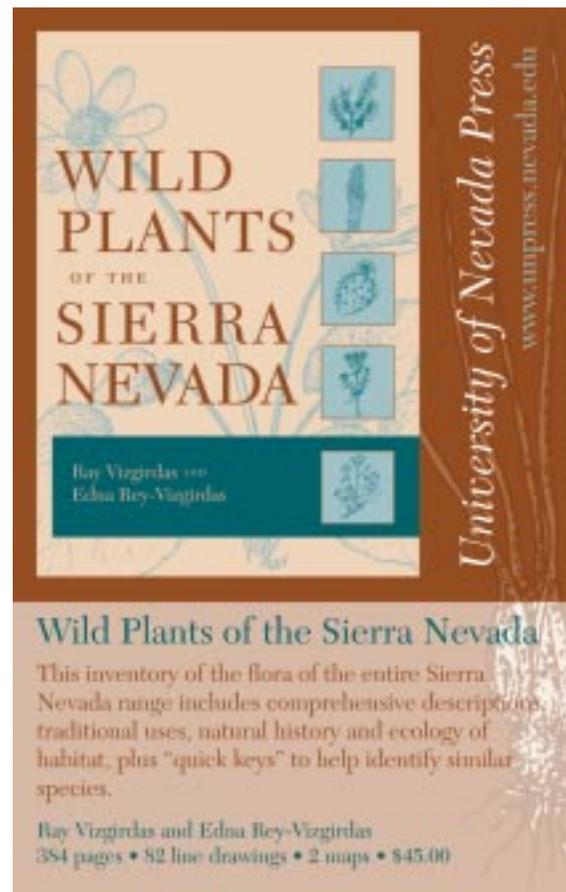
researchers have reported that many factors can affect the reliability of this method (Bielawski and others 1995). AFLP profiles are affected by the DNA preparation protocols, handling of AFLP products, and by analysis (Donini and others 1997; Quagliaro and others 2001). In this study, the quality of goldenseal DNA did not produce any detrimental effect on the F-AFLP banding pattern.

### AFLP Markers and + 3 Primer Screening

Initially, 64 AFLP primer combinations were used for analysis of the goldenseal accessions. Accession WCGA-2 was used to determine the number and size distributions of AFLP bands. Twenty-eight primer combinations resulted in good AFLP profiles with 10 or more scorable polymorphic fragments (Table 1). Band numbers varied from none to 26. Clear band separation began with the 81 bps fragments, and sizes of amplified bands ranged from 35 to 650 bps. Polymorphic AFLP fragments were distributed across the entire range with major distributions between 81 and 300 bps. With few accessions, few bands larger than 500 bps were generated.

### Genetic Divergence and Phylogenetic Relationship

The Tennessee accessions are mapped to be basically identical in all the primer combinations tested (Figure 1). The average similarity between these genotypes was 0.95 (Table 2). The Georgia accession had higher genetic diversities (based on banding patterns of AFLP analysis) than the Tennessee accessions. Their similarity indexes were between 0.50 and 0.65. In the case of the



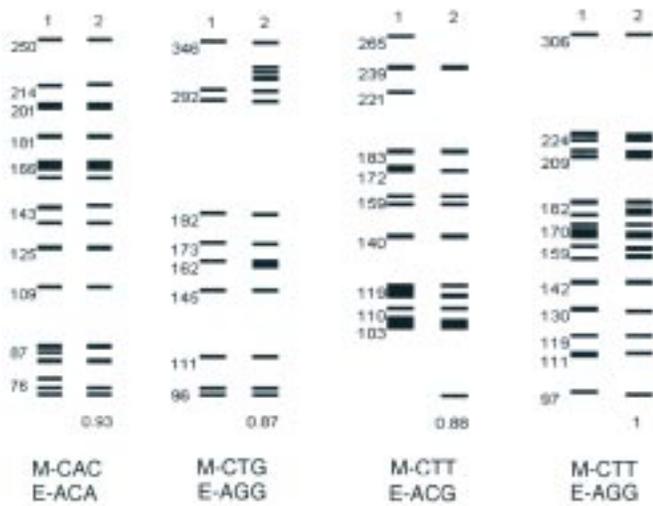


Figure 1. AFLP profiles of goldenseal accessions collected in Tennessee (OCTN). The numbers at the bottom indicate similarity index. The numbers at the top indicate accession number. The numbers on the left side indicate molecular size (bp). The last 3 selective nucleotide sequences indicate the primer combinations used.

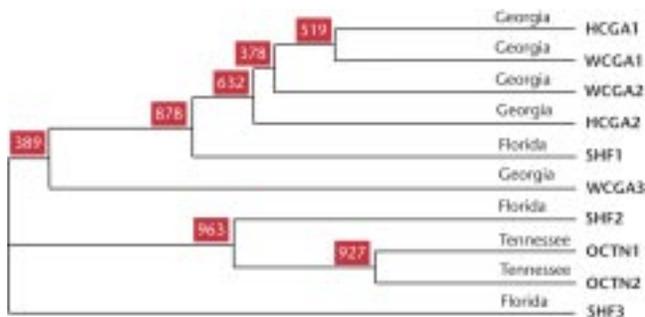


Figure 2. UPGMA dendrogram of 10 goldenseal accessions. Bootstrap values are indicated for 1000 UPGMA searches.

TABLE 2

Genetic similarity between 2 goldenseal accessions from Tennessee.

	M-CAT	M-CTA	M-CAC	M-CAA
E-AAA	—	0.96	—	—
E-AAG	0.88	1.00	—	—
E-ACA	1.00	1.00	—	0.92
E-ACC	—	1.00	—	0.92
E-ACT	0.84	—	—	—
E-AGC	1.00	—	0.93	0.91

Florida accessions, similarity indices varied greatly with primer combinations, ranging from 0.67 to 0.75 for primer combinations of M-CAC + E-AAA and M-CTT + E-ACG, and 0.3 for the primer combination of M-CAC + E-ACA and M-CTT + E-AGG.

Single linkage clustering analysis (Nei and Li 1979) showed that the Tennessee accessions, having a bootstrap value of 93%, belong to one cluster (Figure 2). The bootstrap value is an indication of how consistently the data support a given bipartition, high bootstrap values close to 100% mean uniform support. Close to 100%, or nearly all of the characters informative for this group, agree that it is a group.

All Georgia accessions fell into a large cluster well separated from the Tennessee accessions. The Georgia cluster is divided into 3 subclusters. The Florida accession SHF2 was very closely related to the Tennessee accessions cluster, with a bootstrap value of 96%. Accession SHF1 shared more similarity with the Georgia cluster, with a bootstrap value of 88% (Figure 2). The other Florida accession, SHF3, formed a single cluster. This indicates that it is more distant from all other accessions. The close relationships of 2 Florida accessions to Tennessee and Georgia suggest that these accessions were initiated from plants collected in Georgia and Tennessee.

## SUMMARY

AFLP methodology developed in this study can be used to differentiate genotypes. If F-AFLP molecular markers can be linked to potential medicinal use of specific selections, they would be very useful in breeding programs and for the selection of parental materials. In addition, AFLP molecular markers can be used to track the original source of an accession.

## REFERENCES

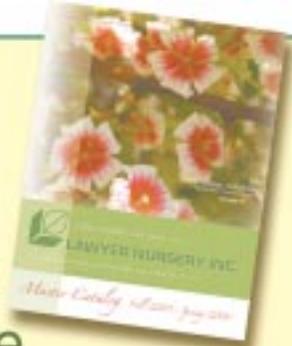
- Aggarwal RK, Brar DS, Nandi S, Huang N, Khush GS. 1999. Phylogenetic relationships among *Oryza* species revealed by AFLP markers. *Theoretical and Applied Genetics* 98:1320–1328.
- Bielawski JP, Noack K, Pumo DE. 1995. Improving amplification of randomly amplified polymorphic markers (RAPD) from vertebrate DNA. *Biotechniques* 18:856–859.
- [COSEWIC] Scientific Committee on the Status of Endangered Wildlife in Canada. URL: <http://raysweb.net/specialplaces/pages/canada-es.html>.
- Coyle HM, Germano-Presby J, Ladd C, Palmbach T, Lee HC. 2002. Tracking clonal marijuana using amplified fragment length polymorphism (AFLP) analysis: an overview. Conference Proceedings: 13th International Symposium on Human Identification, Phoenix, AZ. URL: <http://www.promega.com>.
- Donini P, Elias ML, Bougourd SM, Koebner RMD. 1997. AFLP fingerprinting reveals pattern differences between templates DNA extracted from different plant organs. *Genome* 40:521–526.
- Harding AR. 1936. Ginseng and other medicinal plants. URL: <http://www.ibiblio.org/herbmed/eclectic/harding/hydrastis-cultiv.html>.
- Hoffmann DL. 1995. Goldenseal. Health World Online <http://www.healthy.net/hwlibrarybooks.hoffman/materiamedica/goldenseal.htm>.
- Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Science* 76:5269–5273.
- Quagliaro G, Vischi M, Tyrka M, Olivieri AM. 2001. Identification of wild and cultivated sunflower for breeding purpose by AFLP markers. *Journal of Heredity* 92:38–42.
- Sauvé RJ, Zhou S, Yu Y, Schmid WG. 2005. Randomly amplified polymorphic DNA analysis in the genus *Hosta*. *HortScience* 40:1243–1245.
- [USDA NRCS] USDA Natural Resources Conservation Service. 2005. The PLANTS database, version 3.5. URL: <http://plants.usda.gov> (accessed 23 Nov 2005). Baton Rouge (LA): National Plant Data Center.
- Van de Peer Y, De Wachter R. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Computer Applications in the Biosciences* 10:569–570.
- Vos P, Hogers R, Bleeker M, Reijans M, Van de Le T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23:4407–4414.
- Zhou S, Sauvé RJ. 2002. Use of fluorescent-amplified fragment length polymorphism for species identification in the genus *Pulmonaria*. *Journal of Environmental Horticulture* 20:110–113.

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