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NOTES ON NEGLECTED AND UNDERUTILIZED CROPS

A measure of genetic diversity of goldenseal (*Hydrastis canadensis* L.) by RAPD analysis

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Abstract Goldenseal (*Hydrastis canadensis* L.) is a medicinal plant valued for the treatment of sore eyes and mouths. Although cultivation of the plant has helped meet growing demand, goldenseal is still considered a threatened or endangered species throughout much of its range in North America. In an effort to assess possible conservation strategies for goldenseal genetic resources, levels of genetic diversity within and among cultivated and wild populations were quantified. RAPD analysis was used to examine six cultivated and 11 wild populations sampled from North Carolina, Ohio, Pennsylvania, and West Virginia. The average percentage of polymorphic bands in cultivated and wild populations was low (16.8 and 15.5 %, respectively), and geographic range did not predict the level of genetic diversity. Most of the genetic variation (81.2 %) was within populations; only 3.6 % was partitioned between cultivated and wild populations. Our results differed from a previous study which concluded that genetic differences were greater among than within populations. The results of the current study indicate that, although goldenseal grows clonally and in dense patches, a mixed mating system in which both selfing and outcrossing occur is also operating. We therefore suggest that the *ex situ* conservation of individual plants within populations, chosen carefully to account for clonal propagation *in situ*, is an appropriate strategy for sustaining the genetic diversity of goldenseal.

Keywords AMOVA · Conservation · Genetic resource · Genetic variation · *Hydrastis canadensis* · Medicinal plant · UPGMA

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Introduction

Goldenseal (*Hydrastis canadensis* L.), a valued medicinal plant, is an herbaceous perennial species in the buttercup family (Ranunculaceae). Mature plants, which are 6–14 in. tall, have two or more erect hairy stems that usually end in a branched fork with two leaves. Each plant can produce a single, aggregate fruit that turns red upon seed maturity. The goldenseal plant is found in thick hardwood forests throughout the Northeastern United States and Canada. In the United States, goldenseal grows as far north as Vermont, to as far south as Alabama, and to as far west as Kansas (Davis 1999). Throughout history and depending on locality, the goldenseal plant has had several common

names, including yellow root, orangeroot, ground raspberry, yellow puccoon, wild circuma, eye-balm, yellow paint, wild turmeric, and yellow eye (Davis and McCoy).

The bright yellow root, which is high in the alkaloid berberine, is traditionally used as an antibacterial to treat inflamed mucous membranes of the mouth, throat, and digestive system (Foster and Duke 1990). The US Forest Service listed goldenseal as the second most popular medicinal herb worldwide (Robbins 1999). The majority of goldenseal root comes from wild populations in North America (HRF 2000) that are dwindling due to over-harvesting (Davis 1999). In 1997, goldenseal was listed in Appendix II of the Convention for International Trade on Endangered Species (CITES 1997), a move intended to monitor trade in the plant and curtail harvest practices incompatible with species survival. According to Foster (2011), approximately 113,000 kg of goldenseal are harvested each year. This amount was an increase from the 41,000 kg of goldenseal harvested in 2005 (AHPA 2007), suggesting that demand for the crop is increasing at a rapid rate.

Although goldenseal has been cultivated since the early 1900s (Davis and McCoy 2000), renewed interest in cultivation has arisen in attempts to meet demand. Currently, some goldenseal is being successfully cultivated, but the quantity is only about 23 % of the total goldenseal harvest (Dentali and Zimmerman 2012). The slow growth rate of this species and continued overharvest of wild populations, limits the availability and recovery of the plant in natural habitats. In Ohio, nearly half of all documented goldenseal populations in the plants central habitat area has been overharvested, destroying the plant stands (Mulligan and Gorchow 2004). As a consequence, some unquantifiable amount of genetic diversity within this species has most likely been lost.

Studies of diversity are useful for understanding the genetic structure of populations and for developing conservation strategies targeted at appropriate levels (population, individual, or ecotype). For example, in *Eryngium alpinum* L., an endangered species in the European Alps, the relatively high genetic differentiation among populations indicated that conservation measures should save a maximum number of populations (Gaudeul et al. 2000). In contrast, the endangered *Piperia yadonii* R. Morgan et Ackerman retained only a modest amount of genetic variation among individuals within extant populations, indicating the best conservation method for this species would be through the preservation and expansion of habitat at each site to enable the natural development of populations (George et al. 2009). The use of various molecular marker techniques (RAPD, AFLP, ISSR, and SSR) during the last few decades has provided rapid and reliable information on genetic diversity, allowing such analyses to be undertaken in species such as goldenseal in which no previous genetic work has been conducted.

Life history, geographic range, and breeding system often have significant effects on the partitioning of genetic diversity within and among plant populations (Brown 1989; Hamrick 1983; Loveless and Hamrick 1984). For example, allele frequencies at isozyme loci in inbreeding and outcrossing plant species can be analyzed to examine intraspecific variation in gene diversity (Brown 1978; Schoen and Brown 1991). In comparison with out breeders, inbreeding species show markedly greater variation among populations in average values of Nei's gene diversity statistic. Goldenseal grows clonally, typically in dense patches, although a mixed mating system in which both selfing and outcrossing occur at roughly equal frequencies has been observed (Sanders 2004). Comparing the genetic diversity within cultivated and wild populations is a means of assessing inheritance state in cultivated and wild populations. In general, levels of genetic variation in cultivated populations are significantly lower than in wild populations (Lam et al. 2010; Mandel et al. 2011; Miller and Schaal 2006) in relation to the extent of population bottlenecks that have occurred during the domestication process, a widespread phenomenon in crop species (Doebley et al. 2006).

Because some biological and ecological questions remain unanswered, or, at best, only partially answered in goldenseal (for example, population size, demographics, and genotypic variation), developing cultivation and conservation strategies for the species has been difficult. Few molecular marker studies have been done in goldenseal (Kelley 2009; Zhou and Sauve 2006), however, such knowledge is needed to sustain the species. Our study assessed the level of genetic diversity in goldenseal populations, comparing cultivated and wild populations in an effort to gain insight as to the most appropriate conservation, harvesting, propagation, and cultivation strategies for preserving the species.

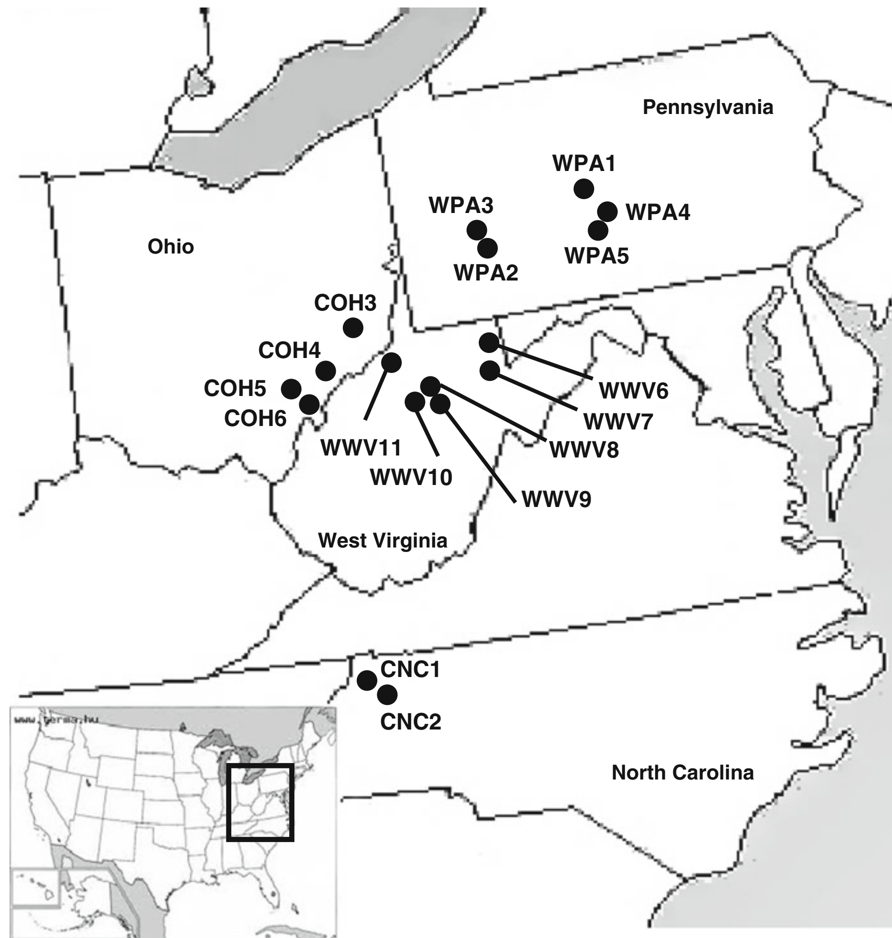

Fig. 1 Geographic distribution of *Hydrastis canadensis* populations sampled in the present study

Materials and methods

Plant materials

Goldenseal plants from 17 populations located in North Carolina, Ohio, Pennsylvania, and West Virginia were used in this study (Fig. 1). Collection sites were documented from Global Positioning System (GPS) data taken at time of sample collection. Populations were classified as either cultivated or wild type (Table 1). At each collection site, leaf tissue from 5 to 10 plants representative of the plant population were randomly gathered from individual plants during a walk through the plant population in August, 2003. The collected leaf tissue was dried in silica gel and stored at room temperature until subjected to DNA extraction by using a modified CTAB method (Xie et al. 1999).

PCR amplification

A total of 10 decamer primers were used in the RAPD analysis (AA1: AGACGGATCC, AA2: GAGACCAGAC, AA3: TTAGCGCCCC, AA4: AGGACTGCTC, AA5: GGCTTTAGCC, AA6: GTGGGTGCCA, AA7: CTACGCTCAC, AA8: TCCGCAGTAG, AA9: AGAT GGGCAG, AA10: TGGTCGGGTG). DNA amplification was done in a RoboCycler Gradient 96 (Stratagene, Agilent Technologies Inc., Santa Clara, CA), using a 20 μL volume containing 20 ng genomic DNA template (1 μL), 2 μL 10 × reaction buffer, 0.4 μL of dNTPs (each 10 mM), 0.4 μL (20 pmol) of primer and 0.1 μL (0.5 U) of Taq DNA polymerase (New England BioLabs Inc., Ipswich, MA). The RAPD markers were amplified under the following PCR conditions: 1 cycle of 94 °C for 2 min; 40 cycles of 94 °C for 30 s, 36 °C for 1 min, and 72 °C for

Table 1 Sampled populations and polymorphic bands in goldenseal

Studied populations[a]	Location	Sample size (No. of plants)	Polymorphic bands[b]	
			No. of bands	%
Cultivated				16.8
CNC1	North Carolina	10	13	15.6
CNC2	North Carolina	10	13	15.6
COH1	Ohio	10	19	22.8
COH2	Ohio	6	7	8.4
COH3	Ohio	10	18	21.6
COH4	Ohio	10	14	16.8
Wild				15.5
WPA1	Pennsylvania	10	16	18.0
WPA2	Pennsylvania	10	14	16.8
WPA3	Pennsylvania	10	10	12.0
WPA4	Pennsylvania	10	12	14.4
WPA5	Pennsylvania	5	8	9.6
WWV1	West Virginia	10	15	18.0
WWV2	West Virginia	8	12	14.4
WWV3	West Virginia	10	12	14.4
WWV4	West Virginia	7	9	10.8
WWV5	West Virginia	10	17	20.4
WWV6	West Virginia	9	18	21.6
Total		155	23	27.7

[a] The population names were assigned to distinguish among the samples and to indicate the geographic origin, but no other relationship to the sample; all samples were collected during August, 2003. Note, the CNC1 and CNC2 samples are a mixture of cultivated plants that originated from several sources of wild plants

[b] A total of 83 bands were detected in the present study

2 min; 1 cycle of 72 °C for 7 min; and a 4 °C holding step.

The PCR products were electrophoresed in 1.8 % agarose gel in TAE buffer. The gel was stained with ethidium bromide and visualized by illumination with UV light with a Fujifilm Luminescent Image Analyzer LAS-3000 (Fujifilm Holdings Corporation, Minatoku, Tokyo, Japan).

Data analysis

RAPD products were scored as 1 for presence and 0 for absence of bands. Genetic diversity was estimated by the percentage of polymorphic bands (PPB), determined by dividing the number of polymorphic bands within a population by the total number of bands surveyed. Within-population diversity values were calculated with Nei's unbiased diversity statistic (Nei 1987). A agglomerative clustering dendrogram, constructed with POPGENE version 1.31 (Yeh et al. 1997) using an unweighted pair group method with arithmetic mean (UPGMA), was chosen to show the relationships among populations based on Nei's genetic distance (Nei 1978). A second, additive-tree clustering dendrogram (Fitch-Margoliash) was also constructed to account for any irregular evolution between cultivated and wild populations.

To describe population structure and variability among populations, the nonparametric Analysis of Molecular Variance (AMOVA) procedure was used as described in Excoffier et al. (1992), where the variation was partitioned among individuals within populations, among populations within groups, among groups (cultivated and wild), and among states (North Carolina, Ohio, Pennsylvania, and West Virginia) with ARLEQUIN software Ver. 3.5.1.2 (Excoffier and Lischer 2010).

Results

RAPD polymorphism

Of the 10 primers that we tested, two (AA5 and AA8) failed to amplify any bands in our samples. From the eight productive primers, we could detect 83 distinct bands. Of those, 23 bands ranging in size from 400 to 1,500 bp were polymorphic among 155 plants

Table 2 Analysis of molecular variance (AMOVA)

Source of variation[a]	df	Sum of squares	Variance components	Percentage of variation	*P* value
Among populations	15	34.36	0.18865	15.25	<0.001
Within groups					
Within populations	96	86.33	0.89924	81.18	<0.001
Cultivated versus wild	1	3.57	0.03949	3.57	0.067
Among states	3	5.06	0.05572	5.06	0.032

[a] The analysis includes total of 155 individual goldenseal samples

(Table 1) and scored for further analysis. Each plant within the 17 populations had a unique genotype, except for two individuals from the wild Pennsylvania population (WPA2), which had identical genotypes at all 23 loci. No population-specific bands were detected, however, one marker (AA6-1) amplified in all populations except those from Pennsylvania. Marker AA7-1 amplified in only four populations from Ohio (COH5 and 6) and West Virginia (WWV6 and 11). Marker AA4-3 had no amplification within three of the Pennsylvania populations (WPA1, 2, and 3), but was present in all individuals of the other two Pennsylvania populations (WPA4 and 5) (data not shown).

For each population and each habitat (cultivated and wild), the average PPB of cultivated and wild populations (16.8 and 15.5 %, respectively) and of each state population (North Carolina = 15.6 %, Ohio = 17.4 %, Pennsylvania = 14.2 %, and West Virginia = 16.6 %) were not significantly different (*t* test).

The genetic structure of populations

An overall assessment of distribution of diversity within populations, among populations within groups, among groups (cultivated and wild), and among states (North Carolina, Ohio, Pennsylvania, and West Virginia), using AMOVA tests conducted twice from the distance matrix (Table 2). The first AMOVA showed more highly significant ($P < 0.001$) genetic differences within populations than among populations and among groups (Table 2). Of the total genetic diversity, 81.2 % was attributable to differences between individuals within a population, only 15.3 and 3.6 % to among population and group differences, respectively. The second AMOVA compared difference among states and indicated that only 5.1 % of the total genetic diversity resided in differences among states (Table 2).

To represent the relationships among populations, geographical differences and habitat (cultivated and wild), cluster analysis (UPGMA) was used to generate a dendrogram based on pairwise distances between populations (Fig. 2). The use of the Fitch-Margoliash cluster analysis produced a clustering pattern similar to the UPGMA. The wild populations that were geographically close (WWV8 and WWV9; WPA4 and WPA5) were separated into the same cluster, however, cultivated populations showed clusters without geographic relevance. Nature and habitat were not clearly separated in UPGMA, which corroborates the results of AMOVA, indicating little genetic differentiation between nature and habitat.

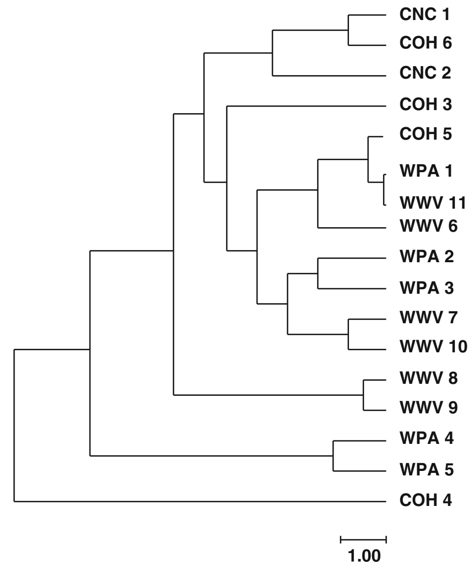

Fig. 2 Dendrogram of 17 wild and cultivated populations of goldenseal. The dendrogram was constructed by using the UPGMA clustering algorithm based on differences at 23 polymorphic RAPD bands

Discussion

The similarity of PPBs and the low number of genetic differences between cultivated and wild populations of goldenseal observed in our study, strongly suggests similar genetic content within the cultivated and wild populations. This result is not surprising given that the original plants in cultivated populations were undoubtedly vegetatively propagated from wild populations. Indeed, the commonality of marker AA7-1 to both the Ohio and the West Virginia populations suggests that the original source of cultivated goldenseal plants in Ohio populations could have come from a wild population in West Virginia or from a similar, but unknown, wild population in Ohio.

The relatively high levels of variance observed within populations suggest that plant reproduction within populations involves seed production. In contrast, the relatively low level of variance among populations suggests that limited pollen movement between populations restricts any genetic flow from one isolated population to another in accordance with Sanders' hypothesis (2004). Support for these limits on genetic variability within goldenseal is strengthened by the presence of identical DNA marker patterns observed within a population. While separate populations could normally be expected to evolve sustainably, the woodland forest habitat of goldenseal is a largely stable environment, and such an environment would reduce the pressure to select a variety of ecotypes.

AMOVA analysis, in which geographically close populations were grouped together, showed similar proportions of total genetic variance as the original AMOVA, suggesting that geographical range could not predict the level of genetic diversity for the goldenseal. Nevertheless, some geographical differences were observed. For example, all of the Pennsylvania populations showed population-specific marker distributions (including a lack of amplification of two markers, AA6-1 and AA7-1). Another marker, AA4-3 amplified in all WPA4 and 5 individuals, but not in WPA1, 2, and 3 plants, most likely due to a genetic bottleneck that occurred in the Pennsylvania populations WPA1, 2, and 3, as opposed to genetic drift that could cause marker-specific differences among these populations.

Our results support a genetic fingerprinting study (using AFLP markers) by Zhou and Sauve (2006) who examined seven goldenseal accessions from three neighboring states (Georgia, Tennessee, and Florida) to the south and west of our study area, observing a genotypic similarity among accessions that ranged from 0.50 to 0.95. Although a limited number of samples were used, Zhou and Sauve (2006) indicated that two sampled Florida goldenseal accessions were closely related to goldenseal accessions collected in Georgia and Tennessee. The accessions in the Zhou and Sauve (2006) investigations, however, do not provide any information on genetic diversity within an accession.

The plants used to establish the North Carolina cultivated populations (NC1 and NC2) were collected from throughout the sampling area used in the current study (Davis 2011, personal communication). Thus, the current data set, although obtained from a limited number of plants growing in a relatively narrow geographic area, could reflect the level of genetic diversity of goldenseal in North America. Such a model is probable due to goldenseal populations being highly isolated with limited pollen flow between populations (Sanders 2004), and diversity being primarily based within populations. These limiting factors make any natural increase in genetic diversity of North American goldenseal highly unlikely.

Because of the endangered nature of this species, available sample populations for this study were restricted on public and private lands to protect habitats and populations. If a larger scale study were feasible, more detailed estimates of genetic diversity levels in goldenseal could be obtained. Nevertheless, the current study at the molecular level provides valuable insight into the limited diversity among natural goldenseal populations in Pennsylvania and West Virginia and suggests the conservation of goldenseal populations is important for maintaining present levels of genetic diversity.

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