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Full Length Research Paper

Effects of anthropogenic activities on genetic diversity and population structure of American ginseng (*Panax quinquefolius* L.) growing in West Virginia

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Using random amplified polymorphic DNA (RAPD) markers, genetic variation and population structure of wild and cultivated American ginseng growing in West Virginia (WV) was assessed. Also, the effects of cultivation intensity and harvest pressure on genetic diversity of ginseng populations were evaluated. Eight primers were used to amplify DNA samples from 468 plants, generating a total of 98 band fragments of which 84 were polymorphic. Overall mean genetic diversity measures were lower in West Virginia populations compared to populations from Pennsylvania and Wisconsin. Among West Virginia populations, mean genetic diversity indices were higher in cultivated than in wild populations but differences were not significant. Means of genetic diversity indices were higher in wild populations from low harvest pressure region [percent polymorphism (*P*) = 33.33%, Nei's (1973) gene diversity (*H*) = 0.1172, and Shannon's index (*I*) = 0.1743] compared to those from high harvest pressure region (*P* = 28.27%, *H* = 0.1019, *I* = 0.1513), however, these indices were not significantly different (*P* > 0.05, Mann-Whitney test). Analysis of molecular variance (AMOVA) however showed significant genetic differentiation (*P* < 0.001) within and among wild populations (48.37 and 54.10% respectively). Cultivated ginseng populations from a region with low cultivation intensity had lower levels of genetic diversity indices (*P* = 29.93%, *H* = 0.0948, *I* = 0.1609) compared to populations from a region with high cultivation intensity (*P* = 60.60%, *H* = 0.2593, *I* = 0.3243), these indices were significantly different (*P* < 0.05, Mann-Whitney test). AMOVA further revealed that in cultivated populations, 53.68 and 42.11% of the total genetic variation was attributed to within and among population differentiation in regions respectively and 4.20% of genetic variation existed between regions. Based on these data, it is evident that genetic diversity of American ginseng is substantially increased with increase in its cultivation intensity. This can be ascribed to higher levels of gene flow associated with sourcing of seeds from various sources to meet cultivation needs of large scale growers. Even though genetic variability among populations experiencing different harvest pressure was not statistically significant in this study, the lower genetic diversity indices exhibited in wild populations from high harvest pressure regions underscores the potential impacts of harvesting pressure on genetic diversity of this medicinal plant. Thus, the data presented in this study will be useful in guiding conservation strategies for this economically important but threatened medicinal species.

Key words: Cultivation intensity, harvest pressure, analysis of molecular variance (AMOVA), random amplified polymorphic DNA (RAPD).

INTRODUCTION

The immediate threats to medicinal plants are anthropogenic activities resulting in habitat loss, habitat degradation and over-harvesting exacerbated by increasing demand for herbs and herbal products both regionally and internationally (Cunningham, 1993; Hamilton, 1997; Kuipers, 1997; Ahmad, 1998; Lange,

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1998). About 75% of medicinal plants used worldwide are collected from the wild (Canter, 2005) and the other 25% are from cultivated sources.

Cultivation of medicinal plants has been on the rise lately, mainly as a response to declining wild populations and the potential economic gains anticipated from the growing demand of herbal products globally. Whereas increasing cultivation of medicinal plants minimizes the pressure on their wild populations, more often, the cultivation process results in change of genetic diversity and structure of plant populations (Doebley, 1989). Such changes in population gene pool associated with the cultivation process are often a concern to plant conservationists as there is always a fear that this will lead to erosion of 'locally' adapted genotypes of a given species, especially if 'non-local' gene pools are used as seed sources for cultivation. For instance, collection of seeds from a narrow source for use in establishing a cultivated population can cause genetic drifts or bottlenecks that can result in populations of relatively low genetic diversity and that have diverged significantly from their wild progenitor gene pools (Zohary, 2004). On the other hand, if cultivated populations are located in close proximity to their wild counterparts, the possibility of gene flow back and forth between cultivated and wild populations is more likely and that will cause reduction in genetic isolation therefore resulting in non-distinct cultivated and wild populations (Otero-Arnaiz et al., 2005).

American ginseng (*Panax quinquefolius* L.), Araliaceae, is a perennial herb native to the eastern deciduous woodlands of North America (Catling et al., 1994). It is among the top 12 commonly used herbs in the United States (O'Hara et al., 1998) and one of the most widely used medicinal herb in the world (Christensen et al., 2006). Growing popularity of ginseng among consumers worldwide has led to its extensive collection from the wild and its increasing cultivation within and outside of its native range (Cruse-Sanders and Hamrick, 2004; Christensen et al., 2006). West Virginia (WV) is at the core of American ginseng's native range, and collection of American ginseng from the wild has been a part of the folklore of West Virginians for hundreds of years (Anon, 2008). In the US, WV ranks second in wild ginseng production (Anon, 2008; WVDOF, 2008). Even though wild American ginseng occurs statewide and ginseng could potentially be cultivated throughout the state, it's harvesting and cultivation is regionalized. According to data records of ginseng production in WV over a 30 year period (1978 to 2006), it is evident that more than half of the total harvested wild ginseng originates from eight counties in the state's southwestern corner, in contrast, 88% of all cultivated ginseng production during this same period came from one county on the northern part of the state (Anon, 2008; WVDOF, 2008). From this data, it can be inferred that ginseng populations in WV are subjected to different levels of harvest pressure and cultivation intensity. Under these varied anthropogenic influences, one may speculate that genetic diversity and population structure will be different or at least are bound to change especially since the species has a long reproductive cycle and its harvesting entails destruction of the entire plant.

American ginseng is listed as a threatened species under Convention of International Trade of Endangered Species (CITES) and therefore, its trade is closely monitored at federal and state levels (Robbins, 1998). Documenting the range of its genetic diversity and population structure, and evaluating how they are influenced by human activities is integral in decision making process to protect this economically important medicinal species. Genetic diversity is a valuable resource from which populations derive short-term adaptation to environmental stochasticity and long-term evolutionary changes (Ellstrand and Elam, 1993), and is a good reservoir from which breeding varieties can be derived.

Genetic diversity and population structure under varied levels of harvest pressure is not extensively explored, and very little is known about how the cultivation process has impacted genetic variability of American ginseng populations growing in WV. The objectives of this study were to assess genetic diversity and population structure of American ginseng populations growing in WV, and evaluate how genetic diversity persist under varied levels of anthropogenic activities (cultivation intensity and harvest pressure). The results presented in this study illuminate the extent of genetic diversity of American ginseng regionally and provide valuable data that would be useful in guiding conservation strategies for this economically important but threatened medicinal species.

MATERIALS AND METHODS

Study species

American ginseng (***P. quinquefloius*** L.) is a long-lived perennial herb with palmate compound leaves and a fleshy tap root. The species is self-fertilized (Schlessman, 1985); however, outcrossing by generalist insects has been reported (Carpenter and Cottam, 1982; Lewis and Zenger, 1983). Plants typically do not flower until they are old enough to produce two leaf whorls, usually in the fourth or fifth year (Carpenter and Cottam, 1982; Lewis and Zenger, 1983; Charron and Gagnon, 1991; Anderson et al., 1993). Seeds usually undergo a dormancy period of 18 to 20 months before germinating in early spring of the second growing season (Lewis and Zenger, 1982; Proctor and Bailey, 1987; Pritts, 1995). The species has no known asexual reproduction using root or stem cuttings (Lewis, 1988).

Sampling

Populations in specifically demarcated regions reflecting different levels of cultivation intensity and harvest pressure were sampled. Regions were demarcated based on ginseng harvest records obtained from West Virginia Department of Forestry (WVDOF, 2008) for the period between 1978 and 2006. Regarding cultivation intensity, two regions were categorized; region 1 (WVCR1), low cultivation intensity, mainly comprised of small scale ginseng farms typically less than 1 acr of land under ginseng cultivation with reported ginseng production averaging less than 10 pounds per

Figure 1. United States map showing sampled populations of *P. quinquefolius*. A map of West Virginia State is enlarged to show population sites and regions where samples were collected. WVCR1 = low cultivation intensity region; WVCR2 = high cultivation intensity region; WVWR1 = low harvest pressure region; and WVWR2 = high harvest pressure region. Sample collection areas are not drawn to scale.

year; and region 2 (WVCR2), high cultivation intensity, characterized with large scale ginseng farms reportedly producing on average 300 pounds or more per year over a 30 year period (Figure 1). With regard to harvest pressure on wild ginseng populations, two regions were categorized; region 1 (WVWR1), a low harvest pressure region, where ginseng collection averages less than 500 pounds per year; and region 2 (WVWR2), a high harvest pressure region, where ginseng collection averages over 500 pounds per year over a 30 year period (Figure 1). Two wild and two cultivated populations from Pennsylvania (PA) and Wisconsin (WI) respectively were also sampled and included in the study for comparison (controls). Wild plants from PA originated from populations that have had a long-time protection from harvest, whereas cultivated samples from WI originated from large scale farm extensively cultivating American ginseng.

Plant materials

Leaf samples from 468 plants representing a total of 26 populations of wild (14) and cultivated (12) American ginseng were collected in 2007 and 2008 from WV, PA, and WI (Table 1). Leaf samples from cultivated populations were donated by growers, whereas samples of wild populations were collected from the wild with the help of ginseng diggers in various regions. At least 9 randomly selected plants within a 50 m radius of a contiguous patch of ginseng plants were sampled. Upon collection, individual leaves were wrapped separately in moist paper towels then placed in Ziploc bags and transported back to the laboratory within 24 h. Once in the laboratory, leaves were rinsed with deionized water then immediately frozen in liquid nitrogen and stored in Ziploc bags at -80°C until DNA extraction.

DNA isolation

Approximately 20 mg of leaf material was placed in 1.5 ml micro centrifuge tube and ground with a sterile disposable plastic pestle (Fisher Scientific, Pittsburgh, PA). DNA extraction was done using a GenElute Plant Genomic DNA Mini Prep Kit (Sigma-Aldrich, St. Louis, MO) following the manufacturer's instructions. Quality of isolated genomic DNA was assessed by measuring its 260/280 nm absorbance ratio using a spectrophotometer (GeneQuat, Pharmacia, LKB Biochrom, England) and by running DNA samples through 1.5% agarose gel in 0.5· TBE (tris borate ethylenediaminetetraacetic acid) buffer. Gels were stained with ethidium bromide (EtBr), then visualized on electronic UV transilluminator (ULTRA-LŪM, Inc., Claremont, CA) and digitally photographed with Canon Powershot G6 camera (Canon USA, Inc.). Only DNA samples with absorbance ratio of > 1.5 and an intact high molecular weight band on the gel were used in RAPD analysis. All DNA samples were diluted to 20 ng/µl with milliQ waterand stored at -20°C until RAPD

Table 1. Population, ecotype, sample size, and population code of populations used in this study.

Population	Ecotype	Sample size	Population code
WV populations			
POP_1	Cultivated	16	WV-C_1
POP_2	"	16	WC-C_2
POP_3		20	WC-C_3
POP_4	"	10	WC-C_4
POP_5	"	10	WC-C_5
POP_6	"	15	WC-C_6
POP_7	"	25	WC-C_7
POP_8	"	20	WC-C_8
POP_9	"	25	WC-C_9
POP_10	"	40	WC-C_10
POP_11	Wild	14	WV-W_11
POP_12	"	18	WV-W_12
POP_13	"	10	WV-W_13
POP_14	"	16	WV-W_14
POP_15	"	15	WV-W_15
POP_16	"	18	WV-W_16
POP_17	"	20	WV-W_17
POP_18	"	12	WV-W_18
POP_19	"	9	WV-W_19
POP_20	"	10	WV-W_20
POP_21	"	25	WV-W_21
POP_22	"	25	WV-W_22
Non-WV populations			
Pennsylvania	Wild	16	PA-W_1
	Wild	20	PA-W_2
Wisconsin	cultivated	21	WI-C_1
	cultivated	22	NC-C_2

analysis.

Primer selection

Initially, 29 decamer primers were selected from a list of those that had been reported successful in RAPD analysis of *P. quinquefolius* in previously published studies. These primers included; UBC-98, UBC-203, UBC-297 (Bai et al., 1997); OPD-03, OPD-05, OPH-04, OPH-05, OPO-15, OPU-10, OPU-15 (Boehm et al., 1999); UBC-06, UBC-18, UBC-81, UBC-164, UBC-177, UBC-210, UBC-227, UBC-262, UBC-326, UBC-398, UBC-419, UBC-464, UBC-497 (Schluter and Punja, 2002), and OPAD-01, OPAD-02, OPAD-11, OPAD-15, OPN-02 , OPN-19 (Lim, 2004). All primers were prescreened for polymorphism and reproducibility using a representative sample from all 26 populations. Eight of the 29 primers exhibited high polymorphism and reproducibility and were therefore selected for further use in RAPD analysis of all samples (Table 2). All primers were synthesized by Operon Biotechnologies (Huntsville, AL).

PCR amplification and gel electrophoresis

Amplification conditions were initially optimized by varying the amount of DNA template and primer concentrations. Upon establishing optimal PCR conditions that yielded discernible and reproducible bands, all samples were amplified twice with duplicate amplifications run on separate days. A HotStarTaq® Master Mix Kit (Qiagen®, Germantown, MD) was used for PCR with each 25 µl mixture containing: 12.5 µl Hotstart mix, 11 µl of RNase free H_2O, 0.5 µl of primer (0.2 µM), and 1 µl of DNA template (~20 ng). Amplifications were done on a single thermo cycler (GeneAmp® PCR System 9700, Applied Biosystems, Carlsbad, CA) with PCR thermocycling based on Schluter and Punja (2002) protocol with some modifications. At the initial cycle, the reaction mixture was heated at 94°C for 15 min to activate the HotStarTaq DNA polymerase, as recommended by the manufacturer, then 10 min at 36°C, and 2 min at 72°C. Subsequent 46 cycles included denaturation at 94°C for 30 s, annealing at 36°C for 1 min, and elongation at 72°C for 1 min. In the final cycle, the reaction was extended for 10 min at 72°C. Ten micro liters of amplified products were loaded into 1.5% agarose gels alongside 1 kb DNA ladder (Promega, Madison, WI) and electrophoresed in 0.5· TBE buffer at 105 V for 40 min. Gels were stained with EtBr, visualized with electronic UV transilluminator and digitally photographed.

Data analysis

Amplification products were scored manually and each band in

Table 2. Primer code, nucleotide sequence, total number of bands, number of polymorphic bands, and size ranges of polymorphic bands generated by each primer used for RAPD analysis of *P. quinquefolius* populations.

Primer	Sequence (5'→ 3')	No. of bands	No. of polymorphic bands	Size ranges(bp) of polymorphic bands
OPD-05	TGAGCGGACA	21	15	300-2000
OPH-04	GGAAGTCGCC	10	9	250-2500
OPH-05	AGTCGTCCCC	8	8	250-2500
OPO-15	TGGCGTCCTT	14	12	300-2000
OPAD-11	CAATCGGGTC	13	12	300-2500
UBC-81	GAGCACGGGG	14	12	250-3000
UBC-98	ATCCTGCCAG	10	8	250-2500
UBC-164	CCAAGATGCT	8	8	250-2000

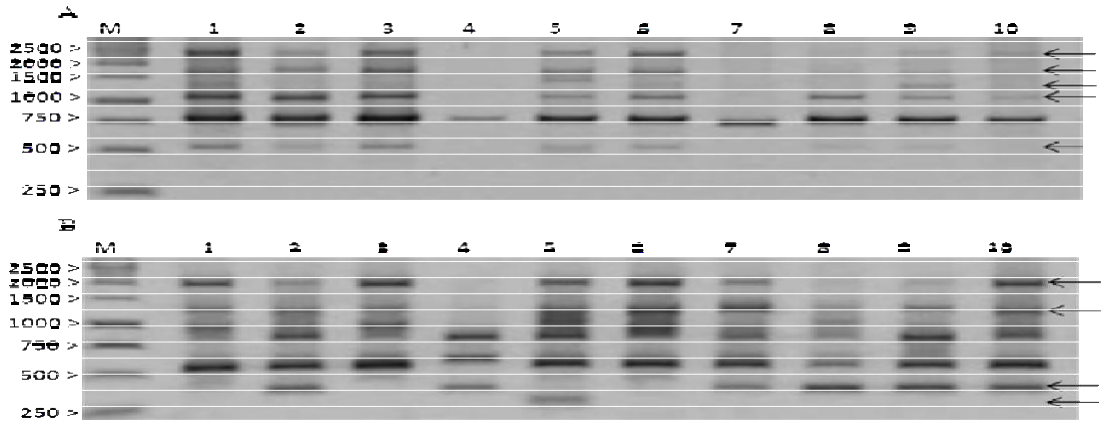

Figure 2. DNA fragments generated by primers OPAD-11(A), and OPO-15 (B) in samples 1-10 of POP_12. M = 1kb DNA ladder, arrows indicate polymorphic bands.

RAPD profile was treated as an independent character (locus) with two states (alleles) scored as either presence (1) or absence (0) of a band at a given size location on the gel picture for each primer. Monomorphic bands across all samples and bands below 250 bp or above 3000 bp were omitted from subsequent statistical analyses. Bands outside of this range (250 to 3000 bp) are considered unreliable for RAPD analysis (Stewart and Porter, 1995). To estimate genetic diversity and population structure, various parameters were estimated using different software programs for population genetics analysis. POPGENE Ver. 1.31 software (Yeh et al., 1997) was used to calculate, percent polymorphic loci (*P*), Nei's (1973) gene diversity (*H*), and Shannon's diversity index defined as $I = -\sum p_i \log_2 p_i$, where p_i is the frequency of a given RAPD band (Lewontin, 1972). The advantage of Shannon's index is that it does not assume that populations are in Hardy-Weinberg equilibrium. Statistical significance of genetic diversity measures among different levels of comparisons was determined using Mann-Whitney test.

Hierarchical population genetic structure and fixation index (Φ_{ST}) values were determined by analysis of molecular variance (AMOVA) using ARLEQUIN Ver. 3.11 software (Excoffier et al., 2005) with 1000 permutations. AMOVA uses phenotypic distances to describe how the RADP variance is partitioned among and within populations, and tests for the significance against the null hypothesis of no population structure (Excoffier et al., 1992; Stewart and Excoffier, 1996). AMOVA was based on Euclidean squared distance matrix which was constructed using AMOVA-PREP software (Miller, 1997).

Genetic relationships of different population groups was determined based on Nei's 1978 genetic distance measure using TFPGA software (Miller, 1998). A phenogram was generated using unweighted pair group method with arithmetic averages (UPGMA) and 1000 bootstraps to measure robustness of different clusters in in the phenogram.

RESULTS

RADP polymorphism

The eight primers used in RAPD analysis of 26 populations (468 samples) generated a total of 98 distinct loci (average 12.25 loci per primer) of which 84 were polymorphic (85.71%), an average of 10.5 polymorphic loci per primer (Table 2). The 84 polymorphic bands were clear, unambiguously scorable, and were between the range of 250 and 3000 bp. Examples of polymorphic bands generated by different primers are shown in Figure 2.

Genetic diversity and structure in all populations

For the 26 populations, mean *P, H,* and *I* values were

Table 3. Summary of within-population genetic diversity.

Group, region, population	n	*P*	*H*	*I*
WV-group				
WVCR1				
WV-C_1	16	36.90	0.1589	0.2275
WC-C_2	16	27.38	0.0829	0.1286
WC-C_3	20	28.57	0.0998	0.1504
WC-C_4	10	36.90	0.1287	0.1925
WC-C_5	10	28.57	0.1066	0.1568
WC-C_6	15	16.67	0.0470	0.0748
WC-C_7	25	34.52	0.1296	0.1916
WVCR2				
WC-C_8	20	64.29	0.2941	0.3691
WC-C_9	25	63.81	0.2508	0.3077
WC-C_10	40	53.71	0.2329	0.2960
WVWR1				
WV-W_11	14	34.52	0.1240	0.1839
WV-W_12	18	39.29	0.1270	0.1914
WV-W_13	10	32.14	0.1177	0.1742
WV-W_14	16	20.24	0.0687	0.1032
WV-W_15	15	35.71	0.1312	0.1932
WV-W_16	18	30.95	0.0923	0.1417
WV-W_17	20	48.81	0.1675	0.2506
WV-W_18	12	25.00	0.1090	0.1560
WVWR2				
WV-W_19	9	27.38	0.1184	0.1697
WV-W_20	10	32.14	0.1130	0.1679
WV-W_21	25	15.48	0.0570	0.0655
WV-W_22	25	38.10	0.1193	0.1820
Non-WV group				
WI-C_1	21	52.58	0.1700	0.2577
WI-C_2	22	52.38	0.1675	0.2525
PA-W_1	16	70.24	0.2028	0.3135
PA-W_2	20	76.19	0.2060	0.3226
Cultivated (12 populations)	20	41.36	0.1482	0.2171
Wild (14 populations)	16	37.59	0.1253	0.1883
All (26 populations)	18	39.33	0.1359	0.2016

n = sample size; *P* = percent polymorphic loci; *H* = gene diversity (Nei, 1973); *I* = Shannon's index (Lewontin, 1972). Group = WV or non-WV; regions: WVCR1 = low cultivation intensity, WVCR2 = high cultivation intensity, WVWR1 = low harvest pressure, WVWR2 = high harvest pressure.

39.33%, 0.1359, and 0.2016 respectively. Among wild populations, mean *P, H,* and *I* values were 37.59%, 0.1253, and 0.1883 respectively and lowest genetic variability was in population WV-W_21 (*P* = 15.48%, *H* = 0.0570, *I* = 0.0655) whereas population PA-W_2 had the highest genetic variability (*P* = 76.19%, *H* = 0.2060, *I* = 0.3226). Among cultivated populations, mean *P, H,* and *I* values were 41.36%, 0.1482, and 0.2171 respectively and lowest genetic variability was in population WV-C_6 (*P* = 16.67%, *H* = 0.0470, *I* = 0.0748) whereas population WV-C_8 had the highest genetic variability (*P* = 64.29%, *H* = 0.2941, *I* = 0.3691) (Table 3).

Table 4. Summary of mean genetic diversity of populations in groups, ecotypes and regions.

Group, ecotype, region	n	*P*	*H*	*I*
WV (22 populations)	389	35.05	0.1267	0.1861
WV cultivated (10 populations)	197	39.13	0.1441	0.2095
WVCR1 (7 populations)	112	29.93	0.0948	0.1603
WVCR2 (3 populations)	85	60.60	0.2593	0.3243
WV wild (12 populations)	192	31.65	0.1121	0.1666
WVWR1 (8 populations)	123	33.33	0.1172	0.1743
WVWR2 (4 populations)	69	28.27	0.1019	0.1513
Non-WV (4 populations)	79	62.85	0.1866	0.2870
Cultivated (2 population)	43	52.48	0.1688	0.2551
wild (2 populations)	36	73.21	0.2044	0.3181

n = sample size; *P* = percent polymorphic loci; *H* = gene diversity (Nei, 1973); *I* = Shannon's index (Lewontin, 1972). Group = WV or non-WV; ecotype = wild or cultivated; regions: WVCR1 = low cultivation intensity region, WVCR2 = high cultivation intensity region, WVWR1 = low harvest pressure region, WVWR2 = high harvest pressure region.

Overall, cultivated populations had higher genetic diversity than wild populations, however, there were no significant differences ($P > 0.05$, Mann-Whitney test) in all genetic diversity indices. The fixation index ΦST analogous to FST (Wright, 1951) obtained by AMOVA using pairwise distances among populations was equal to 0.473 (range 0.082 to 0.678), meaning that 47.3% of total variation was attributed to among population differentiation and the rest (52.7%) of variation to individuals within populations and all values were significantly different ($P < 0.001$) from zero (no differentiation).

Comparison of genetic variations between WV and non-WV populations (groups)

Overall, non-WV populations had significantly higher ($P < 0.05$, Mann-Whitney test) mean genetic variations (P = 62.85%, H = 0.1866, I = 0.2870) than WV populations (P = 35.05%, H = 0.1267, I = 0.1861) (Tables 4 and 5, analysis 1). There was significant differentiation among and within populations in the two groups ($P < 0.001$, AMOVA) (Table 6, analyses 1 and 2). For WV group, among population differentiation accounted for 48.48% of the total variation and the rest (51.52%) was attributed to individual differentiations within populations (Table 6, analysis 1). For non-WV group, 25.33% of the total variation was attributed to among population diffrentiation and 74.67% was due to individual differentiations within populations (Table 6, analysis 2). When total population differentiation was partitioned to a three level hierarchy to account for among groups (WV and non-WV) differentiation, highest genetic differentiation was attributed to individuals within populations (46.50%), among population differentiation accounted for 36.84%, and variation between groups accounted for 16.66% of the total variation (Table 6, analysis 3). UPGMA phenogram based on Nei's (1978) unbiased genetic distances between WV and non-WV groups revealed two well defined clusters separating WV and non-WV populations. Non-WV group formed two distinct sub clusters, one having Pennsylvania (PA) populations and the other having Wisconsin (WI) populations together. These clusters were supported by high bootstrap values suggesting a strong genetic differentiation between groups (Figure 3).

Genetic diversity within and among wild and cultivated populations (ecotypes) in WV

In cultivated ecotype, lowest mean genetic variations was in population WV-C_6 (P = 16.67%, H = 0.0470, I = 0.0748) whereas population WV-C_8 had the highest genetic variability (P = 64.29%, H = 0.2941, I = 0.3691). For wild ecotype, lowest genetic variability was in population WV-W_21 (P = 15.48%, H = 0.0570, I = 0.0655) and population WV-W_17 had the highest genetic variability (P = 48.81%, H = 0.1675, I = 0.2506) (Table 3). Overall, cultivated ecotype exhibited higher mean genetic diversity values (P = 39.13%, H = 0.1441, I = 0.2095) than wild ecotypes (P = 31.65%, H = 0.1121, I = 0.1666) (Table 4), however, differences in these diversity values were not statistically significant (Table 5, analysis 2).

AMOVA revealed significant ($P < 0.001$) molecular variations within ecotypes, with cultivated populations having lower among population differentiation than wild populations (Table 6, analyses 4 and 5). However, when variation was partitioned to account for variation between ecotype, a significant ($P < 0.001$) proportion of variation was attributable to among population differentiation (49.10%) and to individual differentiation within populations (51.72%) but not between ecotypes (P = 0.070). Variation between ecotypes was very small (close to zero), so a negative value was obtained (Table 6, Analysis 6).

Table 5. Mann-Whitney test for differences in mean genetic diversity indices among populations of *P. quinquefolius* in various groups, ecotypes, and regions comparisons.

Analysis	n	*P*	*H*	*I*
Analysis 1: WV vs. non-WV populations (groups)				
WV (22 populations)	18	35.05	0.1267	0.1861
non WV (4 populations)	20	62.85	0.1866	0.2866
Significance		*	*	*
Analysis 2: WV cultivated vs. WV wild populations (ecotypes)				
Cult. (10 populations)	20	39.13	0.1441	0.2095
Wild (12 populations)	16	31.65	0.1121	0.1666
Significance		NS	NS	NS
Analysis 3: WV cultivated populations from low vs. high cultivation intensity regions				
WVCR1 (7 populations)	16	29.93	0.0948	0.1609
WVCR2 (3 populations)	28	60.60	0.2593	0.3243
Significance		*	*	*
Analysis 4: WV wild populations from low vs. high harvest regions				
WVWR1 (8 populations)	15	33.33	0.1172	0.1743
WVWR2 (4 populations)	17	28.27	0.1019	0.1513
Significance		NS	NS	NS

n = average sample size; *P* = percent polymorphic loci; *H* = gene diversity (Nei, 1973); *I* = Shannon's index (Lewontin, 1972). * Significant; NS = not significant at $P < 0.05$.

Genetic diversity of populations in low (WVCR1) versus high (WVCR2) cultivation intensity regions in WV

For populations in WVCR1, *P* values ranged from 16.67 to 36.90%, *H* ranged from 0.0470 to 0.1589, and *I* ranged from 0.0748 to 0.2275 for WV-C_6 and WV-C_1 populations respectively (Table 3). Whereas, for populations in WVCR2, *P* ranged from 53.71 to 64.29%, *H* ranged from 0.2329 to 0.2941, and *I* ranged from 0.2960 to 0.3691 for WV-C_10 and WV-C_8 populations respectively (Table 3). Mean estimates of genetic diversity of populations in WVCR2 were higher (*P* = 60.60%, *H* = 0.2593, *I* = 0.3243) than those of populations in WVCR1 (*P* = 29.93%, *H* = 0.0948, *I* = 0.1603) (Table 4) and were significantly different ($P < 0.05$, Mann-Whitney test) (Table 5, analysis 3).

When genetic differentiation was partitioned to account for between regions variation using AMOVA, it was revealed that a higher proportion of differentiation (53.68%) was attributed to individuals in populations within regions, followed by among populations within regions (42.11%) and differentiation between regions was the lowest (4.20%) but significant (Table 6, analysis 7).

Genetic diversity of populations in low (WVWR1) versus high (WVWR2) harvest pressure regions in WV

For populations in WVWR1, *P* ranged from 20.24 to 48.81%, *H* ranged from 0.0687 to 0.1675, and *I* ranged from 0.1032 to 0.2506 for WV-W_14 and WV-W_17 populations respectively (Table 3). Whereas for populations in WVWR2, *P* ranged from 15.48 to 38.10%, *H* ranged from 0.0570 to 0.1193, and *I* ranged from 0.0655 to 0.1820 for WV-W_21 and WV-W_22 populations, respectively (Table 3). Mean estimates of genetic variation within populations in WVWR1 region were higher (*P* = 33.33%, *H* = 0.1172, and *I* = 0.1743) compared to populations in WVWR2 region (*P* = 28.27%, *H* = 0.1019, and *I* = 0.1513) (Table 4) but were not significantly different ($P > 0.05$, Mann-Whitney test) (Table 5, analysis 4).

Furthermore, AMOVA revealed significant ($P < 0.001$) genetic differentiation among populations within regions and individuals in populations within regions, however, variation between regions was not significant (*P* = 0.791) and resulted in a negative value (Table 6, analysis 8).

DISCUSSION

Genetic diversity and structure of *P. quinquefolius*

Using RAPD markers, this study evaluated genetic variation and structure among 26 populations of *P. quinquefolius*. Mean values of genetic diversity indices showed that cultivated populations had higher levels of diversity compared to wild populations. This observation concurs with a report by Grubbs and Case (2004) for 44

Table 6. Summary of analysis of molecular variance (AMOVA) for groups, ecotypes and regions of *P. quinquefolius* using RAPD markers. Significance tests based on 1000 random permutations.

Source of variation	df	SS	variance components	% of total variance	*P-value*
Analysis 1: WV group					
Among population	21	1938.86	4.96	48.48	< 0.001
Within populations	367	1935.28	5.27	51.52	< 0.001
Analysis 2: Non-WV group					
Among population	3	209.85	3.09	25.33	< 0.001
Within populations	75	684.03	9.12	74.67	< 0.001
Analysis 3: WV vs. non-WV group					
Between groups	1	379.07	2.12	16.66	< 0.001
Among population	24	2148.70	4.69	36.84	< 0.001
Within populations	442	619.31	5.93	46.50	< 0.001
Analysis 4: WV cult. populations					
Among population	9	862.60	4.67	45.30	< 0.001
Within populations	187	1055.33	5.64	54.70	< 0.001
Analysis 5: WV wild populations					
Among population	11	984.85	5.34	52.22	< 0.001
Within populations	180	879.95	4.89	47.78	< 0.001
Analysis 6: WV population ecotypes					
Between ecotypes	1	91.41	-0.08	-0.82	0.070
Among population	20	1847.45	5.01	49.10	< 0.001
Within populations	367	1935.28	5.27	51.72	< 0.001
Analysis 7: WV cult. regions					
Between regions	1	159.56	0.44	4.20	< 0.001
Among population	8	703.04	4.43	42.11	< 0.001
Within populations	187	1055.33	5.64	53.68	< 0.001
Analysis 8: WV wild harvest regions					
Between regions	1	86.93	-0.25	-2.47	0.791
Among population	10	897.92	5.47	54.10	< 0.001
Within populations	180	879.95	4.89	48.37	< 0.001

df = degrees of freedom; SS = sum of squares.

populations of *P. quinquefolius* sampled from different regions across ginseng's native range. On average, genetic diversity values obtained in our study were higher than those reported for 21 populations (Cruse-Sanders and Hamrick, 2004) and 44 populations (Grubbs and Case, 2004) of *P. quinquefolius* using allozymes. This can be attributed to the vast numbers of loci that can be examined with RAPD markers compared to allozymes, therefore, they are able to reveal high degree of DNA level variation. Allozymes are only limited to protein coding regions and may not be representative of genome wide diversity (Stewart and Excoffier, 1996). However it is prudent to note that differences in these values could also be a result of sampling from different populations, even though this study and the other two studies sampled plants from the same geographic area in WV. Genetic differentiations among and within populations were highly significant. According to Wright (1978), F_{ST} values above 0.25 indicate substantial genetic differentiation. In this study mean fixation index Φ_{ST} value was 0.473, implying that the populations we evaluated were highly differentiated genetically. This observation is consistent to that reported previously (F_{ST} = 0.547) for 18 wild populations of *P. quinquefolius* (Mooney, 2007).

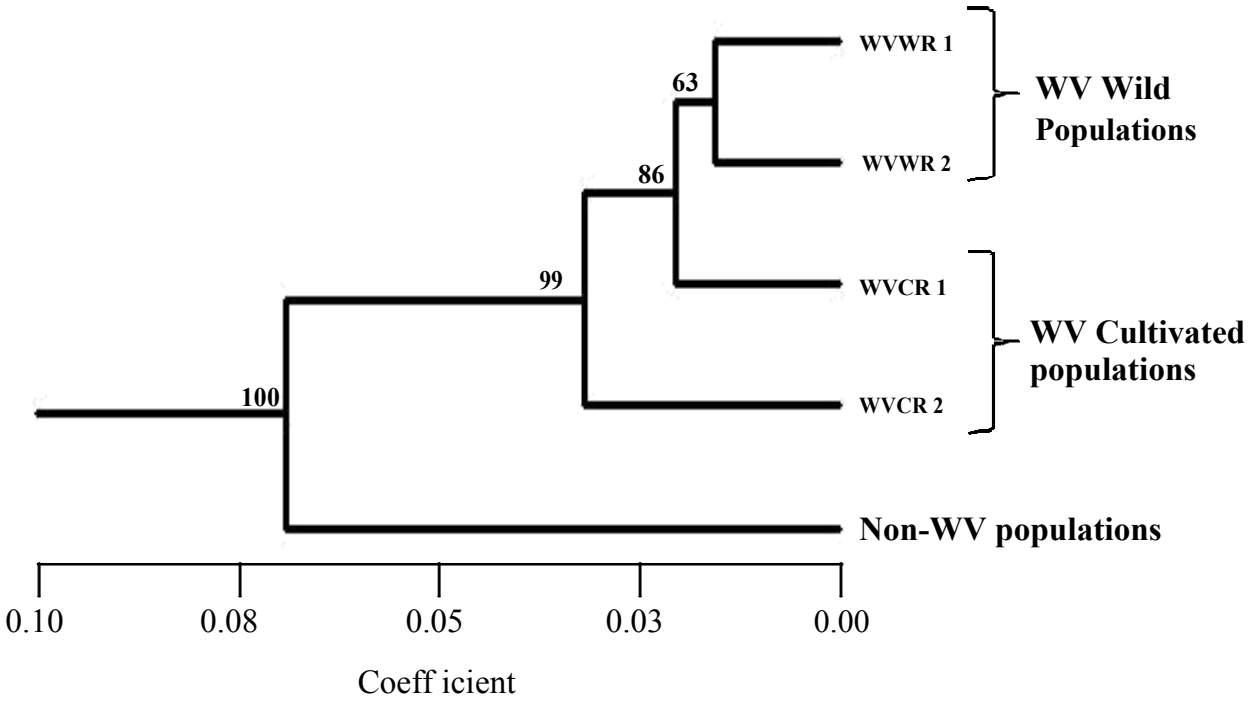

Figure 3. Relationship tree of population groups of *P. quinquefolius* revealed by UPGMA cluster analysis using Nei's 1978 genetic distance measure based on RAPD data. Only bootstraps values greater than 60% are indicated. WVCR1 = low cultivation intensity region; WVCR2 = high cultivation intensity region; WVWR1 = low harvest pressure region; and WVWR2 = high harvest pressure region.

Comparison of genetic diversity between WV and non-WV populations

Overall, we observed lower mean genetic diversity indices for WV populations (*P* = 35.05, *H* = 0.1267, *I* = 0.1861) compared to non-WV populations (*P* = 62.85, *H* = 0.1866, *I* = 0.2866). This could be attributed to harvest pressure associated with the long history of wild ginseng harvesting in WV. American ginseng populations facing harvest pressure have been reported to have reduced genetic diversity when compared to populations in protected areas (low harvest pressure) (Cruse-Sanders and Hamrick, 2004). Indeed, when wild populations in WV were compared directly to wild populations from PA which have not been harvested for several years (protected), measures of genetic diversity in WV wild populations were approximately half that of PA wild populations. The partitioning of genetic structure of WV populations was almost equal for among and within population differentiation (Table 6, analysis 1); however, for non-WV populations, within population differentiation was almost three times that of among population differentiation (Table 6, analysis 2). An almost equal genetic differentiation among and within populations from WV could be explained by lack of significant genetic differences observed among wild and cultivated populations particularly from low cultivation intensity region.

Genetic diversity and structure of population ecotypes in WV

With regard to ecotypes (wild versus cultivated), there were no significant differences in all diversity indices between ecotypes (Table 5, analysis 2). We attributed this lack of significant differences to a high level of genetic similarity observed between wild populations and cultivated populations in low cultivation intensity region (WVCR1). This high level of genetic similarity could be attributed to growers in this region recruiting into their farms seeds collected from wild populations. Although it is highly recommended that ginseng diggers plant seeds at locations where they collect wild roots in order to sustain future ginseng populations, it is not uncommon for diggers to take along with them some wild seeds to start ginseng crops in their own property. After all, these seeds have no associated costs (as opposed to expensive commercial seeds) and establishing ginseng populations on their personal property ensures easy accessibility and most importantly security of their crop from other diggers.

From AMOVA, it was revealed that most of the genetic variation in cultivated ecotype was attributed to individuals within populations, whereas in wild ecotype greater differentiation was partitioned to differentiation among populations. This observation concurs with other reports

on *P. quinquefolius* (Schluter and Punja, 2002; Mooney, 2007). Cultivated populations typically have a high degree of gene flow among population mainly due to movement of seeds between growers as opposed to wild populations which have mostly become isolated because of fragmented habitats therefore have limited gene flow among populations.

Effects of cultivation intensity and harvest pressure on genetic diversity

Significantly higher genetic diversity indices were observed in populations from high cultivation intensity region than in populations from low cultivation intensity regions (Table 5, analysis 3). This could be explained by the higher gene flow in high cultivation intensity region which is a consequence of growers recruiting seeds from a broader source in order to meet their cultivation scale. In contrast, lower gene flow in lower cultivation intensity region could be attributable to small scale growers obtaining seeds from a narrow source or even collecting from a single source in the wild to start their ginseng crop. Surprisingly, harvest pressure did not have a signifi-cant effect on genetic diversity of WV wild populations evaluated in this study (Table 5, analysis 4). However, mean genetic diversity measures were slightly higher in populations from low harvest pressure region (WVWR1) than populations from a high harvest pressure region (WVWR2). This trend is in agreement with both field and simulation studies of *P. quinquefolius* (Cruse-Sanders and Hamrick, 2004; Cruse-Sanders et al., 2005). Lack of significant effect of harvest pressure on genetic diversity of wild populations observed in this study could be a consequence of small sample size especially from high harvest pressure region (only 4 populations, 69 samples were evaluated).

Conservation implications

Genetic variation is a valuable resource from which populations derive short-term adaptation to environmental stochasticities and long-term evolutionary changes (Ellstrand and Elam, 1993). This study indicates that there is a considerable amount of genetic diversity at population level for *P. quinquefolius* growing in WV. Despite not finding significant effects of harvest pressure on genetic diversity in populations we evaluated, it would be unwise to rule it out as driving force behind reduced genetic diversity of wild populations in WV. Further research involving extensive field sampling is needed to assess the effect of harvest pressure on wild *P. quinquefolius* populations growing in WV. For now, it is recommended that good stewardship practices for wild populations continue to be strictly adhered to by ginseng diggers in order to prevent further reduction of genetic diversity in wild populations as their populations decline due to harvesting. Such good stewardship practices include harvesting only mature plants and replanting seeds upon harvesting of roots.

There were no significant differences in genetic diversity between wild and cultivated ginseng from small farms in low cultivation intensity regions. This implies that most of the seeds used by these small farms to cultivate ginseng are likely collected from wild populations within this region. Whereas this shows that some growers are using 'local' seeds to cultivate ginseng thus conserving 'local' gene pool, the potential downside to this is that it will eventually lead to non distinguishable wild and cultivated population genotypes in WV. Data from this study also showed that increase in cultivation intensity resulted in increase in the genetic diversity of American ginseng populations. While this is beneficial as it expands the gene pool, the potential downside to this is that the wide sourcing of seeds beyond the 'local' gene pool to meet cultivation need for large scale growers may end up introgressing 'non-local' genotypes into 'local' gene pool thereby eroding regional specific genotypes. To minimize this, we recommend growers to obtain seeds locally but from different sources within a region to help conserve regional genotypes.

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