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RAPD Analysis of Genome Variability of Planted Ginseng, *Panax ginseng*

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Abstract—Genome variability of 23 ginseng plants (*Panax ginseng*) grown in culture in Primorskii Krai was studied by RAPD method. Eleven arbitrary chosen primers were used to analyze 138 loci of DNA samples, 17 of which appeared to be polymorphic. The OPD-11-1000 fragment was found to be a RAPD marker allowing plants to be differentiated according to their morphotype. Using five primers, it was demonstrated that the genetic polymorphism of the cultivated plants is lower than that in nature (7.6% and 10.6%, respectively). Dendrograms of genetic relatedness are in accord with genetic differences between individuals of planted *P. ginseng* belonging to different morphotypes, and demonstrate close relatedness of one of the morphotypes to wild plants. This morphotype could be recommended for reintroduction into natural habitats.

Key words: true ginseng, *Panax ginseng*, RAPD analysis, genetic variability

INTRODUCTION

In the past, the area occupied by ginseng (*Panax ginseng* C.A. Meyer), a plant famous for its medicinal properties, covered vast expanses in the woods of Eastern Asia (Korea, China, Russian Far East), but it decreased dramatically during the past century [1, 2]. Nowadays it is unlikely to find wild ginseng in Korea or China, and the species is represented by three relatively small and nearly exhausted natural maritime populations [3] and cultivated forms.

Industrial cultivation of ginseng in the Primorskii Krai (Primorie) was launched by a Far-East pioneer M.I. Yankovsky [2]. Nowadays the plant is grown at the "Ginseng" state farm and by a few individuals.

A decrease of the area of normal abundance of ginseng and uncontrolled use of its natural resources resulted in substantial depletion of genetic variability and, consequently, depletion of the genetic potential of the natural population of this rare endemic species. For biodiversity protection, a special regional complex program of reintroduction of the maritime ginseng population was elaborated [4].

Conservation of rare plants can be achieved only if the highest possible genetic potential of a species is retained. Species and populations are usually treated as functional units of evolution, and the genotype structure of these taxa is considered as a stable, evolutionarily established trait. Any disturbance thereof may cause irreversible consequences. It is well known that genetic variability of natural populations is usually higher than that of cultivated ones, because the latter stem from a limited number of plants ("founder effect"), are under the pressure of selection, and utilize only a part of the genetic potential of the species [5–7]. One may speculate, however, that cultivated plants may utilize a certain part of the genetic potential of extinct natural populations. This is quite possible for ginseng because some plantations were brought into being before the sharp decrease of the ginseng area.

To shed light on the present-day genetic status and on the changes in the degree of variability of genomes of cultivated plants, a comparative study of genetic

Table 1. Primers employed in the study of genetic variability of cultivated ginseng

Primer	Nucleotide sequence (5'-3')	Number of registered fragments	Number of polymorphic fragments
OPA-20	GTTGCGATCC	14	5
OPB-12	CCTTGACGCA	13	2
OPC-08	TGGACCGGTG	8	1
OPC-15	GACGGATCAG	12	2
OPD-02	GGACCCAACC	18	0
OPD-07	TTGGCACGGG	19	3
OPD-11	AGCGCCATTG	9	1
OPD-13	GGGGTGACGA	12	0
OPD-20	ACCCGGTCAC	11	1
OPE-11	GAGTCTCAGG	11	2
OPF-05	CCGAATTCCC	11	0
Total:		138	17

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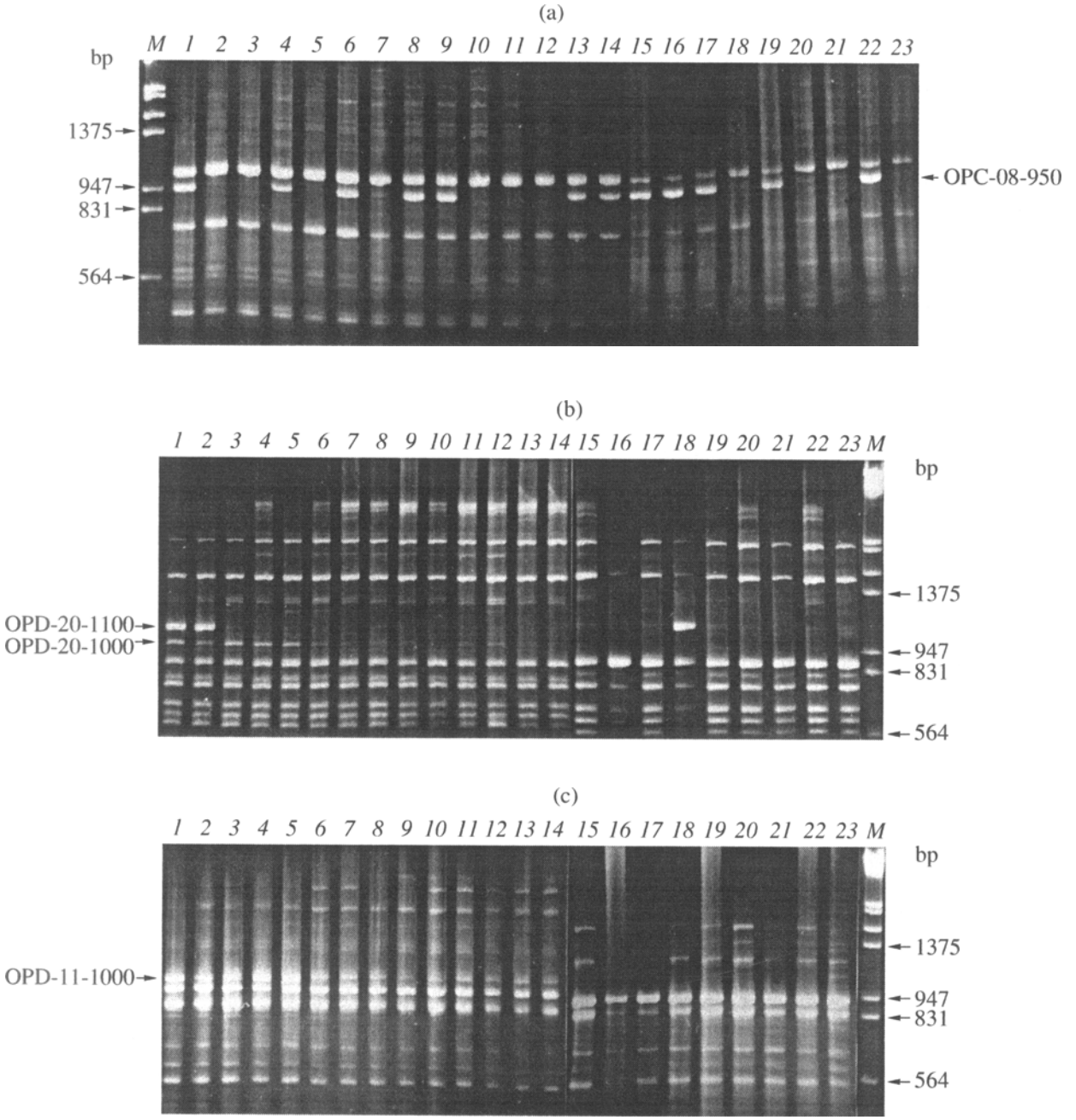

Fig. 1. Ginseng DNA amplification products. (a) OPC-08 primer; M, marker fragments obtained by λ DNA digestion with *Eco*RI and *Hin*dIII; 1–14: cultivated plants of morphotype I; 15–23: cultivated plants of morphotype II. (b) OPD-20 primer, (c) OPD-11 primer; lanes as in (a).

variability of wild and cultivated populations are desirable [7].

RAPD analysis of wild populations in our laboratory has demonstrated that natural populations of ginseng have a low intrapopulation variability [8, 9].

In this paper we present RAPD data on the genome DNA variability of cultivated ginseng grown at Primorskii Krai.

EXPERIMENTAL

Ginseng planted in Dalnegorskii region came either from amateur collections or were gathered in nature near Chuguevo (nos. 24–38), Spassk (nos. 39–49), or Hasan (nos. 50–60). Taking into account the general habit, shape and color of leaves, the plants were subdivided into two morphotypes.

Morphotype I (nos. 1–14) included plants with a relatively short stem, shortened rhizomes, thickened

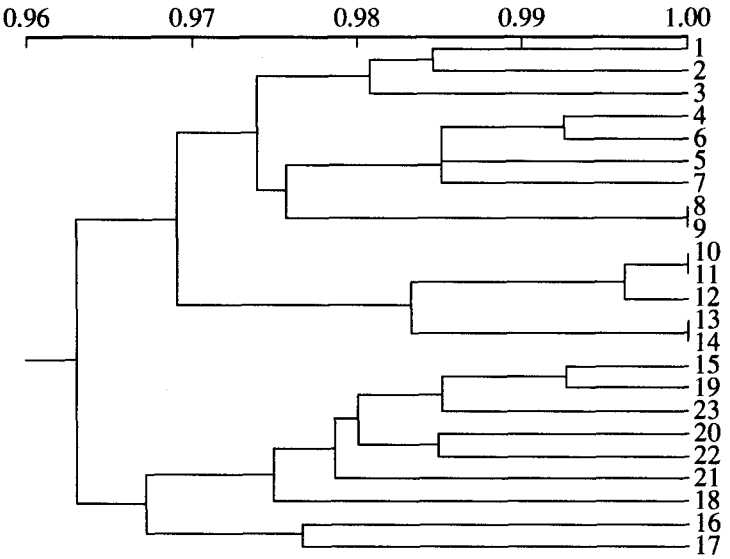

Fig. 2. Dendrogram of genetic relatedness of cultivated ginseng plants constructed basing on data obtained with 11 amplification primers. 1–14: plants of morphotype I; 15–23: plants of morphotype II.

main root, rather large and numerous (more than three) side roots, green leaves with a characteristic yellowish shade, rounded at the base of the leaf central lobe. After first autumn frosts, leaves became yellow.

Morphotype II (nos. 15–23) included plants with longer stems, rhizomes and roots. Side roots were well-developed, but their number was smaller and rarely exceeded three. Leaves were green or dark-green, with extended leaf blades. After first autumn frosts, leaves acquired an orange-reddish color.

DNA isolation and RAPD analysis were performed as described earlier [8, 10]. Amplification products were separated in 2% agarose gels with ethidium bromide. Only distinct and reproducible bands were taken into account in electrophoregram analysis.

UPGMA and NTSYS-ps methods were employed in statistical analysis. The similarity (D) of two samples was calculated as $D = 2N_{ab}/N_a + N_b$, where N_a and N_b is the number of amplified fragments of samples a and b, and N_{ab} is the number of fragments with coinciding electrophoretic mobility [11]. Basing on D matrices, dendrograms of genetic relatedness were constructed.

The percentage of polymorphism of the amplified DNA fragments (P, %) was calculated as the ratio of the number of polymorphic loci to the total number of loci, polymorphy criterion being P_{95}.

RESULTS AND DISCUSSION

Genetic variability of 23 ginseng plants was investigated by RAPD method using 11 decameric primers (Operon Technologies, USA) which had been selected for ginseng DNA PCR [8]. These primers allowed us to study the variability of 138 loci, but only 17 of them (12.3%) appeared to be polymorphic. Primers OPD-02, OPD-13, and OPF-05 did not reveal polymorphic loci and in all samples provided amplification of common, conservative DNA sequences. The maximum amount of polymorphic loci was revealed with OPA-20 (Table 1). In the present study of culti-

Table 2. Polymorphism and the number of alleles per locus in the analyzed populations of ginseng*

Number of samples	Cultivated plants			Wild plants			
	morphotype I (14)	morphotype II (9)	total group (23)	Hasan population (11)	Spassk population(11)	Chuguev population (15)	total group (37)
Polymorphism (%)	3.6	3.6	7.6	3.0	9.1	7.6	10.6
Number of alleles	1.05	1.05	1.08	1.03	1.09	1.08	1.11

* 66 RAPD loci revealed by five primers were analyzed.

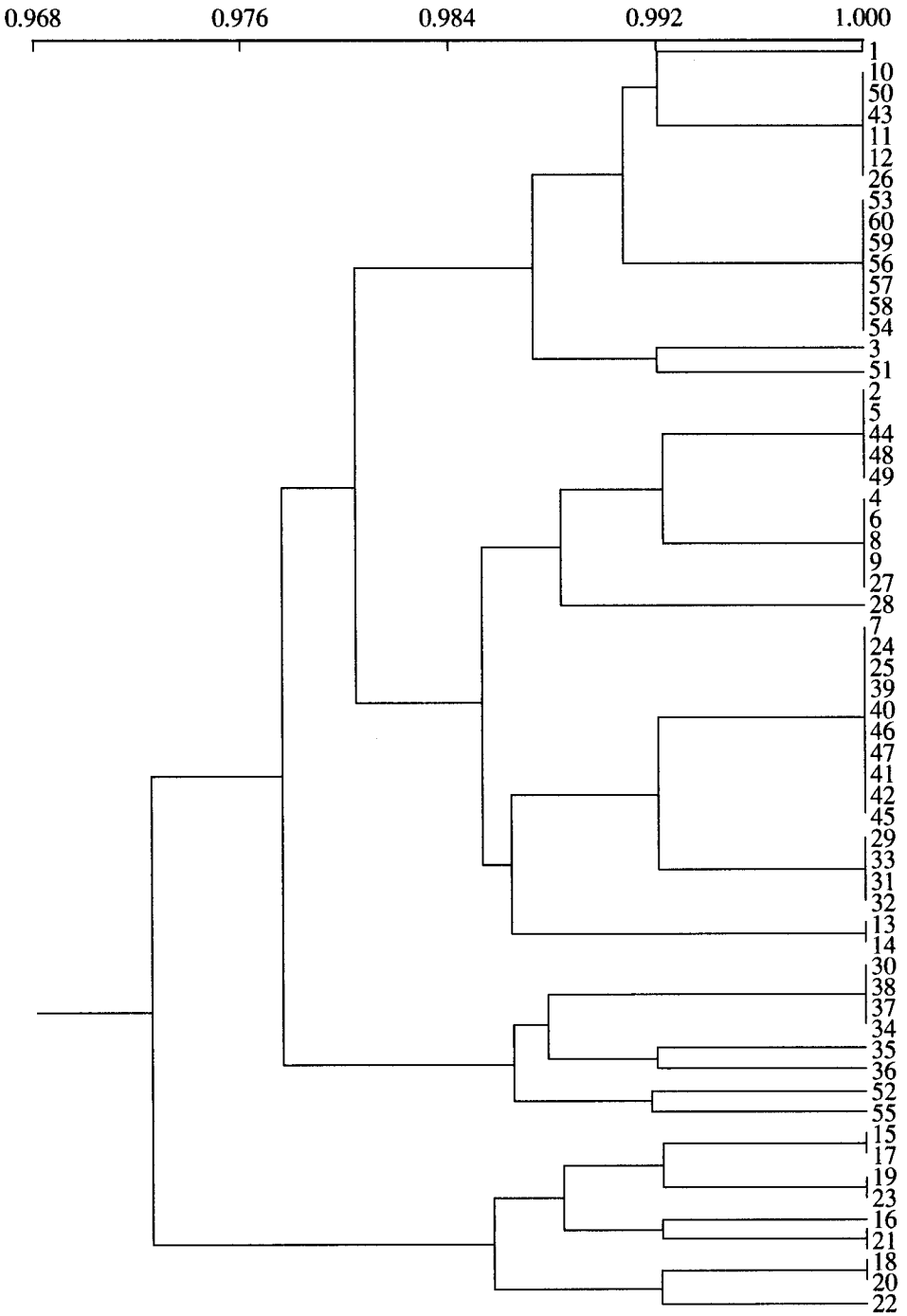

Fig. 3. Dendrogram of genetic relatedness between cultivated and wild ginseng plants constructed basing on the data obtained with five amplification primers. 1–14: cultivated plants of morphotype I; 15–23: cultivated plants of morphotype II; 24–38: wild plants from Chuguev region; 39–49: wild plants from Hasan region; 50–60: wild plants from Spassk region.

vated ginseng, OPC-08 and OPD-07, which had been used in the study of genetic variability of wild ginseng [8], revealed the same polymorphic fragments. The main polymorphic fragment, OPC-08-950, which had been found in two out of 37 wild plants, is present in 12 out of 23 cultivated ones (Fig. 1a). Noteworthily, differences in the RAPD patterns of both cultivated and wild plants are manifested not only as the presence/absence of a fragment, but also as different intensity of electrophoretically identical bands. Amplification products of cultivated plants DNA obtained with OPD-20 are presented in Fig. 1b. Alongside with the most intense polymorphic OPD-20-1100 amplicon (Fig. 1b, lanes *1*, *2*, *18*),

OPD-20-1000 fragment could be observed in patterns of many plants. Its intensity varies from very high (Fig. 1b, lanes *1–5*) to very low (lanes *6–9*, *11–14*, *19*), and it was undetectable in some samples (lanes *10*, *15–17*, *21–23*). In view of this fact, in the present study we did not take into account the OPD-20-1000 amplicon as a polymorphic one, supposing that variations in intensity may reflect a varying number of copies of repetitive DNA in the analyzed samples [12].

Analysis of amplification products of cultivated plant DNAs with primer OPD-11 revealed the OPD-11-1000 amplicon, which could be found only in morphotype I plants and is thus a specific marker of this group (Fig. 1c). In the RAPD patterns of some wild plant DNAs, this fragment may be occasionally present in a small quantity, and it could not be found regularly in repetitive experiments. Hence, it was not taken as a polymorphic one (data not shown). Other primers revealed only one or two polymorphic, poorly represented loci, thus demonstrating the low genetic variability of cultivated ginseng.

Basing on the results obtained with eleven primers, we calculated the Nei distances and constructed a dendrogram of genetic relatedness of the analyzed cultivated plants (Fig. 2). All the samples group into two clusters, each corresponding to plants of either morphotype studied. The genetic distance between plants within clusters is rather small (D = 0.0039–0.0327), and for some pairs it is zero. One may suppose that the morphological differences underlying separation of the plants into two morphotypes are genetically determined.

To compare the genetic variability of wild and cultivated plants, we employed RAPD analysis with five primers: OPC-08, OPD-02, OPD-07, OPD-11, and OPD-13 (Table 2). The genetic polymorphism of 37 wild plants collected in various districts of the Primorskii Krai was 10.6%, whereas in 23 cultivated plants it was 7.6%, which is in accord with the results of allozyme analysis [13]. Garden varieties of ginseng cultivated in Korea were characterized by different coefficients of genetic variability, but it was always smaller than in populations of wild mountain ginseng [14, 15].

The dendrogram illustrating genetic relatedness of wild and cultivated plants (Fig. 3) shows that cultivated plants form two clusters, just as in Fig. 2, corresponding to two morphotypes. All wild plants (nos. 24–60) belong to the morphotype I cluster of cultivated plants (nos. 1–14), grouping according to the habitat. Plants of the second morphotype stand apart, the reason for such a position being obscure. Plants of morphotype I display genetic relatedness to wild plants collected near Hasan, Spassk, and Chuguev. Basing on these observations, one may suppose that cultivated plants of the first morphotype stem from different localities of Primorskii Krai, and these districts may be appropriate for their reintroduction. In general, one may conclude that cultivated plants of the first morphotype are genetically closer to the wild ones and harbor a significant share of the natural genetic variability.

Summing up, it was demonstrated that plants cultivated in Dalnegorskii region have a relatively low level of genetic variability, their genetic polymorphism being lower than that of wild plants of maritime populations (7.6 and 10.6%, respectively). The morphological differences according to which the plants were subdivided into two groups seem to be genetically determined. A RAPD marker OPD-11-1000 was found for cultivated plants of the first morphotype. Plants of this morphotype probably originate from different regions of the natural area of ginseng, and may be employed for reintroduction of the species into its natural habitats alongside with wild plants. However, analysis of morphological and genetic traits of the progeny of cultivated plants should be done beforehand.

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