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# Genetic Variability of *Iris setosa*

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**Abstract**—Genetic variability of *Iris setosa* Pall. ex Link. was studied by the RAPD method. Plants from three different habitats were compared by 135 loci revealed with eight arbitrary primers. The three plant accessions all exhibited a high level of polymorphism, and each was characterized by different frequencies of polymorphic fragments, which probably reflected the geographic isolation of the analyzed populations. The average level of polymorphism detected was 35%.

*Key words*: *Iris setosa*, PCR-RAPD, genetic variability

## INTRODUCTION

*Iris setosa* Pall. ex Link. is one of ten species of the genus *Iris* of the Far Eastern flora. It belongs to the subgenus *Limniris*, subsection *Apogon*, which is considered as one of phylogenetically ancient groups of irises [1]. This mesophyte iris occupies far Northern habitats of Asia and North America. In Far East Russia, it grows in Primorski Krai (Maritime Territory) and Khabarovskii Krai, near Magadan, at Chukotka sea coast, and on Kuril Islands.

As this species is of widespread occurrence and grows in varying environment, it is highly polymorphic [2–4]. Basing on these observations, some authors have suggested to isolate several subspecies and varieties of *I. setosa* [2, 3, 5, 6]. In [7] Fedchenko differentiates two forms of *I. setosa*, i.e., *I. setosa* forma *alpina* Kom. and *I. setosa* forma *serotina* Kom. The chromosome numbers of this species are also variable: samples from Russia have $2n$ = 32–38 [8–10], while for plants in Japan $2n$ = 38 [11], or $2n$ = 52, 54, 56, 57 [5, 11, 12]. The isolated status of endemic varieties of *I. setosa* from Japan was demonstrated by cytological [12] and biochemical methods [5, 13]. Probably, isolation of these forms was a result of hybridization [12, 13]: it is well known that introgressive hybridization played a significant role in the origin of forms and species of irises [1, 14–16].

Primorski Krai is the Southern part of the *I. setosa* area, where it grows either separately, or as background plant at shorthear, herbs-meadows, at common pastures, sea shores, etc. [17], i.e., in very different ecological conditions. The genetic variability of *I. setosa* collected from such different environments is thus of interest.

Variability of seeds and fruits of this species has been studied earlier by comparison of natural and introduced populations of Primorski Krai. It appeared that plants growing in different environment demonstrate substantial variability [4].

In the present paper we compare plants collected at three remote and ecologically different localities so as to determine the intraspecies genetic variability of *I. setosa*. To evaluate simultaneously the variability of many loci, we employed RAPD analysis [18–22].

## EXPERIMENTAL

DNA was isolated from lyophilized leaves [23] of 24 *I. setosa* plants collected at various districts of Primorski Krai: at a meadow located in river Pavlovka valley (400 m above sea level) in the vicinity of settlement Nizhnie Luzhki, Chuguevskii district (Accession I), at a swampy meadow at Shkotovka river outfall (Accession II), and at a meadow at Victorovka river floodlands near Olga settlement (Accession III).

PCR was carried out in Minicycler (MJ Research Inc., USA) as described earlier [24]. Amplification products were separated by electrophoresis in 2% agarose gel with ethidium bromide, visualized in UV light, and photographed. To determine the size of amplicons, DNA of phage was digested with *Eco*RI/*Hin*dIII restriction endonucleases and the fragments obtained employed as markers. A complete amplification mixture devoid of DNA served as control.

In electrophoregram analysis, only distinct and reproducible bands were taken into account. The presence of a band was designated as “1”, and its absence as “0”. Differences in intensity of bands corresponding to amplicons of identical size were not taken into account. Bands with substantially lower intensity were also designated as “0”. Data obtained were sta-

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tistically treated as described in [23]. Basing on *D*-value matrices, dendrograms of similarity of plant DNA amplification products were constructed.

The percentage of polymorphism *P* of amplified DNA fragments was determined as the ratio of the number of polymorphic loci to the total number of loci, basing on polymorphy criterion of 95%.

To quantitate the level of genetic diversity, the Shannon's information measure $H_0$ was used as described in [25]. Estimates of diversity were calculated using the equation

$$H_0 = -\sum p_i \ln p_i,$$

where $p_i$ is the phenotypic frequencies in an individual accession. For the whole sample, values of

$$H_{sp} = -\sum p_k \ln p_k,$$

were calculated, where $p_k$ is the frequency of phenotypes in it. The proportion of intrapopulation diversity was determined as $H_{pop}/H_{sp}$, and the proportion of interpopulation diversity as $(H_{sp} - H_{pop})/H_{sp}$, where $H_{pop}$ is an average of $H_0$ for three accessions.

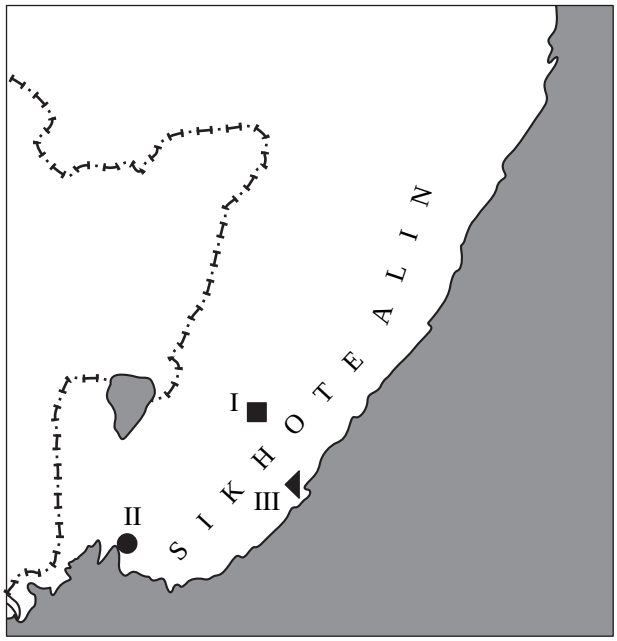


**Fig. 1.** Primorski Krai map. Localities of sample collection are designated.

## RESULTS AND DISCUSSION

To analyze intraspecies genetic variability of *I. setosa*, eight decanucleotide primers of random nucleotide sequence were employed (Table 1). The size of amplification products (DNA fragments) varied from 300 to 2000 bp. Depending on the primer used, the number of bands was different and varied from 13 to 23, allowing us to analyze the variability of 135 amplicons in the accessions of *I. setosa* (Table 1). Each primer gave a reproducible, primer-specific band pattern (RAPD spectrum).

The RAPD spectra obtained with primer OPB-18 are presented in Fig. 2. In this experiment, nine polymorphic fragments were detected. The frequencies of these amplicons in the analyzed accessions are different, and sometimes these differences are quite substantial. The intensity of bands is also variable, probably because the number of copies of the corresponding loci in the analyzed plant DNAs varied [22]. One might conclude that intraspecies variability is characteristic of *I. setosa*. As follows from Fig. 1, the RAPD spectra of all the plants of accession I, with a single exception, include the OPB-$18_{770}$ amplicon, which is absent from amplification spectra of accessions II and III. Hence, this amplicon could be considered as a specific RAPD fragment of plant DNA of accession I.

It is believed that each amplification product corresponds to a certain locus of the analyzed DNA [20, 26]. RAPD loci are represented by only two alleles, the marker one (the fragment is present) and the null one (the fragment is absent). The marker phenotype could correspond to two genotypes, making impossible determination of heterozygosity and related parameters of genetic variability of populations. Therefore, we attempted to estimate the genetic diversity of *I. setosa* populations and to separate it into intra- and interpopulation components by making use of the Shannon's information measure [25]. Data on polymorphism and phenotypic diversity for each primer and for all three accessions are presented in Table 2. On the average, polymorphism appeared to be 35%, which is in accord with the data obtained by allozyme analysis of several plant species [27, 28]. Accession III was the most heterogeneous ($P$ = 41%, $H_0$ = 3.5343), the values of polymorphism and of the Shannon's index appeared to be much lower for acces-

**Table 1.** Properties of primers

| Primer | Nucleotide sequence (5' ⟶ 3') | Number of registered loci | Number of polymorphic loci/polymorphism (*P*, %) |
|---|---|---|---|
| OPA-12 | TCGGCGATAG | 15 | 4/27 |
| OPB-18 | CCACAGCAGT | 20 | 9/45 |
| OPC-01 | TTCGAGCCAG | 16 | 7/44 |
| OPC-14 | TGCGTGCTTG | 17 | 10/59 |
| OPC-15 | GACGGATCAG | 15 | 7/47 |
| OPD-02 | GGACCCAACC | 16 | 9/56 |
| OPD-08 | GTGTGCCCCA | 13 | 6/46 |
| OPD-11 | AGCGCCATTG | 23 | 10/43 |
| Total | | 135 | 62/46 |

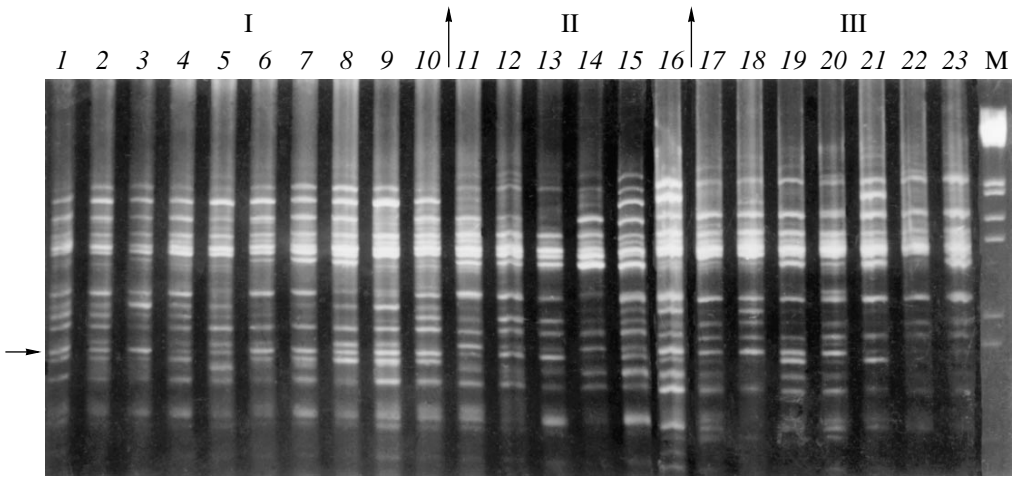


**Fig. 2.** RAPD patterns of *I. setosa* plants obtained with OPB-18 primer. Accession I: 1–10; Accession II: 11–16; Accession III: 17–23. M: *Eco*RI/*Hin*dIII restriction fragments of phage λ DNA. OPB-18 amplicon specific for accession I is indicated by arrow.

sion I ($P$ = 31%, $H_0$ = 2.5436), and accession II was characterized by intermediate values. Indices of intra- and interpopulation diversity vary depending on the primer. On the average, 22% of diversity is accounted for by the interpopulation variability, and 78% by the intrapopulation one.

Basing on these results, dendrograms of genetic relatedness of the analyzed populations of *I. setosa* were constructed (Fig. 3). Analyzed plants formed two clusters. The first one includes all the plants of accession I with the highest genetic relatedness recorded (0.97–0.92), which is probably determined by environmental factors. All other plants belong to the second cluster, which subdivides into two branches. The first branch includes all the plants of accession III (except plant 19), and the second one, all the plants from accession II (except plant 15). The genetic distance between the branches is low, and similarity between individual plants or pairs of plants varies from 0.93 to 0.89, allowing one to conclude that individual variability of plants in accessions II and III is higher than in accession I. The above-mentioned exceptions observed in clusters of accessions II and III could be explained by that the analyzed sets are geographically distant but not completely isolated populations, and one cannot exclude completely an exchange of genetic information between them.

Comparison of dendrograms constructed basing on the amplification data obtained with three, six, and eight primers allows a conclusion that two main clusters are formed even if only three primers are employed. In other words, analysis of the RAPD patterns enables grouping of samples and differentiating them according to populations.

High polymorphism of fruits and seeds of *I. setosa* collected at Shkotovka river floodlands was described

**Table 2.** Polymorphism and genetic diversity of *Iris setosa*

| Primer | Accession I | | Accession II | | Accession III | | $H_{sp}$ | $H_{pop}$ | $\frac{H_{pop}}{H_{sp}}$ | $\frac{H_{sp}-H_{pop}}{H_{sp}}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | $P$, % | $H_0$ | $P$, % | $H_0$ | $P$, % | $H_0$ | | | | |
| OPA-12 | 33 | 2.3242 | 20 | 2.0206 | 27 | 2.1935 | 2.2529 | 2.1794 | 0.9674 | 0.0326 |
| OPB-18 | 35 | 3.1982 | 20 | 2.6948 | 35 | 3.1257 | 4.5274 | 3.006 | 0.6658 | 0.3342 |
| OPC-01 | 31 | 2.4994 | 31 | 2.6358 | 37.5 | 2.7061 | 3.7902 | 2.6138 | 0.6896 | 0.3104 |
| OPC-14 | 35 | 2.8906 | 41 | 4.0826 | 35 | 4.8066 | 4.657 | 3.9266 | 0.8432 | 0.1568 |
| OPC-15 | 13 | 0.8256 | 40 | 3.6858 | 27 | 2.4295 | 3.0612 | 2.3136 | 0.7558 | 0.2442 |
| OPD-02 | 50 | 4.1261 | 44 | 3.6654 | 63 | 5.0839 | 5.3431 | 4.2918 | 0.8032 | 0.1968 |
| OPD-08 | 23 | 1.5470 | 31 | 2.4174 | 54 | 3.6494 | 2.9029 | 2.5379 | 0.8743 | 0.1257 |
| OPD-11 | 26 | 2.9377 | 39 | 4.9942 | 35 | 4.2799 | 5.4082 | 4.0706 | 0.7527 | 0.2473 |
| Mean | 31 | 2.5436 | 33 | 3.2746 | 41 | 3.5343 | 3.9913 | 3.1175 | 0.7811 | 0.2189 |

Note: All designations are given in Experimental.

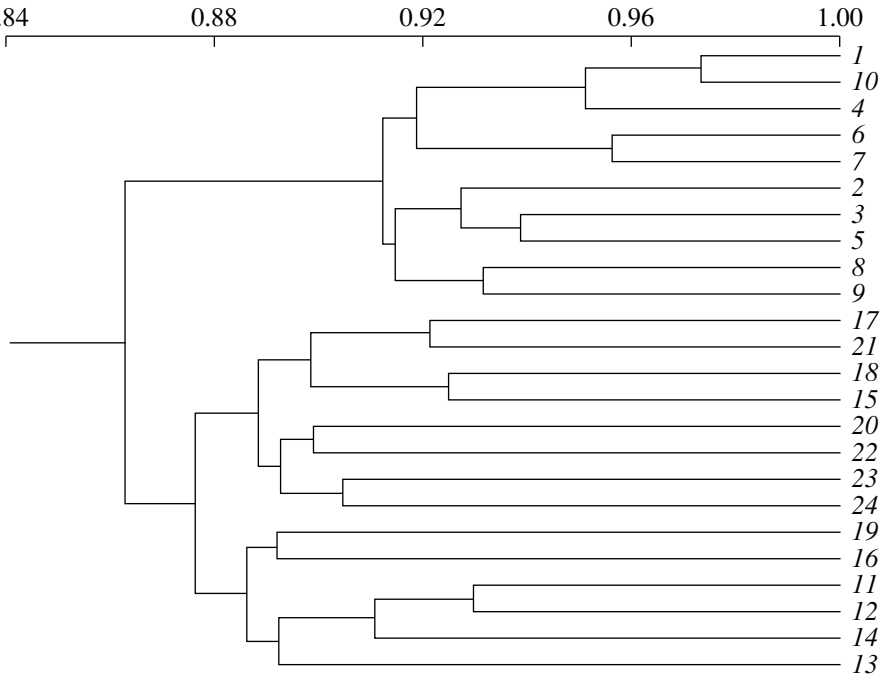


**Fig. 3.** Dendrogram of genetic similarity of *I. setosa* plants constructed basing on data obtained with eight primers. Accession I: 1–10; Accession II: 11–16; Accession III: 17–24.

in [4]. The results presented in this paper are in accord with this observation. Noteworthily, although variability of morphometric traits in accessions I and III has not been studied, the level of DNA polymorphism in these accessions is very high, and genetic differences between plants of accessions II and III are more pronounced than within accession I.

Formerly, polymorphism of Far-Eastern species of the *Iris* genus was demonstrated by using OPD-08 and OPD-11 primers, which were also employed in this study. Genetic distances between the *Iris* species were not less than 0.5, i.e., they were much higher than intraspecies ones. One might conclude that these primers allow not only differentiating species but also revealing the population and individual distinctions and similarities between *I. setosa* plants.

In conclusion, studies of intraspecies genetic variability of *I. setosa* plants from different habitats at the territory of Primorski Krai demonstrated that this species is characterized by high polymorphism. An amplicon was found that is a specific RAPD fragment of DNA of plants from accession I. Other polymorphic fragments are presented in RAPD patterns with different frequencies, and in some cases with different intensities. Three geographically distant accessions of *I. setosa* plants analyzed in the present study correspond to populations with different genetic structure.

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