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Population-Genetic Analysis of *Deschampsia antarctica* from Two Regions of Maritime Antarctica

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Abstract—Comparative analysis of the sequence of the ITS1-2 rDNA region of *Deschampsia antarctica* showed that plants of this species with different genotypes may have migrated into Antarctica after the glacial period. The use of RAPD markers allowed the identification of differences at the level of polymorphisms between populations of *D. antarctica* located in different latitudes and showed a limitation of exchange of genetic material between these populations.

Keywords: Deschampsia antarctica, RAPD-analysis, ITS rDNA, Antarctica.

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Deschampsia antarctica Desv. is the one of two aboriginal flowering plants of Antarctic flora whose exact time of colonization and path into Antarctica are still unknown. In this connection, it is necessary to search for markers in order to clarify these issues. The aim of this study is the evaluation of genetic polymorphisms of *D. antarctica* from two regions of maritime Antarctica using the methods of RAPD analysis and comparison of nucleotide sequences of ITS rDNA.

MATERIALS AND METHODS

Total, 15 specimens of *D. antarctica* from maritime Antarctica were used; 9 of these were collected in the neighborhood of the Argentine islands and the others on King George Island (an archipelago of the South Shetland Islands). The coordinates of the plant collection sites have been published in previous studies [1, 2]. DNA was extracted according to [3]. Thirty ten-nucleotide primers were used for RAPD analysis. PCR, separation of products and result analysis were undertaken according to previously described methods [3]. Calculation of polymorphous loci, the genetic distances according to Jaccard with following clusterisation of all samples by the UPGMA method, and the Shannon diversity index and molecular variance (AMOVA) were carried out using the FAMD program v. 1.21 (beta) [4].

A region of the 18S-25S rRNA nuclear gene, including ITS1, the 5.8S rRNA gene, and ITS2 was amplified with specific primers to the terminal regions of genes 18S and 25S rRNA [5]. The products were cloned using the pBluescript II SK(+) vector. The DNA sequences were determined using an fmol DNA Cycle Sequencing System kit (Promega, USA) on an ABI PRISM 3100-AVANT Genetic Analyzer. The sequences were deposited into GenBank under accession numbers GU181209–GU1811220. The comparison of nucleotide sequences of rDNA and construction of dendrograms was carried out using the PAUP 4.0b10 program [6].

RESULTS AND DISCUSSION

Analysis of rDNA polymorphisms. The sequence of the cloned region of rDNA carrying ITS1, 5.8S rDNA, and ITS2 from 12 plants of *D. antarctica* collected in different regions of maritime Antarctica was determined. The length of the analyzed fragment (595 bp) was similar for all clones, coinciding with the size of this region of rDNA from *D. antarctica* from South America (archipelago Tierra del Fuego and the Falkland Islands) represented in GenBank (AM41213–AM041215). ITS1 and ITS2 had lengths of 217 and 215 bp, respectively. The sequences of clones had 96.3% similarity; a more of variable nucleotides were found in ITS1 (6.5%), less in ITS2 (2.3%) and the fewest variable nucleotides were found in the 5.8S rRNA gene (1.8%).

Dendrograms reflecting the similarity of sequences of the ITS1-2 region of *D. antarctica* from different populations (Fig. 1a) have been built by the methods of neighbor-joining and maximum parsimony. *D. laxa, D. elongate* and *D. tenella* from South Amer-

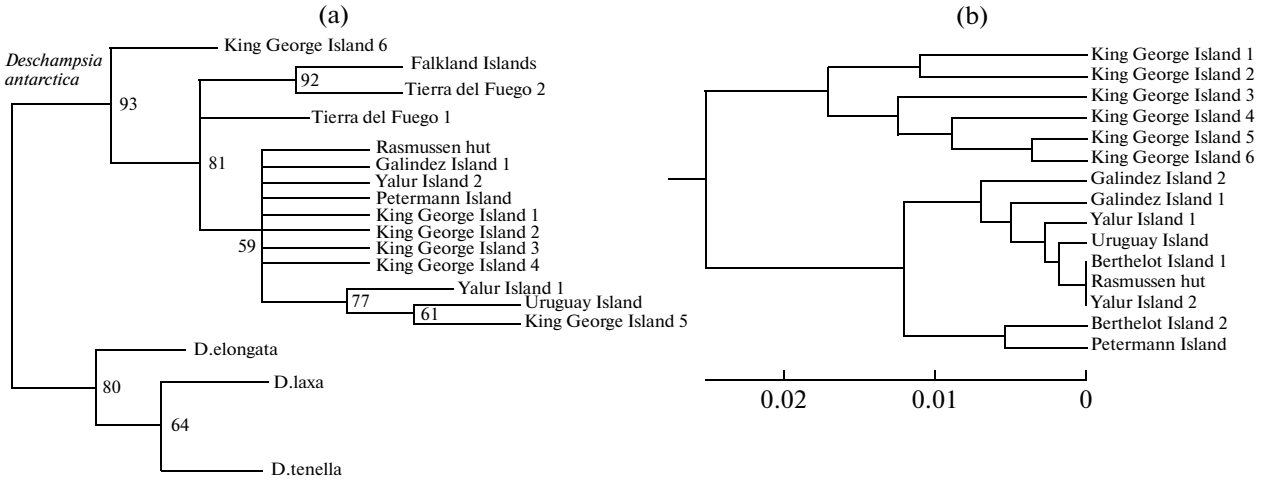

Fig. 1. (a) Dendrogram built by the method of neighbor joining for the ITS1–2 region rDNA of *D. antarctica* and relative species; (b) the values of bootstrap nodes are shown. Dendrogram of genetic similarity was built by the UPGMA method on the basis of Jaccard coefficients and the result of RAPD analysis.

ica and New Zealand, whose ITS1–ITS2 sequences are in GenBank (AM041238, AM041230 and AM041244) were used as the outgroup. The dendrograms built by both methods were nearly identical and differed in their values of bootstrap support of single branches. The study results show with the high reliability that the studied members of *D. antarctica* belong to one monophyletic group.

Comparison of sequences has shown that *D. antarctica* has at least four variants of ITS1–ITS2, which differ by single nucleotides. Variant 1, which is specific for plants in South America, was evolved first. Variant 2, which was derived from variant 1 and differed in one nucleotide substitution in the ITS1, has been identified in most populations of Antarctica. Variant 3, which was found in three Antarctic samples (Yalur 1 Island, Uruguay Island, and King George Island 5) arose from variant 2 and differed in two substitutions in ITS1 and ITS2. The most peculiar one is variant 4 from sample 6 from King George Island, which is placed on the dendrogram in the basal position (Fig. 1a). It differs from most similar sequences from South America (Tierra del Fuego 1) in eight nucleotide substitutions; seven of these are in the ITS1 and one is in the ITS2.

It is believed that the formation of the modern flora of Antarctica occurred after the last glaciation, due to the migration of plants from the subantarctic. The study data indicate that metapopulations of *D. antarctica* from King George Island have three variants of rDNA (no. 2, 3, and 4) and in plants from Argentine Islands only two variants (no. 2 and 3) occur; these differ from variant 1, which is specific for Tierra del Fuego and the Falkland Islands. There are two explanations for these data, first, that the polymorphism of the ITS1-2 region in Antarctic populations of *D. antarctica* was caused by repeated plant migration from genetically different populations out of Antarctic and secondly, Antarctic variants of ITS1-2 appeared on the Antarctic territory after migration. The second explanation is more logical for evolutionary derivatives of variant 2 and 3, which differ only by single mutations. The appearance of variants 2 and 3 probably occurred at the early colonization stages of Antarctic, because today both variants are widely distributed. The fact that unique variant no. 4 has been found on King George Island confirms the concept that the formation of the gene pool of *D. antarctica* in Antarctic occurred on the basis of a small number of initial genotypes. The final explanation of the origin of variant 4 requires additional studies of a larger number of plants from different geographic regions.

RAPD analysis Totally, 289 amplicons have been obtained, while 28 (9.7%) were polymorphous. The plants are grouped on the dendrogram to the clusters according to their geographic origin (Fig. 1b). Analysis of molecular variance (AMOVA) has shown that geographical distances of population located at a distance about 450 km explain half (58%) of the genetic polymorphism cases in the analyzed plant selection. The clear dividing of samples into two groups is evidence of the limitation of genetic material exchange between these groups. Distribution of the samples within the group according to the place of collection was not observed, which indicates the absence of substantial barriers of reproductive isolation within each geographical group. The last fact can be explained by the transfer of plants and seeds of *D. antarctica* by birds over short distances between islands.

Deviations in the level of genetic polymorphism were found between geographical groups of *D. antarctica* populations where the polymorphous plants from Argentine Islands made up 3.4% and plants from King George Island, 6.2%; the Shennon index, which char-

acterizes genetic diversity, was 0.015 and 0.024, respectively. These distinctions are more contrasting if we take the size of the territory of sample collection into account. The plants from King George Island were collected on a small site close to the polish "Arctowski" station, except one sample (no. 6) from the neighborhood of the Brazilian "Comandante Ferraz" station at a distance of 9 km. The samples of the second group were collected from several islands, where the distance between most widely separated islands (Berthelot Island and Petermann Island) is about 17 km. There are two reasons for the deviations at the level of genetic polymorphism between plant groups from King George and Argentine Islands. The first is later habitation of these islands and the appearance of the founder effect. This agrees with the presence of a unique variant, ITS1-2, found on King George Island, which indicates that the gene pool of this metapopulation probably formed on the basis of more diverse material as the result of crossing between plants with different origins.

The other cause of the low genetic diversity of plants from Argentine archipelago is more severe climatic conditions (this region is located 330 km farther from the equator), whose influence appears as more severe natural selection or periodic reduction of population magnitude, which results in a decrease of genetic heterogeneity.

CONCLUSIONS

Comparative analysis of sequences of the ITS1–2 rDNA region has shown the presence of variants in the Antarctic populations of *D. antarctica*, which are different from plants of Tierra del Fuego and Falkland Islands in specific mutations. The rDNA polymorphism found in the Antarctic populations may be explained by both the joining of different genotypes, which occurred as the result of some migrations or the later appearance of new mutations. The RAPD method showed that the studied plants belong to two groups on the basis of their geographical origin and distinctions in the level of genetic polymorphism between populations located at different latitudes.

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