ISSN 0095-4527, Cytology and Genetics, 2017, Vol. 51, No. 6, pp. 422–431. © Allerton Press, Inc., 2017.
Original Ukrainian Text © D.O. Navrotska, I.O. Andreev, I.Yu. Parnikoza, K.V. Spiridonova, O.O. Poronnik, N.Yu. Miryuta, G.Yu. Myryuta, O.M. Zahrychuk, N.M. Drobyk, V.A. Kunakh, 2017, published in Tsitologiya i Genetika, 2017, Vol. 51, No. 6, pp. 12–21.

# Comprehensive Characterization of Cultivated in vitro *Deschampsia antarctica* E. Desv. Plants with Different Chromosome Numbers

**D. O. Navrotska[a], I. O. Andreev[a], I. Yu. Parnikoza[a], K. V. Spiridonova[a], O. O. Poronnik[a], N. Yu. Miryuta[a], G. Yu. Myryuta[a], O. M. Zahrychuk[b], N. M. Drobyk[b], and V. A. Kunakh[a, *]**

[a] *Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, 03143 Ukraine*
[b] *Ternopil Volodymyr Hnatiuk National Pedagogical University, Ternopil, 46027 Ukraine*
**e-mail: kunakh@imbg.org.ua*

Received January 24, 2017

**Abstract**—*D. antarctica* E. Desv. plants cultivated in vitro were analyzed for the chromosome number, leaf length, and efficiency of callus formation. Most of the studied genotypes had a typical diploid chromosome number. However, a hypotriploid and a plant with B chromosomes demonstrated mixoploidy caused by the presence of a significant proportion (up to 15–25%) of aneuploid cells. Jaccard genetic distances between the plants determined from the data of ISSR- and IRAP-PCR analyses were within the range of 0.0323 to 0.1803. Furthermore, genetic distances between the specimens with atypical karyotype and diploids did not exceed the paired distances within the group of diploid plants. Variations in the leaf length and growth parameters of the plants were characterized. Plants with different chromosome numbers differed in the leaf length and efficiency of callus formation. Obtained results may indicate relationships between the chromosome number, studied morphometric parameters, and efficiency of callus formation in the analyzed *D. antarctica* plants.

*Keywords*: *Deschampsia antarctica* E. Desv., leaf length, efficiency of callus formation, chromosome number, ISSR-PCR, IRAP-PCR

**DOI:** 10.3103/S009545271706010X

## INTRODUCTION

*Deschampsia antarctica* E. Desv. is one of the two vascular plants of Antarctic. Although this species' range includes the south of South America and the Subantarctic, the question of the factors contributing to the exclusive distribution of *D. antarctica* in maritime Antarctic lacks an unambiguous answer. Detailed studies of plants of this species did not reveal unique adaptation mechanisms. Probably, the adaptation of the species is associated with the physiological responses of adaptation formed during the gradual change of conditions in Antarctica as well as the successful interaction with other organisms–the components of terrestrial communities [1, 2]. All of this make relevant the comprehensive study of the possible association between the genetic and morphometric parameters of *D. antarctica* plants.

It is known that genomic variability can increase under adverse conditions, providing increased diversity and adaptive potential to a population. Existence under adverse conditions leads to physiological stress that may induce changes in the number, type, structure, and differential staining of chromosomes [3]. Although it is believed that plants resistant to stress that inhabit the polar regions have reduced morphological plasticity [4], Antarctic vascular plants exhibit a variety of morphological forms specific to the local environment [5]. The intensity of growth processes and plant clonal reproduction and regeneration also may play a major role in adaptation.

Antarctic terrestrial ecosystems existing under difficult environmental conditions are considered the most vulnerable, and vascular plant population in these ecosystems are limited and protected. In spite of this, any use of these plants for experimental research or as a biotechnological or medicinal raw material is possible only based upon conditions of artificial growth. Thus, the production and a comprehensive description of plants suitable for in vitro cultivation is an important task. Therefore, the aim of this study was a comprehensive analysis of genetic and morphological characteristics and efficiency of callus formation of *D. antarctica* plants cultivated in vitro.

## MATERIAL AND METHODS

### *Plant Material*

*D. antarctica* plants were obtained from seeds collected in the region of the Argentine Islands (the location of the Vernadsky Research Base). Information

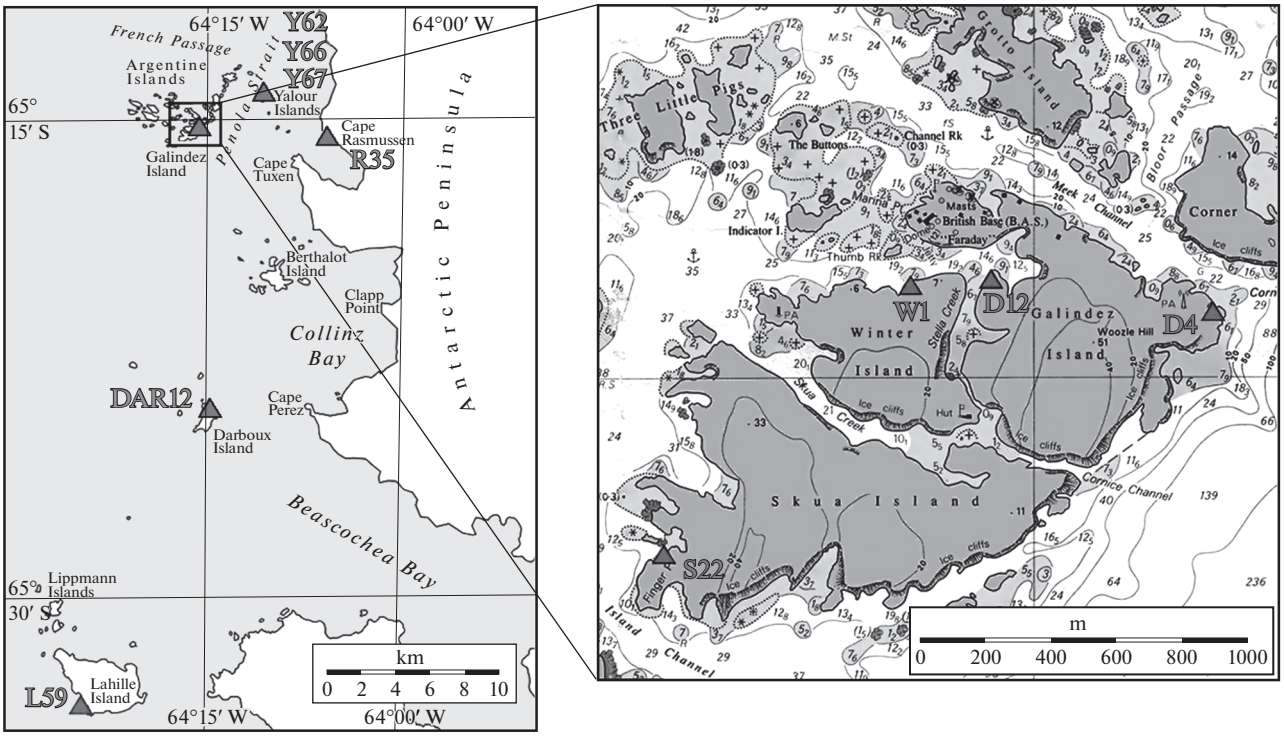


**Fig. 1.** Map of the location of the seed collection areas from which studied *D. antarctica* plants were obtained. The names and coordinates of localities are shown in Table 1.

about the locations and the the year of seed collection are shown in Fig. 1 (see inset) and Table 1.

The material was collected during the IX (2004/05), XI (2006/07), XII (2007/08), XIV (2009/10), XVII (2012/13), and XVIII (2013/14) Ukrainian Antarctic expeditions. The seeds were germinated according to the conditions described in [6]. Aseptic seedlings were harvested and cultivated on the Gamborg and Eveleigh (B5) medium [7]. The plants were multiplied by micropropagation and cultivated at a temperature of 17–19°C and a 16-h photoperiod. Plants, grown in vitro during 1–4 years were used for the study; their cytogenetic and molecular-genetic characteristics during the cultivation were stable [8].

### *Cytogenetic Analysis*

For cytogenetic analysis, the plant roots 1–1.5 cm length were used, which were kept in ice water (0°C) for 24 h for cell synchronization and accumulation of

**Table 1.** Information on the place and time of collection of *D. antarctica* seeds used for the production of the examined plants

| Genotype | Location and date of collection of seeds |
|---|---|
| G/D4-1 | Galindez Island (monitoring site D4), Penguin Point 65°14.916′ S, 64°14.293′ W, 2013 |
| G/D12-2a | Galindez Island (monitoring site D12), 65°14.845′ S, 64°15.156′ W, 2007 |
| G/D12-1 | Galindez Island (monitoring site D12), 65°14.845′ S, 64°15.156′ W, 2014 |
| Y62 | Great Yalour Island, 65°14.039′ S, 64°09.761′ W, 2005 |
| Y66 | Great Yalour Island, 65°14.039′ S, 64°09.761′ W, 2005 |
| Y67 | Great Yalour Island, 65°14.039′ S, 64°09.761′ W, 2005 |
| S22 | Skua Island (Finger Point), 65°15.296′ S, 64°16.441′ W, 2008 |
| R35 | Cape Rasmussen, 65°14.819′ S, 64°5.156′ W, 2005 |
| W1 | Winter Island, 65°14.851′ S, 64 °15.482′ W, 2014 |
| DAR12 | Darboux Island, 65°23.707′ S, 64°12.905′ W, 2007 |
| L59 | Lahille Island, 65°33.167′ S, 64°23.249′ W, 2010 |

**Table 2.** Characteristics of primers and their products in the studied *D. antarctica* plants

| Primers | Nucleotide sequence | Number of PCR loci | |
|---|---|---|---|
| | | total | variable |
| | ISSR | | |
| UBC-04 | $(AC)_8AG$ | 5 | 1 |
| UBC-807 | $(AG)_8T$ | 11 | 1 |
| UBC-811 | $(GA)_8C$ | 10 | 1 |
| UBC-840 | $(AG)_8YA$ | 11 | 2 |
| | IRAP | | |
| 642 | TTTGAAAACTGGCGGCAACG | 8 | 3 |
| 866 | ACCAGCCCGGGCCGTCGACC | 8 | 2 |
| 1681 | ATACCTCGGAGGCGCTGCACCTG | 10 | 6 |
| Total | | 63 | 16 |

mitoses. The samples were fixed in a mixture of ethanol : acetic acid at a ratio of 3 : 1 for 24 h. After a day, the fixator was changed to fresh. The fixed material was stored at –20°C. Root maceration was performed by a mixture of enzymes: 2% (w/v) Cellulase "Onozuka" RS and 20% (v/v) Pectinase from *Aspergillus niger* (Sigma, USA), after which squashed preparations were made and stained with DAPI (Serva, Germany).

Fluorescent in situ hybridization (FISH) was carried out in accordance with [9]. As a probe, *A. thaliana* telomeric repeats of HT100.3 $(TTTAGGG)_n$ [10] were used. Preparations were analyzed using an Olympus Provis AX70 epifluorescence microscope with a Hamamatsu C5810 CCD camera. Only metaphase plates in which the number of chromosomes could be precisely counted were considered.

### *Molecular Genetic Analysis*

DNA was isolated by cetyltrimethylammonium bromide (CTAB) method as described by Doyle et al. [11]. Polymerase chain reaction (PCR) was performed in a mixture of 20 μL containing 30 ng of DNA, 0.2 mM dNTP, 1.25 U Taq polymerase (Amplisens, RF), 1 × $(NH_4)_2SO_4$ buffer (Fermentas, Lithuania), and 1 μM primer. Four ISSR and three IRAP primers with the nucleotide sequences presented in Table 2 were used. PCR was carried out in a Tertsik MC2 thermocycler (Biotechnology, Russia) with the following temperature regime: 2 min at 94°C, 35 times (20 s at 94°C, 30 s at 53°C, 90 s at 72°C), 5 min at 72°C.

The PCR products were separated by electrophoresis in a 1.5% agarose gel in 1× SB buffer (5 mM $Na_2B_4O_7$, pH 8.5) followed by visualization in UV light after staining with ethidium bromide. As a marker of molecular weights, a DNA marker (100 bp + 1.5 Kb + 3Kb, NPO Sibenzyme, Russian Federation) was used. Statistical data processing was carried out using the FAMD 1.3 program [12].

### *Investigation of Morphometric Parameters*

The length of the leaves was measured in 2-month-old plants grown in vitro. All leaves of each plant were measured. Leaf length (cm) values were divided into classes: first–lower than 3.9; second–4.0–5.9; third–6.0–7.9; fourth–8.0–9.9; fifth–10.0–11.9; sixth–12.0–13.9; seventh–14.0–15.9; eighth–16.0–17.9; ninth–more than 18.0 cm. Based on these data, distributions based on the Gaussian model were plotted with an accuracy from 0.93 to 0.99. Standard methods of descriptive statistics were used for the comparison: determination of the mean value, standard deviation, and Mood's median test [13].

### *Investigation of the Efficiency of Callus Formation*

For the induction of callus formation, explants with the length of 5–8 mm from roots and shoots were used. Explants were cultivated on nutrient media: Murasige and Skoog (MS) [14], Gamborg and Eveleigh (B5) [7], and B5 with a half concentration of macro- and microsalts (B5/2), supplemented with different concentrations of cytokinin 6-benzylaminopurine (BAP) and auxins 2,4-dichlorophenoxyacetic acid (2,4-D) or 1-naphthylacetic acid (NAA). Conditions of callusogenesis and the cultivation of the obtained calluses were described in the study [15]. The ability of the six genotypes, G/D12-2a, DAR12, L59, R35, S22, and Y66 (Table 3), to callus formations was investigated. In each experiment, 90–100 explants of three to five plants of each genotype obtained by microclonal propagation in vitro were used.

Callusogenesis percentage (CP) was determined according to the formula: $CP = Nk/N \times 100\%$, where $Nk$ is the number of explants that formed callus; $N$ is the number of planted explants.

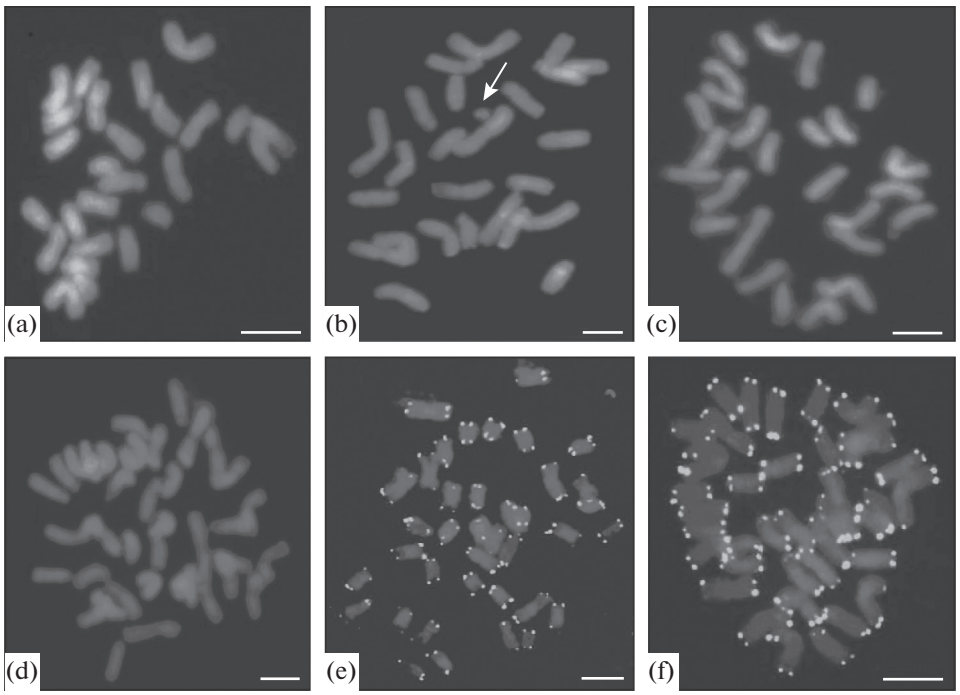


**Fig. 2.** Metaphase plate of cells of root apical meristem of *D. antarctica* plants. Genotypes: (a) DAR12, $2n = 26$; (b) DAR12, $2n = 26 + 1B$; (c) S22, $2n = 26$; (d) Y66, $2n = 36$; (e) Y66, $2n = 37$; (f) Y66, $2n = 38$. Scale: 10 μm. A B chromosome is indicated by an arrow. In Figs. 2e and 2f, FISH localization of telomeric repeats on chromosomes is shown. The photo was taken at the Department of Plant Anatomy and Cytology of the University of Silesia in Katowice (Katowice, Poland).

## RESULTS AND DISCUSSION

### *Cytogenetic Analysis*

It was established that *D. antarctica* plants of the studied genotypes, namely G/D12-2a, G/D4-1, G/D12-1, L59, R35, S22, W1, and Y62, from the Argentine Islands region of the maritime Antarctic have chromosome number ($2n = 26$) that is typical of the species (Fig. 2, see inset, and Table 3). Such results are consistent with data obtained earlier for plants of this species from other Antarctic regions [16–18].

Cells with B chromosomes ($2n = 26 + 0\text{-}2B$) were found in the DAR12 genotype from Darboux Island (Figs. 2a–2b, Tables 3, 4). The number of additional chromosomes in the karyotype was one to two and up to three in rare cases. The presence of additional chromosomes in *D. antarctica* DAR12 plants was determined by in situ hybridization with telomeric and centromeric repeated sequences, described in detail in [18]. Unlike the remaining diploids, this genotype revealed a significant proportion (up to 15%) of aneuploid cells [19].

Plants of the Y66 genotype had a hypotriploid chromosome number $2n = 36–38$ (Figs. 2d–2f, Table 4) and contained a significant number of aneuploid cells (up to 25%) as well as a small percentage of diploid and haploid cells (Table 4). As was established previously, a karyotype with 38 chromosomes, which was characteristic of 43.7% plant cells of this genotype, was formed as a result of the Robertsonian fusion of homologous chromosomes of the 12th pair [18]. The triploid nature of Y66 genotype was determined by FISH analysis with localization of the sequences of 5S rRNA and 45S rRNA genes. Analysis of other samples from the population of the Great Yalour Island revealed diploid (Y62) and mixoploid (Y67) genotypes (Tables 3, 4).

Increased instability of *D. antarctica* genome manifested as the appearance of aneuploidy was also discovered by other researchers for samples from King George Island, where individual plants contained cells with 26 and 28 chromosomes [16]. Variability in the number of chromosomes was also described in the closely related species *Deschampsia caespitosa* (L.) Beauv. Cells of root apical meristem of seedlings of this species, along with diploid, contained aneuploid cells with chromosome number ranging from 15 to 26 [19].

Additional chromosomes were also found in the karyotypes of other *Deschampsia* species, in particular *Deschampsia caespitosa* (L.) Beauv. ($2n = 26 + 0\text{-}2B$) and *D. wibeliana* (Sond.) Parl. ($2n = 26 + 0\text{-}5B$) [20]. In most cases, the B chromosome could be observed in individuals living under suboptimal and stress conditions. Some authors suggest that such conditions may increase the adaptive potential of plants, for example, drought or low temperature tolerance [21–23].

Triploid and tetraploid cytotypes with 39 (*Deschampsia alpina* (L.) Roem & Schultes) and 52 chromosomes

**Table 3.** Cytogenetic, morphometric characteristics and efficiency of callus formation by *D. antarctica*

| Locality | Genotype | Cytogenetic characteristics | | | | Leaf length | | | Callusogenesis percentage, $CP_{aver\ (\%)}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | number of studied | | | chromosome number (2*n*) | number of studied | | X ± S, cm | explant type | | X |
| | | plants | roots | methaphases | | plants | leaves | | roots | shoots | |
| Galindez Island | G/D12-2a | 3 | 18 | 61 | 26 | 55 | 404 | 7.6 ± 2.4 | 42.7 | 12.9 | 27.8 |
| | G/D4-1 | 1 | 15 | 21 | 26 | 14 | 79 | 6.6 ± 1.9 | | | |
| | G/D12-1 | 1 | 9 | 24 | 26 | 39 | 231 | 6.1 ± 1.8 | | | |
| Lahille Island | L59 | 1 | 3 | 32 | 26 | 38 | 307 | 8.0 ± 2.5 | 38.6 | 20.1 | 29.3 |
| Cape Rasmussen | R35 | 3 | 16 | 37 | 26 | 20 | 109 | 8.9 ± 1.9 | 37.5 | 12.6 | 25.1 |
| Skua Island | S22 | 3 | 18 | 45 | 26 | 21 | 150 | 6.7 ± 1.3 | 29.1 | 11.2 | 20.1 |
| Winter Island | W1 | 1 | 7 | 17 | 26 | 28 | 199 | 7.3 ± 1.8 | | | |
| Great Yalour Island | Y62 | 1 | 7 | 67 | 26 | 48 | 276 | 5.9 ± 1.5 | | | |
| | Y67 | 1 | 6 | 29 | 26 | 40 | 285 | 5.7 ± 1.4 | | | |
| | Y66* | 4 | 28 | 52/40 | 36/38 | 20 | 138 | 10.5 ± 1.6 | 45.6 | 20.5 | 33.1 |
| Darboux Island | DAR12* | 3 | 24 | 56/12 | 26 + 0-2B | 26 | 192 | 9.4 ± 2.0 | 21.7 | 9.5 | 15.6 |

X is mean value, S is standard deviation, and $CP_{aver}$ is averaged value for different nutrient media. The detailed cytogenetic characteristics of Y66 and DAR12 genotypes are given in Table 4.

**Table 4.** Cytogenetic characteristics of myxoploid *D. antarctica* plants

| Chromosome number | Number of metaphases, M (%) ± S | | |
|---|---|---|---|
| | Y66 | Y67 | DAR12 |
| 13 | 1.1 ± 0.8 | 2.2 ± 2.2 | 3.1 ± 1.7 |
| 16 | 0.5 ± 0.5 | | 1.0 ± 1.0 |
| 18 | 1.1 ± 0.8 | | 1.0 ± 1.0 |
| 21 | 0.5 ± 0.5 | | 5.1 ± 2.2 |
| 23 | 1.6 ± 0.9 | | 6.1 ± 2.4 |
| 26 | 2.2 ± 1.1 | 95.6 ± 3.1 | 57.1 ± 5.0 |
| 26 + 1-2B | | | 8.3 ± 2.3 |
| 27 | 0.5 ± 0.5 | | |
| 28 | 1.6 ± 0.9 | | |
| 33 | 7.1 ± 1.9 | | |
| 34 | 0.5 ± 0.5 | | |
| 35 | 0.5 ± 0.5 | | |
| 36 | 28.4 ± 3.3 | | |
| 37 | 1.6 ± 1.6 | | |
| 38 | 43.7 ± 3.7 | | |
| 39 | 4.9 ± 1.6 | 2.2 ± 2.2 | |
| 42 | 0.5 ± 0.5 | | |
| 45 | 0.5 ± 0.5 | | |
| 46 | 0.5 ± 0.5 | | |
| 48 | 0.5 ± 0.5 | | |
| 52 | 0.8 ± 0.6 | | |

X is mean value and S is standard deviation. In the analysis, the following number of plants was investigated: Y66—four plants, 28 roots, 183 metaphases; Y67—one plant, seven roots, 45 metaphases; DAR12—three plants, 24 roots, 98 metaphases.

(*D. brevifolia* R. Br., *D. mackenzieana* C. Neufeld, *D. mildbraedii* Pilg., *D. orientalis* (Hulten) B.S.) were also found for other representatives of the genus *Deschampsia* (*D. brevifolia*, *D. mackenzieana*, and *D. orientalis* recognized now as synonyms of *D. cespitosa*) [24–26]. The population of tetraploid *D. antartica* plants ($2n = 52$) was recently found in Chile in the territory of South America (Chubut Province) [17].

Now, the ecological and adaptive importance of the existence of chromosomal races with different levels of ploidy in *Deschampsia* species is unknown. It is possible that their formation is due to the peculiarities of habitats: diploid cytotypes grow predominantly under normal environmental conditions, whereas tetraploids usually occur in habitats with suboptimal conditions, which indicates their increased ability to populate new territories [27]. Plants with different levels of ploidy were also found in other *Deschampsia* species, mainly in several taxa (subspecies) of *Deschampsia caespitosa* (L.) Beauv. complex, including diploid, triploid, and tetraploid cytotypes [25, 27]. This fact allows us to draw a parallel between the chromosomal forms of the northern and southern groups of Deschampsia: although part of *D. antarctica* inhabits the Antarctic region with specific conditions, the whole spectrum of chromosomal variability described for the genus *Deschampsia* and for a closely related species *D. caespitosa* was detected for this species.

### *Molecular Genetic Analysis*

Genetic variation of plants was studied by PCR analysis with ISSR and IRAP primers. In total, 63 amplicons were obtained for were obtained for 11 plants under study, among which 16 (25.4%) were polymorphic (Table 2).

Jaccard genetic distances between plants were calculated on the basis of the data of PCR analysis. Values of distances were within the range of 0.0323–0.1803 (Table 5). The most different were W1 and DAR12 genotypes, the genetic distance between them was 0.1803. The lowest value of the Jacquard coefficient (within the range of 0.0323–0.0645) was found for the group of genotypes R35, S22, Y62, Y66, Y67, which includes hypotriploid and typical diploids, originating from close islands in the studied region (Fig. 1). At the same time, the genotype with a diploid chromosome number from Winter Island (W1) was genetically distant from its geographical neighbors and from other diploid plants.

The genetic distances between the diploid genotypes fluctuated within wide limits – from 0.0476 to 0.1746. The values of the genetic distances between diploids and plants with an atypical chromosome number DAR12 and Y66 were not beyond this range (Table 5).

### *Investigation of Morphometric Parameters*

The length of the leaf of cultivated *D. antarctica* plants varied within 2–19 cm. The highest mean leaf length was detected for plants of DAR12 ($2n = 26 + 0$-2B) and Y66 ($2n = 36$–38) genotypes (Table 3). According to the Gaussian model, leaves of 10.0–11.9 cm were dominant in these plants (Figs. 3, 4). Plants of the diploid L59 genotype were characterized by a predominance of leaves with a length from 6.0 up to more than 18.0 cm and showed positive asymmetry of distribution. In plants of G/D4-1 and Y62 genotypes (diploids) and Y67 (mixoploids), most of the analyzed leaves had a length of 4.0–5.9 cm. Leaves from 6.0 to 7.9 cm predominated in plants of diploid genotypes S22, G/D12-1, R35, and W1 genotypes, while leaves with the length of 4.0–9.9 cm predominated in G/D12-2a (diploid) plants.

To analyse the relationship between the "leaf length" value and the place of growth, a pairwise comparison of the genotypes of common geographic origin and different islands was carried out. For diploid plants from the Galindez Island, similarity between G/D4-1 and G/D12-1 genotypes and the distinction from G/D12-2a genotype were found. For plants from

**Table 5.** Genetic distance according to Jacquard between *D. antarctica* plants calculated according to the results of ISSR and IRAP analysis

| Genotype | W1 | DAR12 | S22 | Y62 | Y66 | Y67 | R35 | L59 |
|---|---|---|---|---|---|---|---|---|
| W1 | 0.000 | | | | | | | |
| DAR12 | 0.1803 | 0.0000 | | | | | | |
| S22 | 0.0847 | 0.0984 | 0.0000 | | | | | |
| Y62 | 0.1290 | 0.0806 | 0.0484 | 0.0000 | | | | |
| Y66 | 0.1452 | 0.0968 | 0.0645 | 0.0476 | 0.0000 | | | |
| Y67 | 0.1475 | 0.0983 | 0.0968 | 0.0484 | 0.0641 | 0.0000 | | |
| R35 | 0.1746 | 0.1270 | 0.0952 | 0.0476 | 0.0323 | 0.0645 | 0.0000 | |
| L59 | 0.1639 | 0.1148 | 0.1129 | 0.0645 | 0.0806 | 0.0500 | 0.0806 | 0.0000 |

Great Yalour Island, the difference between the diploid and mixoploid Y62 and Y67 genotypes was not found, while hypotriploid Y66 had significantly larger leaf sizes.

The pairwise comparisons of plants of diploid genotypes from different islands did not reveal differences between G/D12-2a and L59; R35 and L59; S22 and G/D4-1; G/D12-1 and Y62; G/D12-1 and Y67. DAR12 plants with B chromosomes and Y66 hypothyroid were different from the remaining genotypes. On the whole, differences between genotypes by leaf length were found in 47 among 55 pair-wise comparisons.

Morphological parametrs, including the leaf length, are important for adaptation of plants. The leaf length of the species is a genetically determined characteristic and depends, among other parameters, on the level of ploidy, as was shown for monocotyledonous and dicotyledonous plants [28, 29]. However, this index may vary depending on the growth conditions. Since the investigated plants were cultivated under standardized conditions, it can be assumed that the identified differences were due to the genetic component, in particular cytogenetic characteristics of plants.

### *Efficiency of Callus Formation*

*D. antarctica* plants of all genotypes under study were able to form callus. Shoot and root explants formed calli on B5, B5/2, and MS media supplemented with combinations of different concentrations of BAP (0.09–2 mg/L) and 2,4-D (0.5–1 mg/L) or 2 mg/L NAA. The first signs of callus formation were observed 7–25 days after the beginning of the experiments. Evaluation of the the efficiency of the callus formation revealed the dependence of this parameter from the original genotype of the donor plant, the mineral composition of the nutrient medium, the ratio and concentration of growth regulators, and the type of explants (Tables 4, 6).

The highest frequency of callus formation was recorded for plants of Y66 genotype ($2n = 36–38$): the averaged data for root and shoot explants on all studied nutrient media were 45.6 and 20.5% respectively. Explants of DAR12 genotype ($2n = 26 + 0$-2B) had the lowest callus formation efficiency: mean data for all tested nutritional media were 21.7% for root explants and only 9.5% for shoot explants. Plants of diploid genotypes (G/D12-2a, R35, S22, L59) showed medium values of the frequency of callus formation among the studied samples (Table 4). The use of B5 medium was found to be the most effective for induction of callus formation from root and shoot explants. On this medium, the formation of the callus from both the root and shoot explants occurred in 7–10 days: the percentage of callus formation in some cases was 100%, the callus was characterized by a loose consistency and light yellow color. For S22 and L59 genotypes, a two times decreased concentration of macro- and microsalts in B5 medium contributed to better callus formation from both types of explants (Table 6).

The ratio and concentration of growth regulators of 2,4-D, NAA, and BAP in the nutrient medium sig-

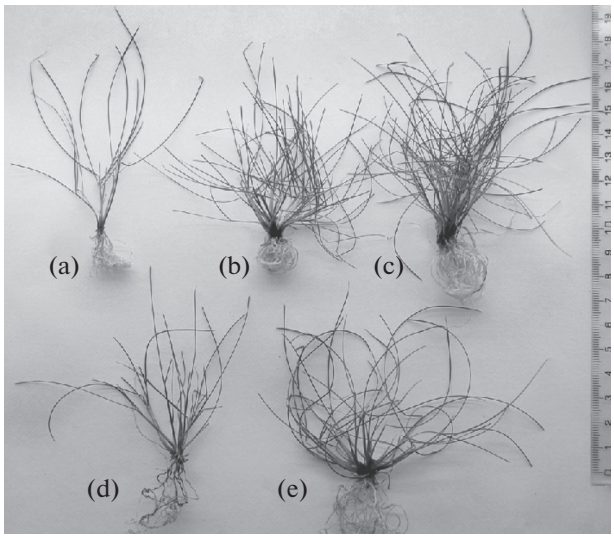


**Fig. 3.** Appearance of *D. antarctica* plants of different genotypes cultivated under in vitro conditions: (a) DAR12 ($2n = 26 + 0$-2B); (b) G/D12-2a ($2n = 26$); (c) R35 ($2n = 26$); (d) S22 ($2n = 26$); (e) Y66 ($2n = 36–38$).

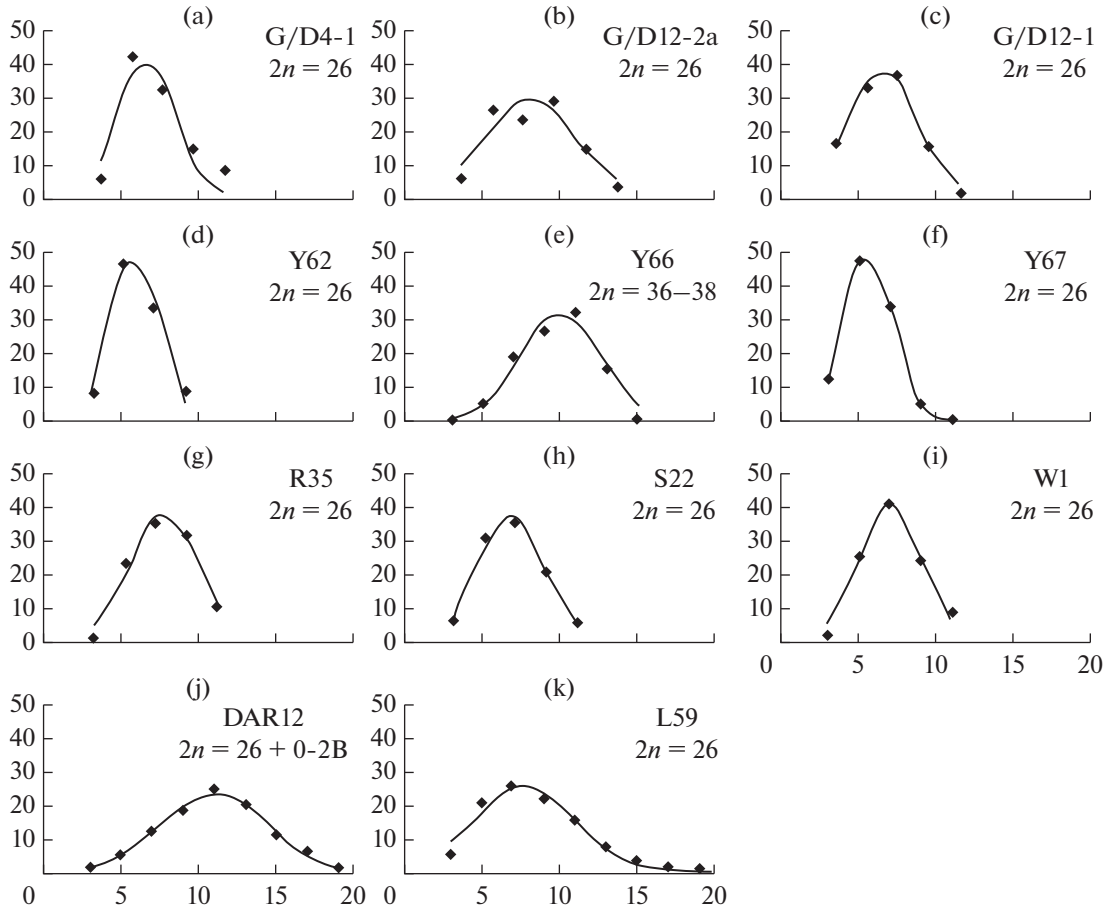


**Fig. 4.** Frequency distributions (Gaussian model) of leaf length (on the horizontal axis, cm) of *D. antarctica* plants of different genotypes cultivated in vitro: (a–c) plants from Galindez Island, (d–f) plants from Great Yalour Island, (f–k) plants from Cape Rasmussen and Skua, Winter, Darboux and Lahille Islands, respectively. Vertically: number of leaves, %.

nificantly affected the callus formation. For S22, L59, and Y66 genotypes, the concentrations of growth regulators in nutrient medium of 0.5 mL/L 2,4-D and 0.1 mL/L BAP were optimal for callus formation. For G/D12-2a (root explants) and R35 genotypes, two times increased auxin concentration was required, and two times increased concentrations of both growth regulators was needed for explants of DAR12 plants (Table 6).

Callus formation ability and its efficiency in the studied genotypes depended also on the type of explant. The percentage of callus formation from root explants ranged from 4.3% (DAR12) to 100% (G/D12-2a). The formation of the callus from shoot explants was less efficient than that from root explants: CP was within the range of 9–65%. The highest ability to form callus was characteristic for shoots explants from plants of Y66 and L59 genotypes, and the lowest ability was found for the DAR12 genotype.

The mean values of the efficiency of callus formation from both types of explants on different variants of the nutrient media were the highest for plants of Y66 genotype ($2n$ = 36–38): 33.21%; they were the lowest for plants of DAR12 genotype ($2n$ = 26 + 0-2B): 15.6%. For other investigated diploid genotypes, mean values of the efficiency of callus formation were within the range 20.1–29.3%.

Obtained data indicate a possible association between the intensity of callus formation and chromosome number of the donor plant and the type of explant. It is known from the literature that efficiency of callus formation in some cases may be higher in polyploid plants than in diploid ones, while, in other cases, they decreased with an increase of ploidy [3].

## CONCLUSIONS

The variability of cytogenetic, molecular genetic, and morphometric parameters of cultured in vitro *D. antarctica* plants with different chromosome number from the Argentine islands region of maritime Antarctic was characterized. It was shown that unlike

**Table 6.** Rate of callus formation, %, from root and shoot explants of *D. antarctica* plants on different variants of nutrient media

| Genotype | Explant type | Variants of nutrient media | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | VIII |
| G/D12-2a | Roots | – | 32.5 ± 4.7 | 28 ± 4.4 | 9 ± 2.8 | 41 ± 4.9 | 75 ± 4.3 | 100 | 56 ± 5 |
| | Shoots | 30 ± 4.5 | – | – | – | 50 ± 5 | – | 23.7 ± 4.2 | – |
| DAR12 | Roots | 4.3 ± 2 | 17 ± 3.7 | 12.4 ± 3.3 | 45 ± 4.9 | 16.4 ± 3.7 | 8.4 ± 2.8 | 40 ± 4.9 | 30 ± 4.5 |
| | Shoots | – | 13 ± 3.3 | – | 33 ± 4.7 | – | – | 30 ± 4.5 | – |
| R35 | Roots | 53.3 ± 5 | 58.3 ± 4.9 | 30.2 ± 4.6 | 33.3 ± 4.7 | 18.8 ± 3.9 | 24.6 ± 4.3 | 59.2 ± 3.1 | 22.6 ± 4.2 |
| | Shoots | – | 32.5 ± 4.7 | 16.4 ± 3.7 | – | – | 12.4 ± 3.3 | 39.7 ± 4.8 | – |
| S22 | Roots | 43 ± 4.9 | 40 ± 4.8 | 62.5 ± 4.8 | 10 ± 3 | 16.4 ± 3.7 | 52.3 ± 4.9 | 10 ± 3 | 8.4 ± 2.8 |
| | Shoots | 20 ± 4 | – | 36.4 ± 4.8 | – | 13 ± 3.3 | 20 ± 4 | – | – |
| Y66 | Roots | 30 ± 4.6 | 62 ± 4.8 | 40 ± 4.9 | 60 ± 4.9 | 52 ± 5 | 43 ± 4.9 | 48 ± 5 | 30 ± 4.5 |
| | Shoots | – | 65 ± 4.8 | 30 ± 4.6 | – | 34 ± 4.7 | 16.4 ± 3.7 | 18.8 ± 3.9 | – |
| L59 | Roots | 41.7 ± 4.9 | 50 ± 5 | 70.4 ± 4.6 | 38.4 ± 4.9 | 18.8 ± 3.9 | 26.4 ± 4.4 | 32.5 ± 4.7 | 30.4 ± 4.6 |
| | Shoots | 20 ± 4 | – | 65 ± 4.8 | 24.6 ± 4.3 | 9 ± 2.8 | 12.8 ± 3.3 | 16.4 ± 3.7 | 12.8 ± 3.3 |

Note: Variants of nutrient media: I—MS with 1 mg/L 2,4-D and 0.1 mg/L BAP; II—B5 with 0.5 mg/L 2,4-D and 0.1 mg/L BAP; III—B5/2 with 0.5 mg/L 2,4-D and 0.1 mg/L BAP; IV—B5 with 1 mg/L 2,4-D and 0.2 mg/L BAP; V—B5 with 0.9 mg/L 2,4-D and 0.09 mg/L BAP; VI—B5/2 with 0.9 mg/L 2,4-D and 0.09 mg/L BAP; VII—B5 with 1 mg/L 2,4-D and 0.1 mg/L BAP; VIII—B5 with 2 mg/L NAA and 0.1 mg/L BAP.

the diploid genotypes (G/D12-2a, G/D4-1, G/D12-1, L59, R35, S22, W1, Y62; $2n = 26$) plants of the hypothyroid genotype (Y66; $2n = 36–38$) and the genotype with B chromosomes (DAR12; $2n = 26 + 0$-2B) showed mixoploidy with the presence of aneuploid cells. The genetic distances between plants with different chromosome numbers based on PCR markers did not exceed those between the diploid samples. The variation range of the leaf length of cultivated plants was 2–19 cm. The mean leaf length was the highest in plants of Y66 and DAR12 genotypes. The efficiency of callus formation of the investigated plants varied in a significant range, the extreme values of which differed more than two times. The highest value of this index was detected for Y66 plants; the lowest value was detected for DAR12 plants. Callus formation on shoot explants was less intensive than on the root explants.

## ACKNOWLEDGMENTS

We are grateful to Prof. Robert Hasterok and Dr. Alexander Betekhtin (University of Silesia in Katowice, Poland) for the opportunity to perform molecular cytogenetic analysis of plants and provided assistance.

The research was carried out in cooperation with the National Antarctic Scientific Center of the Ministry of Education and Science of Ukraine within the framework of the State Target Scientific and Technical Program of Research in Antarctica for 2011–2020.

## REFERENCES

1. Parnikoza, I.Yu., Kozeretska, I.A., and Kunakh, V.A., Vascular plants of the Maritime Antarctic: origin and adaptation, *Am. J. Plant Sci.*, 2011, vol. 2, no. 3, pp. 381–395.
2. Ozheredova, I.P., Parnikoza, I.Yu., Poronnik, O.O., Kozeretska, I.A., Demidov, S.V., and Kunakh, V.A., Mechanisms of Antarctic vascular plant adaptation to abiotic environmental factors, *Cytol. Genet.*, 2015, vol. 49, no. 2, pp. 139–145.
3. Kunakh, V.A., *Biotechnology of Medicinal Plants. Genetic, Physiological and Biochemical Bases*, Kyiv: Logos, 2005.
4. Grime, J.P., *Plant Strategies, Vegetation Processes, and Ecosystem Properties*, 2nd ed., Chichester: John Wiley & Sons, 2001.
5. Gielwanowska, I., Specyfika rozwoju antarktycznych roslin naczyniowych *Colobanthus quitensis* (Kunth) Bartl. i *Deschampsia antarctica* Desv., in *Rozprawa habilitacyjna*, Olsztyn: Wydawnictwo Uniwersytetu Warminsko-Mazurskiego, 2005.
6. Zahrychuk, O.M., Drobyk, N.M., Kozeretska, I.A., Parnikoza, I.Yu., and Kunakh, V.A., Introduction in culture in vitro of *Deschampsia antarctica* Desv. (Poaceae) from two regions of Maritime Antarctica, *Ukr. Antarct. J.*, 2011/2012, nos. 10–11, pp. 289–295.
7. Gamborg, O.L. and Eveleigh, D.E., Culture methods and detection of glucanases in culture of wheat and barley, *Can. J. Biochem.*, 1968, vol. 46, no. 5, pp. 417–421.
8. Spiridonova, K.V., Andreev, I.O., Zahrychuk, O.M., Navrotska, D.O., Twardovska, M.O., Drobyk, N.M., and Kunakh, V.A., Genetic stability of micropropagated plants of *Deschampsia antarctica* Desv. during long-term in vitro culture, *Plant Physiol. Genet.*, 2016, vol. 48, no. 6., pp. 498–507.
9. Jenkins, G., and Hasterok, R., BAC "landing" on chromosomes of *Brachypodium distachyon* for comparative genome alignment, *Nat. Protoc.*, 2007, vol. 2, no. 1, pp. 88–98.

10. Hajdera, I., Siwinska, D., Hasterok, R., and Maluszynska, J., Molecular cytogenetic analysis of genome structure in *Lupinus angustifolius* and *Lupinus cosentinii, Theor. Appl. Genet.*, 2003, vol. 107, no. 6, pp. 988–996.
11. Doyle, J.J. and Doyle, J.L., A rapid DNA isolation of fresh leaf tissue, *Phytochem. Bull.*, 1987, vol. 19, no. 1, pp. 11–15.
12. Schluter, P.M. and Harris, S.A., Analysis of multilocus fingerprinting data sets containing missing data, *Mol. Ecol. Notes*, 2006, vol. 6, no. 2, pp. 569–572.
13. Pollard, J.H., *A Handbook of Numerical and Statistical Techniques*, Moscow: Finansy i statistika, 2008.
14. Murasige, T. and Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiol. Plant.*, 1962, vol. 15, no. 3, pp. 473–497.
15. Zagrychuk, O.M., Herts, A.I., Drobyk, N.M., and Kunakh, V.A., Callus formation and regeneration of *Deschampsia antarctica* Desv. (Poaceae) in culture in vitro, *Biotechnol. Acta*, 2013, vol. 6, no. 6, pp. 77–85.
16. Cardone, S., Sawatani, P., Rush, P., Garcia, A.M., Poggio, L., and Schrauf, G., Karyological studies in *Deschampsia antarctica* Desv. (Poaceae), *Polar Biol.*, 2009, no. 32, pp. 427–433.
17. Gonzalez, M.L., Urdampilleta, J.D., Fasanella, M., Premoli, A.C., and Chiapella, J.O., Distribution of rDNA and polyploidy in *Deschampsia antarctica* E. Desv. in Antarctic and Patagonic populations, *Polar Biol.*, 2016, vol. 39, pp. 1663–1680.
18. Amosova, A.V., Bolsheva, N.L., Samatadze, T.E., Twardovska, M.O., Zoshchuk, S.A., Andreev, I.O., Badaeva, E.D., Kunakh, V.A., and Muravenko, O.V., Molecular cytogenetic analysis of *Dschampsia antarctica* Desv. (Poaceae), Maritime Antarctic, *PLoS One*, 2015, vol. 10, no. 9, p. e0138878.
19. Navrotska, D.O., Twardovska, M.O., Andreev, I.O., Parnikoza, I.Yu., Betekhtin, A.A., Zahrychuk, O.M., Drobyk, N.M., Hasterok, R., and Kunakh, V.A., New forms of chromosome polymorphism in *Deschampsia antarctica* Desv. from the Argentine Islands of the Maritime Antarctic region, *Ukr. Antarc. J.*, 2014, no. 13, pp. 185–191 (in Ukrainian).
20. Nkongolo, K.K., Deck, A., and Michael, P., Molecular and cytological analysis of *Deschampsia caespitosa* population from Northern Ontario (Canada), *Genome*, 2001, vol. 44, no. 5, pp. 818–825.
21. Plowman, A.B. and Bougourd, S.M., Selectively advantageous effects of B chromosomes on germination behaviour in *Allium schoenoprasum* L, *Heredity*, 1994, vol. 72, pp. 587–593.
22. Rosato, M., Chiavarino, A.M., Naranjo, C.A., Hernandez, J.C., and Poggio, L., Genome size and numerical polymorphism for the B chromosome in races of maize (*Zea mays* ssp. *mays*, Poaceae), *Am. J. Bot.*, 1998, vol. 85, no. 2, pp. 168–174.
23. Kunakh, V.A., Supernumerary or B-chromosomes in plants. Origin and biological implication, *Visn. Ukr. Tovar. Genet. Selekts.*, 2010, vol. 8, no. 1, pp. 99–139.
24. Rothera, S.L. and Davy, A.J., Polyploidy and habitat differentiation in *Deschampsia caespitosa, New Phytol.*, 1986, vol. 102, no. 3, pp. 449–467.
25. Chiapella, J. and Probatova, N., The *Deschampsia caespitosa* complex (Poaceae: Aveneae) with special reference to Russia, *Bot. J. Linn. Soc.*, 2003, vol. 142, no. 2, pp. 213–228.
26. Chiapella, J., A molecular phylogenetic study of *Deschampsia* (Poaceae: Aveneae) inferred from nuclear ITS and plastid trnL sequence data: support for the recognition of *Avenella* and *Vahlodea, Taxon*, 2007, vol. 56, no. 1, pp. 55–64.
27. Nygren, A., Studies on vivipary in the genus *Deschampsia, Hereditas*, 1949, vol. 35, no. 1, pp. 27–32.
28. Stebbins, G.L., *Chromosomal Evolution in Higher Plants*, London: Edward Arnold, 1971.
29. Madlung, A., Polyploidy and its effect on evolutionary success: old questions revisited with new tools, *Heredity*, 2013, vol. 110, no. 2, pp. 99–104.

*Translated by V. Mittova*