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NEW FORMS OF CHROMOSOME POLYMORPHISM IN *DESCHAMPSIA ANTARCTICA* DESV. FROM THE ARGENTINE ISLANDS OF THE MARITIME ANTARCTIC REGION

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Cytogenetic analysis of *D. antarctica* plants from the Argentine Islands of the Maritime Antarctic region was performed. Chromosome number 2n = 26 was determined for most of the samples. New forms of chromosome polymorphism for the species were demonstrated for the first time. In particular, the plants from Darboux Island were found, to have some cells that contained one supernumerary B-chromosome along with 26 chromosomes of a regular set. The plants from Great Yalour Island were determined to be mixoploid – the number of chromosomes ranged from 13 to 39. The occurrence of new and unknown earlier karyotypic forms of *D. antarctica* might have resulted from increased genome instability due to the extreme environmental conditions in the Argentine Islands region.
Key words: *Deschampsia antarctica* Desv., chromosome number, B chromosome, mixoploidy, chromosomal variability.

Нові форми хромосомного поліморфізму *Deschampsia antarctica* Desv. з регіону Аргентинських островів Прибережної Антарктики

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Реферат. Проведено цитогенетичний аналіз рослин *D. antarctica* з регіону Аргентинських островів Прибережної Антарктики. Для більшості досліджених зразків встановлено хромосомне число 2n = 26. Вперше для виду показано нові форми хромосомної мінливості. Зокрема, в клітинах апікальної меристеми рослин з острова Дарбо, окрім 26 хромосом основного набору, виявлено одну додаткову B-хромосому. Для рослин з о. Великий Ялур показано наявність міксоплоїдії: розмах мінливості за числом хромосом становив від 13 до 39 хромосом. Поява нових, невідомих раніше форм хромосомної мінливості *D. antarctica* може бути наслідком підвищеної геномної нестабільності, зумовленої екстремальними умовами зростання в районі Аргентинських островів.

Новые формы хромосомного полиморфизма *Deschampsia antarctica* Desv. из региона Аргентинских островов Прибрежной Антарктики

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Реферат. Проведен цитогенетический анализ растений *D. antarctica* из региона Аргентинских островов Прибрежной Антарктики. Для большинства исследованных образцов установлено хромосомное число 2n = 26. Впервые для вида показаны новые формы хромосомной изменчивости. В частности, в клетках апекальной меристемы растений с острова Дарбо, кроме 26 хромосом основного набора, обнаружена одна дополнительная B-хромосома. Для растений с о. Большой Ялур показано наличие миксоплоидии: размах изменчивости по числу хромосом составлял от 13 до 39 хромосом.

Появление новых, неизвестных ранее форм хромосомной изменчивости *D. antarctica* может быть следствием повышенной геномной нестабильности, обусловленной экстремальными условиями роста в районе Аргентинских островов.

1. Introduction

Antarctica is a region with the most severe climatic conditions on Earth. One of the top priorities for Antarctic research is to learn how Antarctic life evolved and survived (Kennicutt et al., 2014). Low temperatures, strong winds, low air humidity (as low as 5% in some Antarctic oases), snow storms, soils that are poor in organic compounds – all of these features create an extreme environment for plant life. Therefore, the study of *Deschampsia antarctica* Desv., one of only two vascular plant species that are native to Antarctica, is especially important. The unique success of Antarctic hairgrass in this region, in contrast to other plant species that have not been able to spread to the south beyond the sub-Antarctic zone, still remains a phenomenon that does not have a reasonable explanation (Parnikoza et al., 2011). One of the components of this success may be the mechanisms of genomic variation, which under adverse conditions provide increasing diversity and consequently increase the adaptive potential of a population.

Chromosome polymorphism in *D. antarctica* was shown for the first time by Cardone et al. (2009) in a cytogenetic study of plants from the area of the Argentine Antarctic station Jubany (now Carlini Base), which is located on King George Island. In some plants, along with diploid cells with 2n = 26, they found cells that have 13 and 28 chromosomes. The proportion of mixoploid plants was about 37%.

It is well known that unfavorable environmental conditions can lead to physiological stress, followed by growth of genomic variability, which can be manifested by the frequency of homologous recombination in somatic cells (Kunakh, 2005; Boyko, Kovalchuk, 2011), the activation of mobile genetic elements (Rebollo, 2010; Kunakh, 2013) and other changes. Variability occurs in the alteration of the number, type, shape and banding pattern of chromosomes at the karyotype level. However, data about changes in the karyotypic structure that are caused by environmental factors are extremely scarce. In order to shed light on this issue, we performed a study of the possible impact of the severe climate conditions in Antarctica on the karyotypic stability of *D. antarctica*. In order to do this, we carried out a cytogenetic analysis of plants from the Argentine Islands region of Maritime Antarctic, which are located about 350 km further south than the previously studied King George Island for the first time.

2. Materials and Methods

Plants used for the study were grown *in vitro* from seeds that had been collected from five localities in the Argentine Islands region (location of the Ukrainian Antarctic Station Academician Vernadsky) in Maritime Antarctic (table 1).

Rough and detailed maps of the sites where seeds were collected are shown in fig. 1. (Fig. 1–4 see the color paste 4.)

In order to obtain aseptic seedlings, dry seeds were sterilized and germinated as described in Zahrychuk et al. (2011/2012). The seedlings that were obtained were cultivated on a B5 agar nutrient medium (Gambourg, Eveleigh, 1968) that was supplemented with 0.1 mg/L α-naphthaleneacetic acid.

Apical meristems from roots that were 1-1.5 cm long were used for the cytogenetic analysis. For cell cycle synchronization and the accumulation of mitotic divisions, roots were kept in ice-cold water for 24 h. The roots were then fixed in methanol : glacial acetic acid at a ratio of 3 : 1 for 24 h at RT. Fixed roots were stored at –20°C. Root tips were macerated in an enzyme mixture comprising 2% (w/v) cellulase “Onozuka RS” (Serva) and 20% (v/v) pectinase from *Aspergillus*

Table 1

Information about the locations of the places where *D. antarctica* was sampled

Locality	Place where material was collected	Geographical coordinates	Year in which the seeds were collected
Darboux Island	Northern part of the island in the vicinity of a rocky grotto	S 65°23.707′, W 64°12.905′	season 2006/2007
Galindez Island	Caroline Point	S 65°14.955′, W 64°15.181′	season 2006/2007
Great Yalour Island	One of the fragments of the local population in the northeastern part of the island	*S 65°14.039′, W 64°9.761′*	season 2006/2007
Lahille Island	Fragment of a large population of the species on the southern coast	S 65°33.167′, W 64°23.249′	season 2009/2010
Rasmussen Cape	Culmination of a rocky plateau in the area of the cross	S 65°14.819′, W 64°5.156′	season 2004/2005

niger (Sigma) at 37°C, then squashed and stained with 0.5 mg/mL DAPI (4',6-diamidino-2-phenylindole, Serva).

Preparations were examined under a wide-field Olympus Provis AX70 epifluorescence microscope with a Hamamatsu C5810 CCD camera. Only metaphase spreads in which the number of chromosomes could be precisely counted were considered and subjected to statistical analyses (Plohynskyy, 1970).

3. Results and discussion

A cytogenetic analysis of plants from five localities in Maritime Antarctic revealed 2n = 26 chromosomes in their karyotypes that ranged in size from 3 µm to 10 µm (fig. 2). These results are consistent with the earlier data, where the same diploid chromosome number was reported for *D. antarctica* from the area of the Falkland and South Shetland Islands (Argentine Antarctic Station Jubany (now Carlini Base) (Moore, 1967; Cardone et al., 2009).

The results of the cytogenetic study of *D. antarctica* plants, which are presented in detail in table 2 show that plants of *D. antarctica* from three localities, i.e. Galindez, Lahille Islands and Rasmussen Cape have 2n = 26 chromosomes. Plants from the other two locations (Darboux and Great Yalour Islands) were determined to be mixoploid. Furthermore, a variation in the chromosome number was observed among individual plants.

The chromosome number of *D. antarctica* from Darboux Island ranged from 13 to 27 (table 2). This group of plants had a modal chromosome number of 26. The proportion of aneuploid cells ranged from 7.7 to 26.7%. Metaphases with 27 chromosomes (fig. 2, *c*) were found in *D. antarctica* from Darboux Island, which contained one supernumerary (B-chromosome) in addition to the regular set of 26 chromosomes (A chromosomes).

The range of variation in the number of chromosomes in *D. antarctica* from Great Yalour Island was found to be even wider and was from 13 to 39 chromosomes; these values correspond to the haploid and triploid chromosome set of this species (table 2). The modal chromosome number was 26 for two plants, while the third one demonstrated a predominance of cells with 36 and 39 chromosomes, although cells with 37 and 38 chromosomes were also found.

B chromosomes have also been found in other species of the genus *Deschampsia*, namely in *D. caespitosa* – 2n = 26 + 0-2 Bs and *D. wibeliana* – 2n = 26 + 0-5 Bs (Nkongolo et al., 2001). The occurrence of B chromosomes was also reported among the cereals – in rye 2n = 2x = 14 + Bs (Houben et al., 1996; Hasterok, 2002), maize 2n = 2x = 20 + Bs (Chiavarino et al., 1998) and other species (Kunakh, 2010). While the occurrence of B chromosomes is relatively common among

Table 2

Cytogenetic analysis of *D. antarctica* from the Argentine Islands region

Locality	Number of genotypes	Number of roots	Number of meta-phases	Number of chromo-somes*	Modal number of chromo-somes	Number of metaphases with modal count, %
Galindez Island	2	6	19	26(19)	26	100
		1	8	26(8)	26	100
Rasmussen Cape	1	6	12	26(12)	26	100
Lahille Island	1	3	32	26(32)	26	100
Darboux Island	2	5	14	26(12), 27(1)	26	92.3
		5	15	13(3), 18(3), 20(1), 26(8)	26	53.3
Great Yalour Island	3	6	17	26(16), 28(1)	26	94.1
		6	13	36(4), 37(2), 38(3), 39(4)	36, 39	30.8; 30.8
		7	45	13(1), 26(43), 38(1)	26	95.6

**Note*. The number of metaphase spreads with a given chromosome number is indicated in parentheses.

angiosperms, supernumerary chromosomes are generally found in plants that are growing in suboptimal or extreme conditions (Chiavarino et al., 1998; Kunakh, 2010). Therefore, there have been some hypotheses that have suggested a relationship between B chromosomes and the adaptive capacity of the plants, which could, for example, increase their resistance to abiotic factors, such as, drought and low temperatures. It cannot be ruled out that the occurrence of supernumerary chromosomes in a plant's karyotype might contribute to a better ability to colonize new ecological niches and that a high degree of the ecological plasticity of a species is associated with an increased occurrence of individuals with B chromosomes (Kunakh, 2010).

Mixoploidy has been observed in numerous plant species, e.g. *Plukenetia volubilis* L. (Cai et al., 2013), *Santalum album* L. (Zhang et al., 2010), many Brassicaceae (Snowdon, 2007; Kunakh et al., 2008; Ockendon, 2008) and species of *Bromus* L. (Joachimiak et al., 2001). In the meristems of various species, the proportion of cells with a chromosome number that differs from a diploid set can reach 77%. It has been suggested that mixoploidy is mainly a manifestation of an organism's reaction to adverse environmental conditions (Kunakh, 2005). It has been also hypothesized that mixoploidy may increase the adaptive capacity of plants.

The phenomenon of aneuploidy that was revealed in *D. antarctica* from Darboux Island may have resulted from the peculiarities of the biology and reproductive system of the species. *D. antarctica* has been shown to be able to spread by both vegetative and sexual propagation. Heterogeneity and partial (up to 25%) pollen sterility may indicate the ability of apomictic seed formation (Kravets, 2011/2012). A high level of aneuploidy has been reported in species that reproduce vegetatively (or by apomixis) (Kozyrenko et al., 2002).

The open question is what could be the reason for the occurrence of new forms of chromosome polymorphism in *D. antarctica* from the Argentine Islands region, which had not previously been observed in the South Shetland Islands (Cardone, 2009). In contrast to King George Island, the Argentine Islands region has a more severe, and therefore, a less favorable environment for vegetation in general and for *D. antarctica* in particular. This is not only due to its more southward location, but also because of the relief features. The habitats of *D. antarctica* in this area are confined to small (up to 0.54 km^2) rocky islands that rise no more than 65 m a.s.l. and the coastal oases of the Antarctic Peninsula. The areas that are available for colonization by plants are only located on the north- and northwest-facing slopes at the top of some hillocks that are isolated by depressions where snow does not melt even in summer. There are no extended flat areas available for vegetation in the Argentine Islands region that are typical of King George Island (fig. 3) where *D. antarctica* forms populations of thousands of individuals (Kozeretska et al., 2010).

The lack of suitable habitats and unfavorable soils, in which the rock substrate can reach 40-60% of the surface, impede the growth of *D. antarctica* populations in the Argentine Islands region. For instance, one of the typical populations on Galindez Island comprised only 227 plants in 2014 (fig. 4, *a*).

The populations of the Argentine Islands region can get access to slightly larger resources for growth only in favorable conditions, as is exemplified by a population of several thousand individuals from Rasmussen Cape and a population of about one thousand individuals from the southern part of Lahille Island (fig. 4, *b*).

However, even in the case of the most successful populations, the habitats of *D. antarctica* are limited to sites among rocks and in soil-filled cracks in the bedrock on rocky terraces that are mainly covered by bryophytes. Regardless of the degree of success and size, all of the localities that were studied are typical rocky habitats that are exposed to extreme environmental conditions for most of the year. The more extreme environmental conditions in the Argentine Islands region compared to the more northern regions can lead to genome instability and, as a result, to an increase in genetic variability. Increased genetic heterogeneity that at the chromosome level can be manifested as the appearance of mixoploidy, or even individuals with an altered karyotype, may enhance the adaptive potential of population. Furthermore, individuals with a different number of chromosomes that arise spontaneously in a population provide genetic material for the emergence of new forms, races and ultimately even species. Variations in the chromosome number such as polyploidy, mixoploidy, aneuploidy are considered to be one of the driving forces of plant evolution (Sedelnykova et al., 2013). The aforementioned makes it necessary to extend research into the intra-population chromosomal polymorphism in *D. antarctica* in order to clarify the phenomenon of the prevalence of various forms of chromosome variability in the Maritime Antarctic region that differ in extreme environmental conditions.

4. Conclusions

Data about a chromosome number 2n = 26 and the occurrence of mixoploidy that had previously been reported by other researchers were confirmed for *D. antarctica* from the Argentine Islands region of the Maritime Antarctic. New forms of chromosome polymorphism of *D. antarctica*, which are associated with mixoploidy, aneuploidy and the occurrence of supernumerary, most likely the B chromosome, were observed for the first time. The appearance of new forms of chromosome polymorphism in *D. antarctica,* which were not reported earlier on the South Shetland Islands, may result from an increase in genome instability in the harsher environmental conditions of the Argentine Islands region, which is located 350 km to the south.

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