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ВПЛИВ VEGF НА ПРОДУКЦІЮ ЕНДОТЕЛІАЛЬНИМИ КЛІТИНАМИ

Було встановлено, що VEGF та анти-VEGF мають протилежний ефект на ендотеліальних клітинах. VEGF стимулює вироблення NO та інгібує апоптоз, а анти-VEGF зменшує вироблення NO і індукують апоптоз.

Ключові слова: ендотеліальні клітини, апоптоз, VEGF.

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ВЛИЯНИЕ VEGF НА ПРОДУКЦИЮ ЭНДОТЕЛИАЛЬНЫМИ КЛЕТКАМИ

Было установлено, что VEGF та анти-VEGF имеют противоположный эффект на эндотелиальные клетки. VEGF стимулирует выработку NO и ингибирует апоптоз, а анти-VEGF уменьшает выработку продукции NO и индуцируют апоптоз

Ключевые слова: эндотелиальные клетки, апоптоз, VEGF.

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CLONING AND MOLECULAR-GENETIC ANALYSIS DESCHAMPSIA ANTARCTICA MITOCHONDRIAL SEQUENCES

First mitochondrial DNA was isolated from *Deschampsia antarctica*. Mitochondrial DNA sequences cloned into the pUC19 vector. Based on this vector a of recombinant clones pDac was created. Nucleotide fragments of the *D. antarctica* mitochondrial genome were sequenced and analyzed. The presence of mitochondrial DNA genes *D. Antarctica*: first and fifth NADH dehydrogenase subunits, 26S ribosomal RNA was found. Bioinformatic analysis of mitochondrial DNA sequences of *D. Antarctica* was made. The high (90%) homology with the mitochondrial genomes of representatives of the family Gramineae (*T. aestivum*, *O. sativa*, *B. oldhamii* etc.) was showed.

Key words: *Deschampsia antarctica*, mitochondrial genome, *Antarctica*, sequece.

Introduction: Subkingdom Embryophyte is represented in Antarctica flora only by two species: *Deschampsia antarctica* (Poacea) and *Colobanthus quitensis* (Caryophyllaceae). The science interest conditioned due to their adaptive abilities to survive in the hard climate conditions (low temperatures, high insensitive sunlight during the polar day etc).

Searching thought National Centre of National Center for Biotechnology Information databases showed, that nowadays is known 228 nucleotides sequences (without any mitochondrial DNA), only 86 proteins, 4 references on markers and genome mapping. The biomedical literature database showed only 33 articles about *D. antarctica* and references in 19 books. Therefore, *D. antarctica* isn't investigated enough.

Studies of physiological and biochemical mechanisms of plants stress adaptation to low temperature has the great theoretical and practical significance. Especially with regard to overcoming low temperature limits for farmed crops. Even a slight growing in frost resistance could cause a significant effect. Thus increasing hardiness of winter wheat by only 2°C could extend its production in the area that is used for spring wheat, which yields 25-40% lower.

It is very difficult to increase the adaptive capacity of agricultural plants using traditional breeding methods. Further enhance frost resistance required purposeful change in the genetic apparatus of plants: expression of certain genes, increasing their copies, including cryoprotective genes into the plant genome etc. This in way is impossible

without knowledge of the biochemical mechanisms of plant frost resistance.

The aim of this study was molecular genetic analysis of *Deschampsia antarctica* mitochondrial DNA, search and evaluation of the degree of homology with other members of the family Gramineae, identify possible mechanisms of adaptation to survive in climate of Antarctic region.

Materials and methods: Plants samples were obtained from the 15-th Ukrainian Antarctic expedition to the experimental station "Akademik Vernadsky", located on Cape Galindez Island Marina, 7 km from the western coast of the Antarctic Peninsula.

The plants were put into sterile culture by surface sterilization and cultivated for a long time in vitro [1].

Plant material in the amount of 10 g of fresh leaves of *D. antarctica*, was homogenized in a buffer solution that carried 0.33 M sorbitol, 50 mM Tris, 25 mM EDTA, β-mercaptoethanol 1%. Mitochondrias were isolated in stepwise sucrose density gradient (52% and 30% respectively) by conventional method [2]. Organelles were lysed in a buffer solution that included 50 mM Tris, 25 mM EDTA, β-mercaptoethanol 1%. The isolated DNA was deproteinized. Then it was hydrolyzed by endonuclease HindIII (Thermo Scientific) according to the manufacturer's recommendations. The resulting DNA fragments were cloned into plasmid vector pUC19. After transformation of recombinant plasmids in *Escherichia coli* strain XL-1 Blue, performed the selection of individual colonies and separation of plasmid DNA. These clones are studied through the

analysis of restriction enzymes HindIII, EcoRI, PstI and Sall. Restriction products were separated by electrophoresis in agarose gels. Selected clones were sequenced with primer M13REV. Comparative analysis of fragments already known nucleotide sequences Genetic Bank (GenBank) [3] was carried out using the Basic Local Alignment Search Tool (BLAST) on NCBI.

Results and discussion. As a result of cloning a library of recombinant clones pDac was created (Table 1). Size of cloned sequences ranged from 200 to 4000 bp This occurred the same length fragments, which were separated by mixes of three different restriction endonucleases (Fig. 1). In the first mixture were used enzymes HindIII, EcoRI, PstI,

in the second – HindIII, EcoRI, Sall, the third – HindIII, EcoRI, PstI, Sall [4].

On the next step was sequenced the longest fragments of selected clones using primer M13REV. Sequencing was performed at the Institute of Molecular Biology and Genetics, NAS of Ukraine Department biosynthesis of nucleic acids. Six clones have already sequenced. Two of the six sequenced sequences (clones pDac15 and pDac22) gave unsuccessful results in sequencing, which can be explained by the possible contamination of foreign DNA or insufficient cleaning of mixing. Characterization sequenced sequences presented in Table 2.

Assessing the degree of homology was used by resource leveling Blast NCBI. The data is presented in Table 3.

Tab. 1 The assignment of the recombinant clones pDac using electrophoresis in agarose gel after processing by restrictions mixes

Clone pDac*	HindIII	HindIII, EcoRI, PstI	HindIII, EcoRI, Sall	HindIII, EcoRI, PstI, Sall
252.6	4000, 100	4000, 100	4000, 100	4000, 100
270.9	1700	–	–	–
270.10	1500	1500	1100, 400	1100, 400
295.3	1500	1000	1000	1000
293.7	1350	1000, 350	1350	1000, 350
305	1200, 100	–	–	–
252.1	1200	700, 500	700, 500	700, 500
252.7	1200	700, 500	700, 500	700, 500
252.10	1200	700, 500	700, 500	700, 500
283.5	1000	1000	1000	1000
285.7	1000	1000	1000	1000
307	1000	1000	900, 100	900, 100
205.3	800, 700	–	–	–
270.4	800	600, 200	600, 200	600, 200
290.3	300	–	–	–
290.1	200	–	–	–

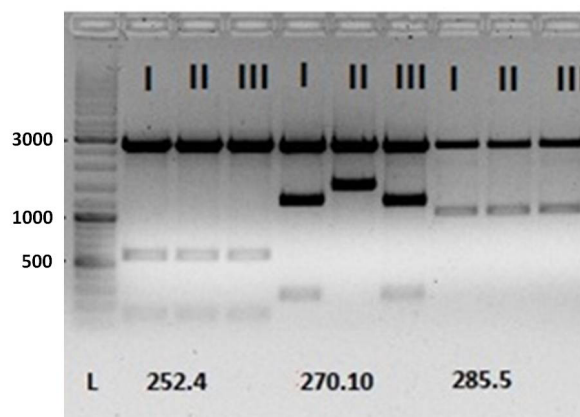


Fig. 1. Restriction analysis of pDac clones L Ladder Mix; arabic numerals show clone number; roman numerals show restriction mix: I HindIII, EcoRI, PstI; II HindIII, EcoRI, Sall; III HindIII, EcoRI, PstI, Sall

During the searching for nucleotide database for clone pDac.270.4 were found 122 adjustment with E-value from 0.00 to 2×10^{-23} . To characterize the plan it is important to consider the most similar matches – almost identical sequences. Thus, there are 122 sequence homologs for research in the database with E-number $\leq 10^{-10}$. These sequences can be considered close family. Clone pDac.270.10

showed 125 matches of E-number from 0.00 to 4.9. For clone pDac.283.5 were found 125 matches with E-value from 0.00 to 4.9. There are 13 sequences with E-value $\leq 10^{-10}$. These sequences can be considered as close family. To clone pDac.295.3 found 193 similar sequences in the database. They all belong to the genomic sequences of four plants: bamboo, sweet flag, sorghum and corn.

Tab. 2. The results of recombinant clones pDac sequencing

Clone pDac	Sequence size (nucleotides)	Gene	The highest homology
3	–	–	–
22	–	–	–
270.4	948	1-st ra 5-th subunits of the NADH dehydrogenase	<i>T. aestivum</i> (98%)
270.10	944	ribosomal RNA 26S and Open Reading Frame	<i>O. sativa</i> (91%)
283.5	935	1-st subunit of the NADH dehydrogenase	<i>B. oldhamii</i> (98%), <i>T. aestivum</i> (97%)
295.3	936	1-st ra 5-th subunits of the NADH dehydrogenase	<i>B. oldhamii</i> (99%), <i>T. aestivum</i> (98%)

Comparative analysis of mitochondrial sequences indicated the presence of the following genes: 26S ribosomal RNA, 1-st, 2-nd and 5-th subunits of NADH dehydrogenase. It was also found a sequence that similar to the open reading frame.

Analyzing the data sequence homology showed close relationships between *D. antarctica* and species from the family

Gramineae (Poaceae). The highest homology was observed to areas of mitochondrial genomes of wheat (*T. aestivum*), rice (*O. sativa*), bamboo (*B. oldhamii*). Also observed affinity to the species *Sorghum bicolor*, *Zea mays* and others.

Tab. 3. Results alignment using nucleotide database

Clone pDac	Plant Specie	Overlap (%)	Number of bases convergence (%)	E-value	Alignment weight
270.10	<i>Sorghum bicolor</i>	95	89	0.0	1117
	<i>Zea mais</i>	76	81	0.0	708
	<i>Oryza sativa</i>	77	87	0.0	1845
	<i>Sorghum bicolor</i>	95	89	0.0	1117
283.5	<i>Bambusa arnhemica</i>	96	97	0.0	1537
	<i>Ferocalamus rimosivaginus</i>	96	97	0.0	1531
	(<i>Sorghum bicolor</i>)	72	94	0.0	2072
	<i>Zea mais</i>	68	98	0.0	1084
295.3	<i>Bambusa arnhemica</i>	97	98	0.0	1605
	<i>Oryza sativa</i>	97	98	0.0	3069
	<i>Triticum aestivum</i>	97	98	0.0	1589
	<i>Ferocalamus rimosivaginus</i>	97	98	0.0	1596

Conclusions: The high degree of similarity of mitochondrial gene sequences between *D. antarctica* and plant crops indicates the close relationships of these species. However, the presence of variability in the sequences suggest that these variable regions may be responsible for the unique properties of *D. antarctica*.

Further analysis of mitochondrial DNA *D. Antarctica* will provide more reliable data on the molecular mechanisms of adaptation to cold, high levels of ultraviolet radiation, will shed light on the factors that induce flowering higher plants and vegetation in coastal Antarctica. It also

will determine affinity *D. antarctica* from different plant species, especially from *Poaceae* family.

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КЛОНУВАННЯ І МОЛЕКУЛЯРНО-ГЕНЕТИЧНИЙ АНАЛІЗ ПОСЛІДОВНОСТЕЙ МІТОХОНДРІАЛЬНОЇ ДНК ЩУЧНИКА АНТАРКТИЧНОГО

Вперше було виділено мітохондріальну ДНК щучника антарктичного. Послідовності мітохондріальної ДНК клонувано у вектор pUC19 і на його основі було створено бібліотеку рекомбінантних клонів pDac. Секвеновано і проведено аналіз нуклеотидних фрагментів мітохондріального геному *Deschampsia antarctica*. Встановлено наявність в мітохондріальній ДНК щучника антарктичного генів 1-ої та 5-ої субодиниць NADH дегідрогенази, 26S рибосомальної РНК. Проведено біоінформатичний аналіз послідовностей мітохондріальної ДНК *D. antarctica*. Показана висока (понад 90%) гомологія з мітохондріальними геномами представників родини Злакових (*T. aestivum*, *O. sativa*, *B. oldhamii* та ін.)

Ключові слова: Антарктида, *Deschampsia antarctica*, мітохондріальний геном, секвенування.

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КЛОНИРОВАНИЕ И МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЙ АНАЛИЗ ПОСЛЕДОВАТЕЛЬНОСТЕЙ МИТОХОНДРИАЛЬНОЙ ДНК ЩУЧНИКА АНТАРКТИЧЕСКОГО

Впервые было выделено митохондриальную ДНК щучника антарктического. Последовательности митохондриальной ДНК клонировано в вектор pUC19 и на его основе создано библиотеку рекомбинантных клонів pDac. Секвенировано и проведено анализ нуклеотидных фрагментов митохондриального генома *Deschampsia antarctica*. Установлено наличие в митохондриальной ДНК щучника антарктического генов 1-ой и 5-ой субъединиц NADH-дегидрогеназы, 26S рибосомальной РНК. Проведено биоинформатический анализ последовательностей митохондриальной ДНК *D. antarctica*. Показана высокая гомология с митохондриальными геномами представителей семейства Злаковых (*T. aestivum*, *O. sativa*, *B. oldhamii* та ін.)

Ключевые слова: Антарктида, *Deschampsia antarctica*, митохондриальный геном, секвенирование.