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В сборнике научных трудов публикуются обзорные и экспериментальные статьи в области молекулярной и прикладной генетики растений, микроорганизмов, животных, человека, отражающие исследования генетических процессов на молекулярном, клеточном, организменном и популяционном уровнях. Особое внимание уделяется наиболее актуальным проблемам геномики, генетической и клеточной инженерии. Публикуются результаты изучения генетических основ селекции растений, животных и микроорганизмов, разработки эффективных биотехнологий для сельского хозяйства, здравоохранения, охраны окружающей среды, биобезопасности.

Сборник предназначен для специалистов, работающих в области генетики, преподавателей, аспирантов и студентов ВУЗов биологического, сельскохозяйственного и медицинского профиля.

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DNA BARCODING TECHNOLOGY IN BELARUS: PROSPECTS AND NEEDS

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The DNA identification of rare and endangered plant species started in 2017 at the Republican DNA Bank. DNA barcoding is the best approach and tool to study flora and fauna and organize the obtained data in a special database. At present, the database contains barcodes (*ITS2, rbcL, psbA-trnH*) of 35 wild plant species growing in the territory of the Republic of Belarus. This is the first effort Belarus has made in compiling a Reference DNA Barcode Library to provide for the identification of rare and endangered plant species in the protected areas and biological collections.

Keywords: taxonomic identification, *ITS2, rbcL, psbA-trnH*, the Red Book of the Republic of Belarus.

Introduction

The Republic of Belarus acceded to the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity in 2014. To implement its Article 17 "Monitoring the Utilization of Genetic Resources", taxa verification of genetic resources should be conducted.

DNA barcoding is the best approach and tool to study flora and fauna and organize the obtained data in a special database. Scientific collaboration and training on the use of DNA barcoding techniques are in dire need for specialists in Belarus. The DNA identification of rare and endangered plant species started in 2017 at the Republican DNA Bank of a Human, Plants, Animals and Microorganisms with a view of being operational and closer to the use of DNA barcoding techniques to assess species diversity and their spread in the total gene pool of plants.

The Republican DNA Bank was established in 2013 at the Institute of Genetics and Cytology, NAS of Belarus, and in 2016, it acquired a status of the National Heritage. DNA Bank is a full-range and multifunctional structure. Its collections are depositories of biological material viable for the long-term scientific study. The nature protection activity of the DNA Bank is based on the study related to the genetic diversity of natural populations of wild species and the molecular genetic identification of species, including their taxonomic status verification.

Natural vegetation covers 67% of the territory of the Republic of Belarus and is represented by 230 types of plant communities formed by 12 thousand species of plants and fungi. From 4100 taxa of the higher plants registered in the Republic, about 1400 (34.15%) are representatives of the native fraction of flora and 2700 (65.85%) are adventive species [1]. Conservation of biological diversity is the national policy priority and is implemented through various mechanisms. The most effective among them is the development of a system of specially protected natural areas occupying 8.72% of the country's territory that corresponds to a national indicator of safety in the ecological sphere [2].

The necessary mechanism includes the inventory and mapping of rare and endangered species,

periodic monitoring of populations' state and determining the number of protected and resource-generating species [3]. Rare and endangered plant species are characterized by the lower ability to survive in the face of climate change and the pressure of anthropogenic factors, leading to the loss of valuable genotypes and a decrease in biodiversity in general. To date, the Red Book of the Republic of Belarus (4th edition, 2015) lists 303 species of wild-growing plants, including 189 vascular plant species falling under national nature conservation categories I, II, III and IV, which correspond to the following international categories: CR (critically endangered), EN (endangered), VU (vulnerable), NT (near threatened), respectively [4].

In our research, we aimed to perform the inventory of botanical resources using a DNA barcoding technique and establishing a Reference DNA Barcode Library of Rare and Endangered Plant Species.

Materials and Methods

DNA barcoding of rare plant species is carried out jointly with the National Coordination Centre on Access to Genetic Resources and Benefit-sharing. Collecting biological material for molecular genetic research on especially protected natural areas was performed by the members of environmental institutions and specialists in botany without removing plants from their habitats. Special methodological recommendations regulating collection, storage and transport of plant material for high-quality DNA isolation with no signs of degradation and impurities have been developed. Along with DNA barcoding, ecological and geographical description of rare plant populations is verified and is together with the morphological plant description entered into the Rare Plant Collection Database [5].

We researched the biological material of 35 rare and endangered plant species collected in the National Park "Narochansky" and in Brest Region (including the National Park "Belovezhskaya Pushcha" and the Transboundary Biosphere Reserve "Western Polesie") in 2016–2018 (Table 1). When collecting plants for their subsequent transfer to the Republican Bank, employees were guided only by the "belonging" of a species to the Red Book of the Republic of Belarus giving no advantage to the members of any particular family.

Table 1

List of plants studied using the DNA barcoding method

No.	Plant species	Conservation category *
1	*Abies alba* Mill.	I (CR)
2	*Anacamptis morio* L.	II (EN)
3	*Anemone nemorosa* L.	**
4	*Anemone sylvestris* L.	IV (NT)
5	*Arnica montana* L.	IV (NT)
6	*Betula nana* L.	II (EN)
7	*Botrychium matricariifolium* (Retz.) A. Br. Ex W. D.J. Koch	II (EN)
8	*Botrychium multifidum* (S. G. Gmel.) Rupr.)	III (VU)
9	*Cardamine bulbifera* L.	IV (NT)
10	*Cephalanthera longifolia* (L.) Fritsh	III (VU)
11	*Cephalanthera rubra* (L.) Rich.	III (VU)
12	*Daphne cneorum* L.	II (EN)
13	*Digitalis grandiflora* Mill.	IV (NT)
14	*Dracocephalum ruyschiana* L.	III (VU)
15	*Gentiana cruciata* L.	I (CR)
16	*Gentiana pneumonanthe* L.	**

Continuation of table 1

No.	Plant species	Conservation category *
17	*Gladiolus imbricatus* L.	IV (NT)
18	*Hedera helix* L.	II (EN)
19	*Iris sibirica* L.	IV (NT)
20	*Laserpitium latifolium* L.	III (VU)
21	*Lathyrus laevigatus* (Waldst. & Kit.) Gren.	III (VU)
22	*Lathyrus palustris* L.	**
23	*Lilium martagon* L.	IV (NT)
24	*Melittis melissophyllum* auct. P.P.	III (VU)
25	*Nuphar lutea* (L.) Sm.	**
26	*Potentilla alba* L.	III (VU)
27	*Prunus spinosa* L.	III (VU)
28	*Pulmonaria mollis* Wulf. ex Hornem.	III (VU)
29	*Pulsatilla patens* (L.) Mill	IV (NT)
30	*Quercus petraea* (Matt.) Liebl.	II (EN)
31	*Salvia pratensis* L.	IV (NT)
32	*Scorzonera austriaca* Willd.	I (CR)
33	*Thesium ebracteatum* Hayne	IV (NT)
34	*Trollius europaeus* L.	IV (NT)
35	*Tulipa sylvestris* L.	III (VU)

Footnote. * — I (CR) — critically endangered; II (EN) — endangered; III (VU) — vulnerable; IV (NT) — near threatened; ** — Plants No. 16, 22 and 25 not included in the 4th edition of the Red Book of the Republic of Belarus, but protected in the territories of adjacent States (Russia, Ukraine)

The plant material (leaves or their apical part, petals) was collected without removing plants from their habitats. For each species, DNA was isolated from three separately growing plants, except for the cases where a plant is currently represented by a single specimen (most often it is vulnerability category I or II) or cannot be collected for some other reasons. The collected plant material was stored before the DNA isolation at 4 °C. Before the DNA isolation, the samples were frozen in liquid nitrogen and comminuted in the porcelain mortar. Total DNA was extracted in line with the extraction protocol of the DNeasyPlant Mini Kit (Qiagen, Germany) as per producer's guidelines. DNA was normalized by adjusting its concentration to 12 ng/μL.

Three marker sequences were used to identify rare and endangered species of wild plants: *rbcL* (ribulose-bisphosphate carboxylase gene), *psbA-trnH* (intergenic spacer (*IGS*) regions of *psbA-trnH*) and *ITS2* (internal transcribed spacer).

The *ITS2* marker is the nuclear sequence region that forms part of a ribosomal cluster and is localized between the structural genes of the *5.8S* and *28S* ribosomal RNA. The *rbcL* gene is the most extensively characterized chloroplast plant gene. The plastid DNA *rbcL* region encodes a large subunit of ribulose-bisphosphate carboxylase — a key enzyme for fixing CO_2 in the dark phase of photosynthesis. The non-coding plastid DNA *psbA-trnH* region is the Intergenic Spacer (*IGS*) region and is located between the gene controlling the synthesis of the D1 protein of photosystem II and the histidine tRNA [6, 7].

Amplification of the marker sequences *rbcL*, *psbA-trnH* and *ITS2* was performed using specific primers (Table 2) in the final volume of the reaction mixture of 10μl (thermal cycler C1000 Touch Thermal Cycler, BioRad, USA).

Table 2

Primers for the marker sequence amplification

DNA barcode	Primer sequences	Expected PCR product size, bp
ITS2	5'-ATGCGATACTTGGTGTGAAT-3' 5'-GACGCTTCTCCAGACTACAAT-3'	~220-260 [9]
psbA-trnH	5'- GTTATGCATGAACGTAATGCTC-3' 5'- CGCGCATGGTGGATTCACAATCC-3'	~509 [10]
rbcL	5'-ATGTCACCACAAACAGAGACTAAAGC-3' 5'-GTAAAATCAAGTCCACCGCG-3'	~654 [10]

The number of DNA introduced into the reaction mixture constituted 12-20 ng/μl. The composition of the PCR mixture was standard (buffer 10x, 25M $MgCl_2$, 2mM dNTPs, 3% DMSO, 10μM of each primer, 5U/μl DNA polymerase). Primer sequences and basic recommendations for amplification conditions are freely available on the website of the International Center for DNA Barcoding — CCDB (Canadian Center for DNA Barcoding) [8]. The annealing temperature of primers to obtain the *ITS2* sequence was 58 °C, *rbcL* — 54 °C and *psbA-trnH* — 55 °C. The amplification results were checked in the agarose gel: 1% for *ITS2* and *rbcL* markers and 2% gel was used for the *psbA-trnH* marker due to the significant variability of amplification products by this marker.

Amplification products were purified using Exonuclease I and Shrimp Alkaline Phosphatase (Thermo Fisher Scientific, USA) enzymes as per producer's recommendations. The terminator reaction was performed using the commercial kit BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) followed by purification of the reaction product with ethanol. Identification of the nucleotide sequence was performed on the automatic genetic analyzer ABI 3500 DNA Analyzer (Applied Biosystems, USA). The chromatograms of sequences were analyzed using ChromasPro 13.3 and saved in the FASTA format. The alignment of target (marker) individual sequences for each plant of the same species to obtain the resulting sequence was performed using the program *MEGA X* (Molecular Evolutionary Genetics Analysis). The resulting nucleotide sequences were compared, using the National Center for Biotechnology Information, the Basic Local Alignment Search Tool (GenBank NCBI), with the DNA sequences stored in International Databases [11].

Results and Discussions

We based on the double loci genotyping scheme using *ITS2* and *rbcL* markers. If the data obtained are not sufficient for accurate plant species identification, then you can additionally use a psbA-trnH marker. As a result of amplification of the *ITS2* marker sequence, the product size in all the cases did not exceed 550 bp and in the case of *rbcL* amplification — 600–650 bp. This does not contradict the data of other authors [12]. It should be noted that as a result of using a pair of primers to the *ITS2* region recommended by the Canadian Center for DNA Barcoding (CCDB), not only *ITS2* is amplified, but also partially *5.8S* and *28S* regions. In this case, the size of the total product is most commonly 400–550 bp, which is twice the size of *ITS2* in angiosperms (220–240 nucleotides [9]). However, this does not interfere with further work.

The amplification products of *ITS2*, *rbcL* and *psbA-trnH* marker sequences were sequenced in the forward and reverse directions to obtain an individual nucleotide sequence of those markers for each plant. Individual sequences were aligned using *MEGA X*. As a result, two or three resulting marker sequences (*ITS2*, *rbcL* and *psbA-trnH*) were obtained for each of 35 species, which were compared with the similar ones recorded in the NCBI BLAST Database. Identification of a nucleotide sequence of a known biological species in NCBI completely coinciding with our sequence enables us to consider our sequence as a reference DNA barcode for a particular plant species in the territory of the Republic of Belarus.

The general scheme of the performed species identification is presented by *Scorzonera glabra Rupr. (austriaca)* used as an example, which belongs to category I of the national nature conservation significance. This is a relict boreal mountain taiga species occurring in Belarus in

an isolated locality — Myadzel District, Minsk Region. It grows as single specimens and in small groups in a very confined area. It is the only population, which includes several dozen well-developed flowering and fruiting individuals [4].

When amplifying DNA of *Scorzonera glabra Rupr. (austriaca)* (2 plants) using the *ITS2* marker, a fragment of ~450 bp in size was received; with *rbcL* ~600 bp. The amplification products of *ITS2* and *rbcL* were sequenced and 2 qualitative sequences of the *ITS2* region (reading length was 375 bp) and 2 qualitative sequences of the *rbcL* region (reading length was 536 bp) were obtained. The resulting *ITS2* marker sequence for *Scorzonera austriaca* from the National Park "Narochansky" by 97% coincided with the sequence AM117047.1 presented in NCBI for the species *Scorzonera austriaca*. Also, the *rbcL* marker sequence by 100% coincided with the sequence GQ436482.1 represented in NCBI for the *Scorzonera austriaca* species.

In the course of the work performed, good replicability of amplification results was demonstrated for all three markers, which corresponds to the criterion of universality [6]. With regard to the sequences' quality, the *ITS2* and *rbcL* markers were better meeting the requirement. The independent application of each of three markers made it possible to identify species of 66% plants under study (23 species). In doing so, *ITS2* showed the maximum contribution — 20 plant species out of 35. Table 3 shows the species identification results of the plants listed in the Red Book of the Republic of Belarus using *ITS2* marker.

ITS sequences are considered as reference sequences due to a number of merits: they are localized in the nuclear genome and are highly

Table 3

Identification results of 20 rare and endangered plant species using the *ITS2* marker

No.	Plant species	Species identity, % *	Marker sequence code **
1	*Abies alba*	99	JN177292.1
2	*Anacamptis morio*	100	Z94092.1
3	*Anemone nemorosa*	91	KX167066.1
4	*Arnica montana*	99	FM177855.1
5	*Betula nana*	99	KX167561.1
6	*Cardamine bulbifera*	99	KX167752.1
7	*Cephalanthera longifolia*	99	AY146447.1
8	*Gentiana cruciata*	99	DQ398634.1
9	*Hedera helix*	99	AM50387.2
10	*Iris sibirica*	100	MF543721.1
11	*Laserpitium latifolium*	100	FJ415131.1
12	*Lathyrus laevigatus*	98	DQ311967.1
13	*Lathyrus palustris*	99	KX166469.1
14	*Lilium martagon*	99	AF088203.1
15	*Melittis melissophyllum*	99	KX165838.1
16	*Nuphar lutea*	100	KX165445.1
17	*Pulmonaria mollis*	99	KT737694.1
18	*Quercus petraea*	99	FM244114.1
19	*Salvia pratensis*	100	KX166755.1
20	*Scorzonera austriaca*	97	AM117047.1

Footnote. * — % Similarity of the received *ITS2* region sequence of the species under study and the sequence represented in NCBI BLAST; ** — Code of the *ITS2* marker sequence in NCBI BLAST

variable; the accumulation rate of nucleotide substitutions and rearrangements in them, that is the rate of their evolution, is higher in comparison with mitochondrial and chloroplast genes; they are highly copyable — there are cases of up to 30 000 copies per cell, which ensures successful amplification of the region even with a small amount of material; point mutations predominate in the *ITS* region of nuclear DNA and long InDel is rarely occurring, and therefore *ITS* sequences in different plants vary insignificantly in length, which is usable for comparing of different species' sequences [7, 13]. The resemblance by *ITS2* in the studied species using NCBI data varied from 91 to 100%; most often that marker sequence coincided with the database by 99%. The obtained results confirm the possibility of using the *ITS2* sequence to identify plants at different taxonomic levels: generic, species and possibly subspecies.

The *rbcL* marker showed the maximum efficacy by the criterion "sequence quality". It is characterized by the most qualitative sequencing: a high reading length, minor errors with the possibility of subsequent manual correction. The similarity of *rbcL* marker sequences in the species under study with the NCBI data was 99–100% in almost all cases, except for *Lathyrus laevigatus*, *Pulmonaria mollis* and *Quercus petraea* species. The result of their genotyping at the *rbcL* locus must be repeated.

The amplification results with Intergenic spacer *psbA-trnH* were not so unambiguous. The marker proved to be the most variable in length — the sizes of the received amplicons varied from 300 to 1000 bp, which corresponds to the literature data on its features [13]. As distinct from the *ITS* region of nuclear DNA, which is characterized by the predominant accumulation of point mutations, structural rearrangements characteristic of the chloroplast genome as a whole occur most frequently in the *psbA-trnH* region of chloroplast DNA. InDel regions found in the *psbA-trnH* spacer most often constitute small inversions. These are insertions and deletions, which determine the difference in size of the *psbA-trnH* spacer in the compared taxa [12, 13]. In the course of the *psbA-trnH* marker amplification, we obtained several products for three species of plants (*Cardamine bulbifera, Gentiana pneumonanthe, Thesiume ebracteatum*) and the selection of another marker is required in such a case.

By the criterion "sequence quality", the *psbA-trnH* marker significantly conceded *ITS2* and *rbcL* despite the optimal reading length up to 732 bp. When using the *psbA-trnH* marker, the sequences with unambiguous nucleotide reading were received, which is consistent with the opinion of other authors that for the *trnH-psbA* fragment the significant variability of this marker by length hampers a sequence alignment procedure [7, 10]. However, for the three species in our study, the resolving power of *psbA-trnH* as an independent marker proved to be quite high. *Arnica Montana, Iris sibirica, Tulipa sylvestris, Botrychium matricariifolium, Hedera helix* and *Potentilla alba* were identified with an accuracy of 99–100% (AM690567.1, MF543660.1, AJ585047.1, KF700595.1, HE966656.1 and GQ384976.1, respectively). However, in other cases (*Cardamine bulbifera*, *Anacamptis morio*, *Pulsatilla patens*) the *psbA-trnH* marker identified plants only to the genus. To efficiently identify rare wild plants, *psbA-trnH* should be used along with other marker sequences.

Thus, the used marker sequences (*ITS2, rbcL, psbA-trnH*) allow to perform the taxonomic identification of rare and endangered plant species. The convergence of DNA analysis with classical methods of botanical research makes it possible to accurately identify wild plants, which allows for the effective inventory of genetic resources. However, in order to increase the resolving power of DNA barcodes, they should be used in an integrated manner. A four-loci panel is planned for use in the future.

Conclusion

Direct analysis of DNA nucleotide sequences will allow to enhance the traditional systems of classification. In Belarus, this is the first effort to compile a Reference DNA Barcode Library with a view of identification of rare and endangered plant species in National Parks and detecting errors or any divergences in the taxa identification. Scientific collaboration and training on the use of DNA barcoding techniques are in dire need for specialists in Belarus.

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References

1. Convention on biological diversity. Fifth National report. Belarus. 59p. (In Russ.) [Electronic resource]: – Mode of access: https://www.cbd.int/doc/world/by/by-nr-05-ru.pdf . – Date of access: 17.04.2019.

2. News of Belarus / Area of especially protected natural territories in Belarus in 2017 increased almost by 13 thousand hectares [Electronic resource]: – Mode of access: http://www.belta.by/society/view/ploschad-osobo-ohranjaemyh-prirodnyh-territorij-v-belarusi-v-2017-godu-vozrosla-pochti-na-13-tys-ga-289996-2018. (In Russ.). – Date of access: 22.10.2018.

3. Buchenkov, I.E. Vegetable resources of Belarus, rational use and protection: short course of lectures / I.E. Buchenkov. – Minsk, MGEU of A. D. Sakharov. – 2012. – 73 p. (In Russ.).

4. Red Book of Republic of Belarus. Plants: the rare and being under the threat of disappearance species of wild-growing plants / Main editorial board: I. M. Kachanovsky (chairman), M. E Nikiforov, V. I. Parfyonov, etc. 4th edition. – Minsk: Belarusian encyclopedia, 2015. – 448 p. (In Russ.).

5. Studying of bioecological features of populations of rare plants in the Brest region, monitoring, sampling and development of cartographic web applications: report on research (final) / Brest State A. S. Pushkin University; head N. M. Matusevich. – Brest, 2018. – 69 p. – (In Russ.).

6. Kress, W. J. A two-locus global DNA barcode for land plants: the coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region / W. J. Kress, D. L. Erickson. – 2007. – PLoS ONE. 2 (6). – e508. – doi:10.1371/journal.pone.0000508.

7. Molecular markers for plant species identification and phylogenetics of plants / T. V. Matveeva [et al.] // Ecological genetics. – 2011. – Vol. 9, № 1. – P. 32–43 (In Russ.).

8. CCDB [Electronic resource] / Canadian Centre for DNA Barcoding. – Mode of access: http://ccdb.ca/. – Date of access: 17.01.2018.

9. Use of *ITS2* Region as the Universal DNA Barcode for Plants and Animals / H. Yao [et al.] // PLoS One. – 2010. – Vol. 5, № 10. – e13102. – doi: 10.1371/journal.pone.0013102.

10. Hollingsworth, P. M. Choosing and using a plant DNA barcode / P. M. Hollingsworth, S. W. Graham, D.P. Little // PLoS One. – 2011. – Vol. 6, № 5. – e19254. – doi: 10.1371/journal.pone.0019254.

11. NCBI BLAST [Electronic resource]: National Center for Biotechnology Information, Basic Local Alignment Search Tool. – Mode of access: https://blast.ncbi.nlm.nih.gov/Blast.cgi. – Date of access: 02.04.2018.

12. A DNA barcode for land plants / CBOL Plant Working Group // Proc. Natl. Acad. Sci. USA. – 2009. – Vol. 106, № 31. – P. 12794–12797. – doi: 10.1073/pnas.0905845106.

13. Identification of Amazonian Trees with DNA Barcodes / M. A. Gonzalez [et al.] // PLoS ONE. – 2009. – Vol. 4, № 10. – e7483. – doi:10.1371/journal.pone.0007483.

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ТЕХНОЛОГИЯ ДНК-ШТРИХКОДИРОВАНИЯ В БЕЛАРУСИ: ПЕРСПЕКТИВЫ И ВОСТРЕБОВАННОСТЬ

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В 2017 году в Республиканском банке ДНК начата генетическая идентификация редких и находящихся под угрозой исчезновения видов растений. ДНК-штрихкодирование является наилучшим методом для изучения флоры и фауны и последующей систематизации результатов в специальной базе данных. В настоящее время база данных содержит штрихкоды (*ITS2, rbcL, psbA-trnH*) 35 диких видов растений, произрастающих на территории Республики Беларусь. Это первая попытка Беларуси составить справочную библиотеку штрихкодов ДНК для идентификации редких и находящихся под угрозой исчезновения видов растений на охраняемых территориях и в биологических коллекциях.

Ключевые слова: таксономическая идентификация, *ITS2, rbcL, psbA-trnH*, Красная книга Республики Беларусь.

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