IRSTI: 34.15.25 https://doi.org/10.26577/eb.2023.v97.i4.08

E. Kyrbassova[1*], A. Sartayeva[1], M. Parmanbekova[1], E. Imanova [1], G. Yernazarova [2]

[1]Kazakh National Women's Teacher Training University, Kazakhstan, Almaty
[2]Al-Farabi Kazakh National University, Kazakhstan, Almaty
*e.kyrbasova@gmail.com

PHYLOGENETIC ANALYSIS OF THE RBCL GENE SEQUENCE OF THE PLANT GENUS *AEGOPODIUM* L.

Herbal plants have been used in the treatment of many diseases since ancient times. The need for medicinal plant research is growing day by day. In this context, the study, identification of effective prospective medicinal plants will be relevant. Genetic taxonomic identification is more important than morphological identification of herbal plants. It is known that one of the markers of chloroplasts used in plant species identification and phylogenetic studies is the sequences of the rbcL gene. The purpose of our work is to study phylogenetic analysis by bioinformatic methods using rbcl markers.

The article presents the result of a phylogenetic analysis conducted to determine the relationship of *Aegopodium alpestre* Ledeb plants. according to the sequence of the rbcL gene with other species of the genus Aegopodium L., as well as with representatives of the Apiacea family from the database.

Key words: rbcL, *Aegopodium L.*, *Aegopodiumalpestre* Ledeb., isolation DNA, phylogenetic analysis.

Э.А. Кырбасова[1*], А.А. Сартаева[1], М.Х. Парманбекова[1], Э.М. Иманова[1], Г.И. Ерназарова[2]

[1]Қазақ ұлттық қыздар педагогикалық университеті, Қазақстан, Алматы қ.
[2]Әл-Фараби атындағы Қазақ ұлттық университеті, Қазақстан, Алматы қ.
*e-mail: e.kyrbasova@gmail.com

Aegopodium L. туысына жататын өсімдіктердің rbcL генінің бірізділіктерінің филогенетикалық талдауы

Көптеген ауруларды емдеуде дәрілік өсімдіктер ерте кезден бастап қолданылып келеді. Дәрілік өсімдіктерді зерттеудің қажеттілігі күннен-күнге артып отыр. Осы тұрғыда, перспективті дәрілік өсімдіктерді зерттеп тиімділерін анықтау және оларды идентификациялау өзекті болып табылмақ. Дәрілік өсімдіктерді морфологиялық идентификациялауға қарағанда, генетикалық жағынан таксономиялық анықтаудың маңыздылығы жоғары. Өсімдік түрлерін идентификациялау мен филогенетикалық зерттеулерде қолданылатын хлоропластық маркелрердің бірі – rbcl генінің бірізіділіктері екені белгілі. Жұмысымыздың мақсаты: rbcl маркерін қолдана отырып, биоинформатикалық әдістердің көмегімен филогенетикалық талдау жасауды зерттеу.

Мақалада преспективті *Aegopodium alpestre* Ledeb. дәрілік өсімдігінің жапырағынан бөліп алынған rbcL генінің секвенсі жасалып, дерекқордағы Aegopodium L. туысына жататын өсімдік түрлерімен және сондай-ақ шатыргүлділер тұқымдасының өкілдерімен туыстық қатынасын анықтауда жүргізілген филогенетикалық талдау нәтижесі берілген.

Түйін сөздер: rbcL, Aegopodium L., *Aegopodiumalpestre* Ledeb., ДНҚ бөліп алу, филогенетикалық талдау.

Э.А. Кырбасова[1]*, А.А. Сартаева[1], М.Х. Парманбекова[1], Э.М. Иманова[1], Г.И. Ерназарова[2]

[1]Казахский национальный женский педагогический университет, Казахстан, г. Алматы
[2]Казахский национальный университет имени аль-Фараби, Казахстан, г. Алматы
*e-mail: e.kyrbasova@gmail.com

Филогенетический анализ последовательности гена rbcL растений рода Aegopodium L.

При многих заболеваниях лекарственные растения используются в лечении с древних времен. Потребность в исследованиях лекарственных растений растет день ото дня. В

перспективных лекарственных растений. Генетическая таксономическая идентификация имеет большее значение, чем морфологическая идентификация лекарственных растений. Известно, что одним из маркеров хлоропластов, используемых в идентификации видов растений и филогенетических исследованиях, является последовательности гена rbcl. Цель нашей работы: изучение филогенетического анализа биоинформатическими методами используя маркера rbcl.

В статье представлен результат филогенетического анализа, проведенного при определении родства растений *Aegopodium alpestre* Ledeb. по последовательности гена rbcL с другими видами рода Aegopodium L., а также с представителями семейства зонтичных из базы данных.

Ключевые слова: rbcL, Aegopodium L., *Aegopodiumalpestre* Ledeb., выделение ДНК, филогенетический анализ.

Introduction

Aegopodium L. is a genus of perennial plants of the Apiaceae family, widely distributed in Europe and Asia. Currently, 12 species of this genus are known [1], and in Kazakhstan, there are two species: common ashweed (*Aegopodium podagraria L.,)* and alpine ashweed (*Aegopodium alpestre Ledeb*.).Representatives of plants of the genus *Aegopodium L.* have long been used in folk medicine to treat various diseases (gout, inflammatory diseases). Also, common ashweed is included in drugs for the prevention and treatment of oncological diseases [2].

The Apeaceae are a large family of angiosperms, including many medically important species. The ability to identify these species and their admixtures is important, but difficult to do due to subtle morphological differences in the fruits and the frequent lack of diagnostic characters in surviving specimens [3].Species identification and assessment of intraspecific genetic polymorphism are the most important tasks not only of modern plant genetics, but also of plant science. To solve these problems, many different methods of search and research of taxonomically significant sections of DNA were developed, which were called molecular or DNA markers. [4].One of these markers is the chloroplast marker rbcL gene [5-7]. DNA barcoding techniques combined with metabolomics, transcriptomics and proteomics can enable the authentication of plant products [8]. Plant barcoding can be used to distinguish species within a genus and to preserve DNA from the same species[9, 10].

DNA barcoding is one of the methods for rapid species identification using a short DNA fragment containing 400-800 bp.The Consortium for the Barcode of Life (CBOL) [11] recommended ribulose bisphosphate carboxylase/oxygenase (RbCl) as one of the candidate loci with high potential for plant barcode generation. This is because the RbCl gene has been well characterized, so primer design can be easily improved. In addition, RbCl has high versatility and high discrimination ability [12, 13]

The work carried out a molecular genetic analysis of *AegopodiumalpestreLedeb*. using modern methods of molecular biology to determine the genetic relationship of this plant with other species of *Aegopodium L.,* also with the *Apiaceae* family.

Materials and Methods

DNA isolation and amplification of the rbcL gene

DNA was obtained by a modified CTAB method from leaves of *Aegopodiumalpestre*Ledeb. plants [14-16]. The detergent CTAB (cetyltriethylammonium bromide) is good at breaking down the cell membrane and separating DNA from polysaccharides.DNA quality and quantity were checked by electrophoresis [17, 18] in a 1.4% agarose gel in 1x TAE buffer (0.04 M-Tris HCl, 0.02 M CH_3COONa, 0.01 M, EDTA, pH 8.0). To prepare a 1.4% agarose gel, take 1.4 g of agarose per 100 ml of 1x TAE buffer. The mixed agarose and buffer were brought to a boil in a microwave oven at high power until the agarose was completely dissolved. The mixture was cooled to 40-50^{0}C and 7 μl of ethidium bromide at a concentration of 10 mg/ml was added, mixed, and poured into the mold. A comb was placed to form wells for applying samples, then the gel was cooled until it hardened.

PCR was performed using MyTaq red mixe (Bioline) [19, 20]. The forward primer used in this study was RBClaf (5'-ATG CCA CAA ACA GAG ACT AAA GC-3') and the reverse primer was RBClar (5'-GTA AAA TCA AGT CCA CCA CG-3') with a total PCR volume of 30 ml. The PCR program was 95^{0}C for denaturation, 55^{0}C for annealing and 72^{0}C for degradation and 72^{0}C for final degradation. The ZymocleanTM DNA Gel Recovery Kit (Zymo Research) is used to obtain purified PCR products.

Phylogenetic analysis

The following programs were used to analyze the obtained data:

1. BLASTn was used to compare the resulting sequence with DNA sequences from GenBank. The program compares user-entered nucleotide or protein sequences with all available nucleotide or protein sequences in the NCBI database, and calculates percentage statistics of the total matching of each pair of compared sequences.

2. Sequences were aligned and analyzed using the Clustal Omega program. Phylogenetic trees were constructed using the Neighbor-Joining (NJ) and Maximum parsimony (MP) methods in the MEGA 11 program. The stability of phylogenetic trees in NJ and MP analyzes was assessed using the bootstrap method.

The neighbor joining (NJ) method in phylogenetic analysis can describe the clarity of species identification; the difference is limited to cluster and node. A sample can be in the same cluster, even if they are from different areas [21].

Results and Discussion

DNA barcoding is one way to contribute to the Barcode of Life database aimed at collecting reference sequences [22, 23]. This work used variation in short, standardized gene regions to identify new species [24]. The first step in DNA barcoding was to extract the total DNA from the sample. DNA was isolated from the leaves of *Aegopodiumalpestre*Ledeb. The next step was to determine the quality and quantity of DNA using a DNA spectrophotometer and agarose gel electrophoresis. We determined the concentration of isolated DNA and its purity using a spectrophotometer (Fig. 1).

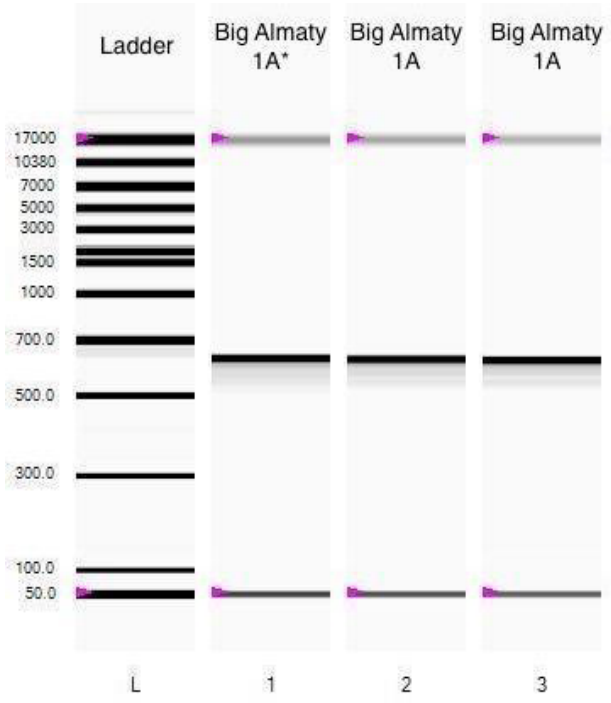


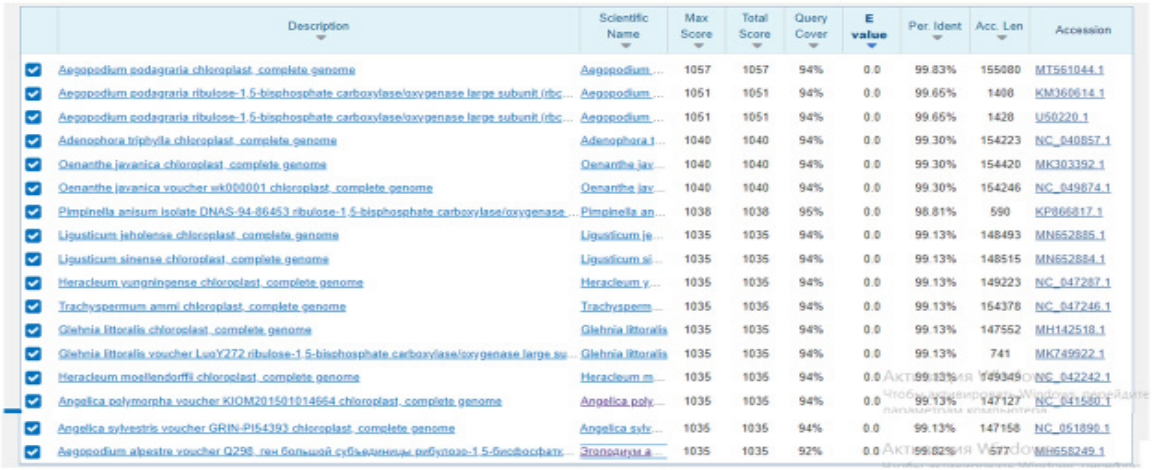
Figure 1 – Electropherogram of the rbcL gene of *Aegopodium alpestre* Ledeb.

After determining the concentration and purity of the rbcL gene of *Aegopodium alpestre* Ledeb. performed sequencing.rbcL is a fragment of the coding region of the chloroplast gene. The rbcL fragment has low species resolution, but in angiosperms it has relatively high species resolution [25].

The RbCl gene that was successfully amplified from the *Aegopodium alpestre* Ledeb plant accession in this study was 610 bp in length.

>A.alpestre
NNNNGNNNCTANGCAGGTGTTGGATTCAAAGCT
GGGGTTAAAGATTACAAATTGACTTATTATACTCCGG
ACTATGAAACCAAAGATACTGATATCTTGGCAGCATT
CCGAGTAACTCCTCAACCCGGAGTTCCACCTGAAGAA
GCGGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTA
CATGGACCACTGTGTGGACCGATGGACTTACCAGCCT
TGATCGTTACAAAGGGCGCTGCTACGGAATCGAGCC
CGTTGCTGGAGAAGAAAATCAATTTATCGCTTATGTA
GCTTACCCATTAGACCTTTTTGAAGAAGGTTCTGTTA
CTAACATGTTTACTTCCATTGTAGGTAATGTATTTGG
GTTCAAAGCCTTGCGCGCTCTACGTCTGGAAGATCTG
CGAATCCCCGTTGCTTATGTTAAAACTTTCCAAGGAC
CGCCACATGGCATCCAAGTTGAGAGAGATAAATTGA
ACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAA
ACCTAAATTGGGGTTATCCGCTAAAAACTACGGTAGA
GCGGTTTATGAATGTCTCCGCGGTGGACTTGATTTTA
CGTCATACNTGGTTTTTCCTGANN

Then, using BLAST, we searched for similar nucleotide sequences (Fig. 2).



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Aegopodium podagraria chloroplast, complete genome	Aegopodium ...	1057	1057	94%	0.0	99.83%	155080	MT561044.1
Aegopodium podagraria ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbc...	Aegopodium ...	1051	1051	94%	0.0	99.65%	1408	KM360614.1
Aegopodium podagraria ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbc...	Aegopodium ...	1051	1051	94%	0.0	99.65%	1428	U50220.1
Adenophora triphylla chloroplast, complete genome	Adenophora t...	1040	1040	94%	0.0	99.30%	154223	NC_040857.1
Oenanthe javanica chloroplast, complete genome	Oenanthe jav...	1040	1040	94%	0.0	99.30%	154420	MK303392.1
Oenanthe javanica voucher wk000001 chloroplast, complete genome	Oenanthe jav...	1040	1040	94%	0.0	99.30%	154246	NC_049874.1
Pimpinella anisum isolate DNAS-94-86453 ribulose-1,5-bisphosphate carboxylase/oxygenase...	Pimpinella an...	1038	1038	95%	0.0	98.81%	590	KP866817.1
Ligusticum jeholense chloroplast, complete genome	Ligusticum je...	1035	1035	94%	0.0	99.13%	148493	MN652885.1
Ligusticum sinense chloroplast, complete genome	Ligusticum si...	1035	1035	94%	0.0	99.13%	148515	MN652884.1
Heracleum yungningense chloroplast, complete genome	Heracleum y...	1035	1035	94%	0.0	99.13%	149223	NC_047287.1
Trachyspermum ammi chloroplast, complete genome	Trachysperm...	1035	1035	94%	0.0	99.13%	154378	NC_047246.1
Glehnia littoralis chloroplast, complete genome	Glehnia littoralis	1035	1035	94%	0.0	99.13%	147552	MH142518.1
Glehnia littoralis voucher LuoY272 ribulose-1,5-bisphosphate carboxylase/oxygenase large su...	Glehnia littoralis	1035	1035	94%	0.0	99.13%	741	MK749922.1
Heracleum moellendorffii chloroplast, complete genome	Heracleum m...	1035	1035	94%	0.0	99.13%	149349	NC_042242.1
Angelica polymorpha voucher KIOM2015010014664 chloroplast, complete genome	Angelica poly...	1035	1035	94%	0.0	99.13%	147127	NC_041580.1
Angelica sylvestris voucher GRIN-PI54393 chloroplast, complete genome	Angelica sylv...	1035	1035	94%	0.0	99.13%	147158	NC_051898.1
Aegopodium alpestre voucher Q298 :ген большой субъединицы рибулоза-1,5-бисфосфатк...	Эгопадиум а...	1035	1035	92%	0.0	99.82%	577	MH658249.1

Figure 2 – Sequence analysis of the rbcl gene of *Aegopodium alpestre* Ledeb. in the BLAST program

As a result of searching for similar sequences using the BLAST program, it was revealed that the nucleotide sequences of *Aegopodium alpestre* Ledeb. coincides with sequences from the database with *Aegopodium podagraria* (MT561044.1) by 99.83%, with *Aegopodium podagraria* (KM360614.1) – 99.65%; with *Aegopodium alpestre* (MH658249.1) – 99.82%, and with some plants of the umbrella family – 99.13%.

The neighbor joining (NJ) method in phylogenetic analysis can describe the clarity of species identification; the difference is limited to cluster and node. A sample can be in the same cluster, even if they are from different areas [7]. The relationship of species based on genetic similarity is shown in the phylogenetic tree.

In order to determine the genetic relationship of *Aegopodium alpestre* Ledeb. with the *Aegopodium* family, including *Aegopodium L.*, a phylogenetic tree was constructed and analyzed using the sequences obtained from the gene database.

In order to reveal the genetic relationship of the plant *Aegopodium alpestre* Ledeb. with the umbrella family, including the genus *Aegopodium L.*, a phylogenetic tree was constructed and analyzed (Fig. 3).

The sequence similarity of the rbcL gene of P. anisum plants (KP866817.1) is higher than that of related genes in the other clade. This is a 90% large branch (marked in green by Large Clade), which is considered a bootstrap analysis that is repeated 1000 times. The plants in this green square all belong to the genus *Aegopodium L.*

Those marked in yellow themselves form one large branch (Clade), but their similarity to the rbcL gene sequences of the *A. alpestre* plants (sequenced by us) is distant.

Boostrap values:
Strong: >90%
Well: <70-90%
Weak: 50 -70%
No: <50%

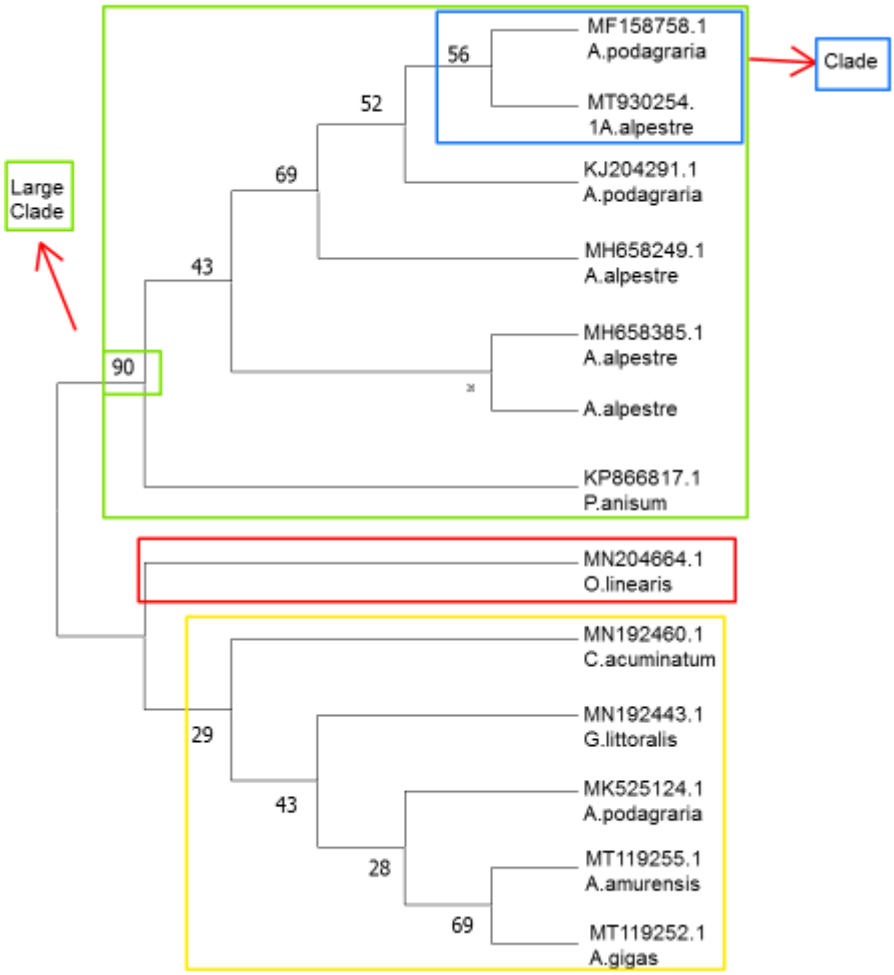


Figure 3 – Tree built using the nearest neighbor method with bootstrap support (Bootstrapped Neighbor-Joining Tree)

Conclusion

The RbCl gene that was successfully amplified from the *Aegopodium alpestre* Ledeb. plant accession in this study was 610 bp in length. The sequence was 99.83% similar to sequences from the *Aegopodium podagraria* database (MT561044.1) and 99.83% similar to *Aegopodium alpestre* (MH658249.1).

Acknowledgment

The authors of the article express their gratitude to Professor of the Mississippi Valley State University (USA) Newsam Abigail for providing methodological assistance in conducting bioinformatics analyses.

Conflicts of Interest

All authors participated in the analysis and interpretation of the results, are familiar with the contents of the article and have no conflicts of interest.

References

1. «Aegopodium L.» *Plants of the World Online. Royal Botanic Gardens, Kew*. Retrieved 2022-12-16.

2. Грудзинская Л.М., Гемеджиева Н.Г., Нелина Н.В., Каржаубекова Ж.Ж. Аннотированный список лекарственных растений Казахстана. – Алматы, 2014. – 200 с.

3. Liu J, Shi L, Han J, Li G, Lu H, Hou J, Zhou X, Meng F, Downie SR. Identification of species in the angiosperm family Apiaceae using DNA barcodes. MolEcolResour. 2014 Nov;14(6):1231-8. doi: 10.1111/1755-0998.12262. Epub 2014 May 14. PMID: 24739357.

4. Нигматуллина Н.В., Кулуев А.Р., Кулуев Б.Р. Молекулярные маркеры, применяемые для определния генетического разнообразия и видоидентификации дикорастущих растений// Биомика, 2018. – Т. 10, №3. – С. 290-318.

5. Manhart JR. Phylogenetic analysis of green plant rbcL sequences. Mol Phylogenet Evol. 1994 Jun;3(2):114-27. doi: 10.1006/mpev.1994.1014. PMID: 8075831.

6. Savolainen V, Chase MW, Hoot SB, Morton CM, Soltis DE, Bayer C, Fay MF, de Bruijn AY, Sullivan S, Qiu YL. Phylogenetics of flowering plants based on combined analysis of plastid atpB and rbcL gene sequences. Syst Biol. 2000 Jun;49(2):306-62. doi: 10.1093/sysbio/49.2.306. PMID: 12118410.

7. Kang, Y., Deng, Z., Zang, R. *et al.* DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests. *Sci Rep* **7**, 12564 (2017). https://doi.org/10.1038/s41598-017-13057-0

8. Mahima K, Sunil Kumar KN, Rakhesh KV, Rajeswaran PS, Sharma A, Sathishkumar R. Advancements and future prospective of DNA barcodes in the herbal drug industry. Front Pharmacol. 2022 Oct 21;13:947512. doi: 10.3389/fphar.2022.947512. PMID: 36339543; PMCID: PMC9635000.

9. Marghali S, Zitouna N, Gharbi M, Fadhlaoui I, Trifi-Farah N. Evolution of rbcL among Lathyrus and Kupicha's classification. Genet Mol Res. 2014 Oct 27;13(4):8729-39. doi: 10.4238/2014.October.27.14. PMID: 25366764.

10. W.John Kress. Plant DNA baecodes: Applications today and in the future// Journal of Systematics and evolution, 2017 https://onlinelibrary.wiley.com/doi/10.1111/jse.12254

11. CBOL Plant Working Group. 2009. A DNA barcode for land plants. PNAS106(31): 12794-12797.DOI: 10.1073/pnas.0905845106

12. StevanusPharmawati M. 2021. Biodiversity and phylogenetic analyses using DNA barcoding rbcL gene of seagrass from Sekotong, West Lombok,Indonesia. Biodiversitas, Volum 22, /1, P: 50-57

13. Jianli Wang et al. Screening of universal DNA barcodes for identifying grass species of Gramineae// Front. Plant Sci., 2022 Sec. Plant Bioinformatics. Volum 13https://www.frontiersin.org/articles/10.3389/fpls.2022.998863/full

14.Schenk JJ, Becklund LE, Carey SJ, Fabre PP. What is the "modified" CTAB protocol? Characterizing modifications to the CTAB DNA extraction protocol. Appl Plant Sci. 2023 Jun 2;11(3):e11517. doi: 10.1002/aps3.11517. PMID: 37342162; PMCID: PMC10278931.

15. Gupta N. DNA Extraction and Polymerase Chain Reaction. J Cytol. 2019 Apr-Jun;36(2):116-117. doi: 10.4103/JOC.JOC_110_18. PMID: 30992648; PMCID: PMC6425773.

16.de Boer H, Rydmark MO, Verstraete B, Gravendeel B (2022) Molecular identification of plants: from sequence to species. Advanced Books. https://doi.org/10.3897/ab.e98875

17. Abdel-Latif, A., Osman, G. Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. *Plant Methods* **13**, 1 (2017). https://doi.org/10.1186/s13007-016-0152-4

18. Arslan M, Tezcan, Camcı H, Avcı MK. Effect of DNA Concentration on Band Intensity and Resolution in AgaroseGel Electrophoresis. Van Sag BilDerg 2021, 14, (3) 326-333. https://dergipark.org.tr/en/download/article-file/1874337

19. Samuel Kwawukume, Frank J. Velez, David Williams, Leqi Cui, Prashant Singh, Rapid PCR-lateral flow assay for the onsite detection of Atlantic white shrimp, Food Chemistry: Molecular Sciences,Volume 6,2023, 100164, ISSN 2666-5662,https://doi.org/10.1016/j.fochms.2023.100164.

20. AlmiraZada, Puspasri S. Susanto, Raden L7 Putri, et al. DNA-FFPE isolation methods and performances of PCR kits// Cell MolBiol (Noisy le Grand) 2018, Volume 64, Issue 13

21. Che J, Chen H-M, Yang J-X, Jin J-Q, Jiang K, Yuan Z-Y, Murphy R W and Zhang Y-P 2012 Universal COI primers for DNA barcoding amphibians: UNIVERSAL COI PRIMERS FOR DNA BARCODING AMPHIBIANS Molecular Ecology Resources 12 247–58.

22. Geary, J. and Bubela, T., 2019. Governance of a global genetic resource commons for non-commercial research: A case-study of the DNA barcode commons. *The Commons Journal*, 13(1), p.205-243.DOI: https://doi.org/10.18352/ijc.859

23.Andreas Kolter and Birgit Gemeinholzer. 2021. Plant DNA barcoding necessitates marker-specific efforts to establish more comprehensive reference databases. *Genome*. **64**(3): 265-298. https://doi.org/10.1139/gen-2019-0198

24. Gutteridge A and Burns M 2013 The Application of DNA Molecular Approaches for the Identification of Herbal Medicinal Products Journal of the Association of Public Analysts

25. Huang X.C., Ci X.Q., Conran J.G., Li J. Application of DNA barcodes in Asian tropical trees—A case study from xishuangbanna nature reserve, Southwest China. *PLoSONE.* 2015;10:e0129295. doi: 10.1371/journal.pone.0129295.

References

1. Abdel-Latif, A., Osman, G. Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. *Plant Methods* **13**, 1 (2017). https://doi.org/10.1186/s13007-016-0152-4

2. «Aegopodium L.» *Plants of the World Online. Royal Botanic Gardens, Kew*. Retrieved 2022-12-16.

3. AlmiraZada, Puspasri S. Susanto, Raden L7 Putri, et al. DNA-FFPE isolation methods and performances of PCR kits// Cell MolBiol (Noisy le Grand) 2018, Volume 64, Issue 13

4.Andreas Kolter and Birgit Gemeinholzer. 2021. Plant DNA barcoding necessitates marker-specific efforts to establish more comprehensive reference databases. *Genome*. **64**(3): 265-298. https://doi.org/10.1139/gen-2019-0198

5. Arslan M, Tezcan, Camcı H, Avcı MK. Effect of DNA Concentration on Band Intensity and Resolution in AgaroseGel Electrophoresis. Van Sag BilDerg 2021, 14, (3) 326-333. https://dergipark.org.tr/en/download/article-file/1874337

6. CBOL Plant Working Group. 2009. A DNA barcode for land plants. PNAS106(31): 12794-12797.DOI: 10.1073/pnas.0905845106

7. Che J, Chen H-M, Yang J-X, Jin J-Q, Jiang K, Yuan Z-Y, Murphy R W and Zhang Y-P 2012 Universal COI primers for DNA barcoding amphibians: UNIVERSAL COI PRIMERS FOR DNA BARCODING AMPHIBIANS Molecular Ecology Resources 12 247–58.

8.de Boer H, Rydmark MO, Verstraete B, Gravendeel B (2022) Molecular identification of plants: from sequence to species. Advanced Books. https://doi.org/10.3897/ab.e98875

9. Geary, J. and Bubela, T., 2019. Governance of a global genetic resource commons for non-commercial research: A case-study of the DNA barcode commons. *The Commons Journal*, 13(1), p.205-243.DOI: https://doi.org/10.18352/ijc.859

10. Gupta N. DNA Extraction and Polymerase Chain Reaction. J Cytol. 2019 Apr-Jun;36(2):116-117. doi: 10.4103/JOC.JOC_110_18. PMID: 30992648; PMCID: PMC6425773.

11. Grudzinskaya L.M., Gemedzhieva N.G., Nelina N.V., Karzhaubekova ZH.ZH.Annotated list of medicinal plants of Kazakhstan. – Almaty, 2014. – 200 p.

12. Gutteridge A and Burns M 2013 The Application of DNA Molecular Approaches for the Identification of Herbal Medicinal Products Journal of the Association of Public Analysts

13. Huang X.C., Ci X.Q., Conran J.G., Li J. Application of DNA barcodes in Asian tropical trees—A case study from xishuangbanna nature reserve, Southwest China. *PLoSONE.* 2015;10:e0129295. doi: 10.1371/journal.pone.0129295.

14. Jianli Wang et al. Screening of universal DNA barcodes for identifying grass species of Gramineae// Front. Plant Sci., 2022 Sec. Plant Bioinformatics. Volum 13https://www.frontiersin.org/articles/10.3389/fpls.2022.998863/full

15. Kang, Y., Deng, Z., Zang, R. *et al.* DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests. *Sci Rep* **7**, 12564 (2017). https://doi.org/10.1038/s41598-017-13057-0

16. Liu J, Shi L, Han J, Li G, Lu H, Hou J, Zhou X, Meng F, Downie SR. Identification of species in the angiosperm family Apiaceae using DNA barcodes. MolEcolResour. 2014 Nov;14(6):1231-8. doi: 10.1111/1755-0998.12262. Epub 2014 May 14. PMID: 24739357.

17. Mahima K, Sunil Kumar KN, Rakhesh KV, Rajeswaran PS, Sharma A, Sathishkumar R. Advancements and future prospective of DNA barcodes in the herbal drug industry. Front Pharmacol. 2022 Oct 21;13:947512. doi: 10.3389/fphar.2022.947512. PMID: 36339543; PMCID: PMC9635000.

18. Manhart JR. Phylogenetic analysis of green plant rbcL sequences. Mol Phylogenet Evol. 1994 Jun;3(2):114-27. doi: 10.1006/mpev.1994.1014. PMID: 8075831.

19. Marghali S, Zitouna N, Gharbi M, Fadhlaoui I, Trifi-Farah N. Evolution of rbcL among Lathyrus and Kupicha's classification. Genet Mol Res. 2014 Oct 27;13(4):8729-39. doi: 10.4238/2014.October.27.14. PMID: 25366764.

20. Nigmatullina N.V., Kuluev A.R., Kuluev B.R.Molecular markers used to determine the genetic diversity and video identification of wild plants// Biomika, 2018. – Vol. 10, No. 3. – pp. 290-318.

21. Samuel Kwawukume, Frank J. Velez, David Williams, Leqi Cui, Prashant Singh, Rapid PCR-lateral flow assay for the onsite detection of Atlantic white shrimp, Food Chemistry: Molecular Sciences,Volume 6,2023, 100164, ISSN 2666-5662,https://doi.org/10.1016/j.fochms.2023.100164.

22. Savolainen V, Chase MW, Hoot SB, Morton CM, Soltis DE, Bayer C, Fay MF, de Bruijn AY, Sullivan S, Qiu YL. Phylogenetics of flowering plants based on combined analysis of plastid atpB and rbcL gene sequences. Syst Biol. 2000 Jun;49(2):306-62. doi: 10.1093/sysbio/49.2.306. PMID: 12118410.

23.Schenk JJ, Becklund LE, Carey SJ, Fabre PP. What is the "modified" CTAB protocol? Characterizing modifications to the CTAB DNA extraction protocol. Appl Plant Sci. 2023 Jun 2;11(3):e11517. doi: 10.1002/aps3.11517. PMID: 37342162; PMCID: PMC10278931.

24. StevanusPharmawati M. 2021. Biodiversity and phylogenetic analyses using DNA barcoding rbcL gene of seagrass from Sekotong, West Lombok,Indonesia. Biodiversitas, Volum 22, /1, P: 50-57

25. W.John Kress. Plant DNA baecodes: Applications today and in the future// Journal of Systematics and evolution, 2017 https://onlinelibrary.wiley.com/doi/10.1111/jse.12254