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Molecular Genetic Identificatin of Plants of the Genus Artemisia L. Growing in Southern Regions of Kazakhstan

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Abstract

The purpose of this research study is to carry out a molecular genetic identification of the plant species of the genus *Artemisia L.* growing in Southern Kazakhstan. Such methods as barcoding, PCR analysis, sequencing and electrophoresis were involved. In the course of DNA barcoding, universal plastidial markers for plants were used: rbcL, matK and trnH-psbA. Three samples of *Artemisia L.* plants were studied for further determination of genetic relationship between species, and identification thereof. Molecular genetic passportization was done based on the obtained results. Molecular genetic analysis enabled identification of the species-level for *Artemisia L.* samples, and selection of the most promising plants. It was demonstrated that all samples belong to the same species, namely *Artemisia annua L.* The longest genetic distance between contrast samples is 0.9%. The advised methodology may serve as a model for genetic passportization of resource plant species, as well as an approach to assessing the state of gene pools in population.

Keywords: genetic analysis; molecular markers; DNA markers; genetic passportization; *Artemisia L.*

1. Introduction

Botany being part of the biodiversity conservation system and the source of new technologies, currently represents one of the priority areas for scientific development. Fundamental research is in full swing worldwide addressing the issues of systematics, floristics, and genetics for rational use and protection of plants (Pavlov, 1961; Aubakirova et al., 2014; Chen et al., 2010; Graham et al., 2010).

In consideration of highly informative content of DNA molecules, a number of molecular genetic analyses of cultivated plants using molecular markers, known as DNA markers, is rapidly on the rise in the global scientific practice (Drobot and Matveeva, 2015; Leonova, 2015; Chase, 2003). Such methods of DNA molecular analysis can be used for genetic passportization of plant varieties instead of the low-informative methods of protein marker analysis (Cho et al., 2004; Hajibabaei et al., 2007; Kaliyeva et al., 2015).

At present, the molecular or DNA marker detection technologies have become a relevant standard for plant identification, and are increasingly and widely used around the world (Valentini et al., 2009). Their introduction provides for accurate and quick identification of genetic diversity of populations, subspecies, species, as well as differentiation of higher taxonomic ranks: genus and family. Besides, it makes it possible to create the genetic fingerprints of varieties and, from the cost perspective, to determine economically valuable criteria as early as at the initial stage of selection at the level of DNA (Delabays et al., 2001; Graham et al., 2010). Such methods can become the basis for genetic passportization of varieties, lines and hybrids of various cultivated plants. Study and conservation of plant genetic resources, including medicinal plants, is the key aspect of sustainable development of agroindustry in any country. The loss of such resources and, consequently, of genetic diversity, represents a harsh reality of today (Mezhnina and Urbanovich, 2016; Rzepka-Plevneš et al., 2009).

Among the most effective and inexpensive methods for detecting intraspecific polymorphism are such analyses as ISSR (Inter Simple Sequence Repeats, with its primer comprising a microsatellite sequence); SSR (Simple Sequence Repeats: microsatellite markers); and SNP (Single Nucleotide Polymorphism). SNP analysis is the most informative of them, and it is likely to experience wide acceptance in genetic testing and breeding of cultivated plants. It is acknowledged, that different types of markers are suitable for studying polymorphism both within one species and within higher taxonomic ranks (genus, family, etc.) (Omasheva et al., 2016; Sukhareva and Kuluev, 2018; Pavlov, 1961).

A range of papers discuss that rbcL gene of the chloroplast genome can be used for phylogenetic analysis (Cai et al., 2017; Alrawashdeh, 2011; Ferreira et al., 2005; Kavrakova et al., 2015). RbcL gene underwent in-depth research, and is now widely used in the phylogenetic analysis of plants (Omasheva et al., 2016; Boronnikova, 2009; von Cräutlein et al., 2011). The resolution of this gene is quite low. In this respect, for the purposes of the phylogenetic analysis, it is used in conjunction with other markers, such as matK and trnH-psbA.

MatK gene is one of the fastest evolving plastidial genes of plants; and together with other markers used in

barcoding, it is responsible for increasing resolution in line with the plant identification (Pavlov, 1961; Hall, 1999; Kaliyeva et al., 2014).

TrnH-psbA gene represents the most variable fragment of plastidial DNA, and can be successfully used for the phylogenetic analysis of plants (Chen et al., 2010; PS et al., 2017).

Passportization of varieties is particularly required for the purposes of certification and commercial distribution of seeds. Besides, the methods underlying genetic passportization can be successfully adapted by plant breeders in their breeding activities: for instance, for early detection of genetic markers of valuable phenotypic properties, as well as for consolidation and preservation of achievements in selection. The methods of genetic passportization are used worldwide, as well as in seed production and control of purchased seed lots in terms of their compliance with the declared grade because fraud is not uncommon there.

Methods of electrophoretic determination of polymorphism of proteins for passportization of cultivated plant varieties are still popular in Russia and in other countries (Omasheva et al., 2016; Mower et al., 2007). Nonetheless, protein markers are currently considered obsolete owing to a great number of drawbacks. They are more and more seldom used in the course of genetic analysis of cultivated plants (Kanukova et al., 2019). More promising for genetic passportization of agricultural plants are considered DNA markers-based methods. There is an obvious shift in passportization of varieties from the protein polymorphism analysis to a system enabling detection of polymorphic DNA loci in Russia over the past few years (Drobot and Matveeva, 2015; Oladosu et al., 2015). Unfortunately, in the present-day Russia, selection of cultivated plants often takes place solely on the grounds of phenotypic. Owing to this, the creation of new varieties takes long. In order to improve efficiency of breeding activities and reduce the time required to create a variety, application of advanced methods of genetic analysis seems relevant.

ITS2-amplicon sequencing based on a new generation of NGS sequencing technology is the most preferred method for DNA barcoding of plant compounds. This method enables management of highly degraded DNA in the multicomponent plant compounds, and makes it possible to determine species membership for all ingredients in multicomponent phyto preparations (Leonova, 2015). Various studies focused on the genetic transformation containing rol B and rol C genes (Chase, 2003). Random amplified polymorphic DNA (RAPD) markers were applied in a number of studies to investigate genetic variability of wild medicinal plants growing in Jordan. In order to plot a dendrogram and similarity matrix, 10 primers displaying polymorphous bands were used (Trifonova et al., 2016). ISSR amplification was applied to study polymorphisms of microsatellite sequences in genome, and to estimate genetic diversity (Kaliyeva et al., 2015).

One of the immediate priority of public health service is to extend the range of medicines used by introducing new plant products into traditional medicine. Long-term medical practice proves that phyto preparations are not inferior in terms of their effectiveness to synthetic analogues for treatment of a number of diseases, and given the absence of side effects and contraindications, they are even superior to synthetic analogues.

Among various medicinal plant species growing in Kazakhstan, *Artemisia L.* genus is of particular interest. The plants of this genus stand out from other medicinal plants used in traditional and folk medicine. It is famous for its anti-inflammatory, hypolipidemic, antimalarial, antitumor, bronchodilating, anticonvulsant properties (Kocherina and Chesnokov, 2016; Boyko, 2013). In accordance with the obtained patent, the method for extraction of essential oil from *Artemisia porrecta* was demonstrated (Rakhimberdiyeva et al., 2020). Essential oil obtained from *Artemisia L.* has a bactericidal and bacteriostatic action, and provides an antimicrobial effect in treating inflammatory airway diseases.

The first round of clinical trials with regard to the extract from *Artemisia L.* plants proved the effectiveness of this agent to treat SARS-CoV-2, i.e., the American scientists succeeded in stopping the spread of the virus in cells in laboratory conditions using the plant. The study outcome was published on the bioRxiv website. *Artemisia annua* is widely used in a number of countries to treat tropical malaria, and waseven proved helpful to treat SARS-CoV-1, the virus that caused an outbreak of SARS in the years of 2002-2003. The scientists confirmed that in the form of hot aqueous solution, the extract manifests antiviral activity against SARS-CoV-2. Moreover, *Artemisia L.* plants are active against the virus after its penetration into cells. In the future, the researchers will have to continue the trials in order to understand whether it is possible to create a drug based on the ground in powder dried leaves of the plant. According to their assumptions, the drug can become an inexpensive and safe remedy against a new coronavirus, and can find its application in the cases when vaccination is constraint (Balandina, 2021).

Molecular genetic identification of artemisia species shall ensure the selection of the economically important ones and selection thereof. Passportization of rare relict plant species is another compulsory step to implement gene pool conservation of these species at the population level.

The purpose of this research was to conduct a molecular genetic identification of the plant species of *Artemicia L.* genus growing in South Kazakhstan – *Artemisia karatavica (Krasch. & Abolin ex Poljakov)*, *Artemisia cina Berg ex Poljak*., and *Artemisia porrecta (Krasch. ex Poljakov)* – by molecular marking their genomes at the population level to assess the state of population gene pool.

2. Materials and Methods

The molecular genetic research study was conducted in 2019.

The material for the study comprised three species of *Artemicia L.* genus: *Artemisia karatavica Krasch. & Abolin ex Poljakov* (Artemisia karatavica), *Artemisia cina Berg ex Poljak*. (Artemisia cina) and *Artemisia porrecta Krasch. ex Poljakov* (Artemisia porrecta) from the family Asteraceae. The collection of plants took place in various areas of vegetation in the Turkestan region of Kazakhstan: Shardarinsky district (Komsomol village); Baydibek district (Shakpak village); Aryssky district (Darmino

village). The collected study samples entered their flowering stage.

DNA purification. For the purposes of purification, there were used three samples of each species of *Artemisia L*: *Artemisia karatavica Krasch. & Abolin ex Poljakov* (Artemisia karatavica), *Artemisia cina Berg ex Poljak*. (Artemisia cina), and *Artemisia porrecta Krasch. ex Poljakov* (Artemisia porrecta). Genomic DNA was purified from the aboveground parts of *Artemisia L*. using CTAB and TE buffers (Sukhareva and Kuluev, 2018) of certain modifications. 100 mg of leaves were pulverized in a cooled pounder in presence of 1 ml of extraction buffer (100 mM Tris-HCl pH 8.0; 20 mM EDTA, pH 8.0; 1, 4M NaCl, 2% CTAB, PVP and 2-mercaptoethanol were added before use to reach the resulting concentration of 2 and 0.2% respectively). The resulting homogenate was incubated at 60°C for 30 minutes, and then extracted with chloroform. 0.5 volumes of 5M of chloride of sodium and 2 volumes of ethanol were added into a water phase. The compound was incubated at 4°C for 15-20 minutes, and then centrifuged for 15 minutes at 13000 g. The sediment of DNA was washed with 70% ethanol, and dissolved in 100 µl double-distilled water. DNA was treated with RNA. The assessment of DNA quality and amount was done by electrophoresis with 1% agarose gel in 1xTAE buffer, and by absorption at wavelengths of 260 and 280 nm by spectrophotometry employing Nanodrop 2000 (Thermo Scientific, USA).

DNA barcoding: The following markers were used to barcode DNA of artemisia: two plastidial DNA genes (rbcL and matK), and one spacer region (trnH2/psbA) (Hajibabaei et al., 2007).

Amplification of PCR of 20 µl in volume was done in Eppendorf Vapo.protect Mastercycler thermal cycler (USA) and containing as follows: 2 µl of 10xTAQ buffer (750 mM TrisHCl, pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 2.5 µl of 25 mM $MgCl_2$, 0.4 µl of 10 mM compound of deoxynucleotide triphosphates (dNTP), 0.4 µl of 10 mM direct and reverse oligonucleotides of one of the markers, 12.3 µl of deionized sterile water and 1 TAQ polymerase unit. The concentration of genomic DNA was 40-60 ng/20 µl. The annealing point was selected depending on the marker sequence. The synthesis was carried out at the following temperature: one cycle at 94°C for 4 minutes; 35 cycles consisting of the following stages: 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 1 minute; and the final cycle at 72°C for 10 min.

DNA quality and quantity testing was done by electrophoresis with 1.4% agarose gel in 1xTAE buffer (0.04 M-Tris HCl, 0.02 M CH_3COONa, 0.01 M EDTA, pH 8.0). 1.4 g of agarose per 100 ml of 1xTAE buffer was measured to prepare 1.4% agarose gel. The agarose mixed with buffer was boiled in a microwave oven at high power to complete the dissolution of agarose. The compound was cooled down to 40-50°C, and then 7 µl of ethidium bromide was added into 10 mg/ml concentration, stirred and poured into a reservoir. Dividing strips were placed in the reservoir to form wells needed for sample application. Then the gel was cooled to its hardening.

At the end of the amplification program, 5 µl of each PCR reaction were tested by electrophoresis with 1% agarose gel containing ethidium bromide, and visualized in UV light using BioRad gel documentation system. The remnants of PCR reaction were cleared through GeneJet PCR Purification Kit column (Thermo Scientific, USA). Once purified, PCR concentration of the product was measured. At that, per every sequencing reaction there were taken 10 to 20 ng depending on the product length.

The quality of DNA samples was also tested by PCR amplification of 18S ribosomal DNA using the primers as follows:

5′-GAGAAACGGCTACCACATCCAAGG-3′;

5′- rCCATGCACCACCACCCATAGAATC-3′.

The assumed amount of product reached 870-bp. PCR was done in the resulting volume of 25 µl, containing 0.2 µm of deoxyribonucleotide triphosphate, 0.2 µm of each primer, 0.5 UTaqDNA polymerase (Thermo Scientific, USA), 2.5 µm, and 40 ng of DNA in 1xTAQ Buffer with $(NH_4)_2SO_4$ (750 Mm of Tris-HCl, pH 8.8, at 25°C, 200 µm of $(NH_4)_2SO_4$ and 0.1% (v/v) Tween 20). Amplification: initial denaturation at 94°C for 2 min; 25 cycles at 94°C for 30 sec, annealing at 67°C for 15 sec, and synthesis at 72°C for 15 sec. The final elongation was done at 72°C for 10 min.

Sequencing was done by ABIPrizm 310 genetic analyzer (Applied Biosystems, USA) according to the instruction manual. The comparison of nucleotide sequences was accomplished using DNAMAN and BioEdit software complexes (Delabays, 1997).

Selection of markers and PCR conditions. Three ISSR markers were used to assess genotypes. The material for the assessment comprised 10 samples. In all further experiments, DNA with the indicators of absorption relation ranging from 1.68 to 1.87 at wavelengths of 260/280 was used (SmartSpecPlus spectrophotometer, Bio-Rad).

PCR was done in the volume of 20 µl containing 2 µl 10xTAQ of the buffer (750 Mm of TrisHCl, pH 8.8, 200 µm $(NH_4)_2SO_4$, 0.1% of Tween 20), 2.5 µl of $MgCl_2$, 0.4 µl of 10 mM compound of deoxyribonucleotide triphosphate (dNTP), 0.8 µl 10 mM of oligonukleotid of the used marker, 12.7 µl of deionized sterile water and 1xTAQ of polymerase. The concentration of genomic DNA was 40-60 ng/20 µl. Amplification was done according to the following sequence: one cycle at 94°C for 2 minutes; 35 cycles consisting of the following stages: 94°C for 40 sec, 44°C for 45sec, 72°C for 1.5 minutes; and the final cycle at 72°C for 15 min.

Electrophoresis of the amplification products obtained using ISSR-PCR was done with 2% agarose gel. After staining with ethidium bromide, UV visualization followed using BioRad gel documentation system. In order to control the amplified DNA, GeneRulerTM 1kb (Fermentas, USA) marker was used.

Phylogenetic analysis. Sequence alignment was achieved using UGENE software. Plotting of genetic trees using nucleotide DNA barcoding sequences was done in NCBI-blast module.

When measuring the genetic distance, the maximum likelihood technique or maximum composite likelihood technique was used.

Only DNA fragments of good reproduction in repeated experiments were taken into account. The intensity of the fragments was disregarded. The obtained data were computer-processed using PopGen32 software, as well as the specialized GenAlEx6 macros for MS Excel.

3. Results

At the outcome of sequencing three samples of *Artemisia L*. the nucleotide sequences of rbcL, matK and trnH-psbA for each sample were obtained (Figure 1). Numbers 1 to 3 in the figure are rbcL, matK and trnH-psbA samples respectively.

When comparing the nucleotide sequences of rbcL gene with the known sequences of rbcL from NCBI genetic database, the studied samples formed a cluster containing eudicots of the greatest genetic affinity with various species of *Artemisia L*. (Figure 2).

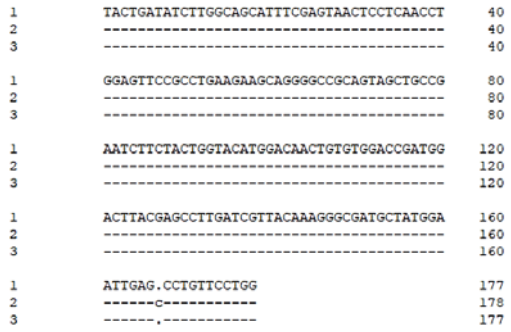

Figure 1. Comparison of nucleotide sequences of rbcL for three samples of Artemisia L.

Comparison of the nucleotide sequences of rbcL for three studied samples was done with the sequences of NCBI database (National Center for Biotechnology Information).

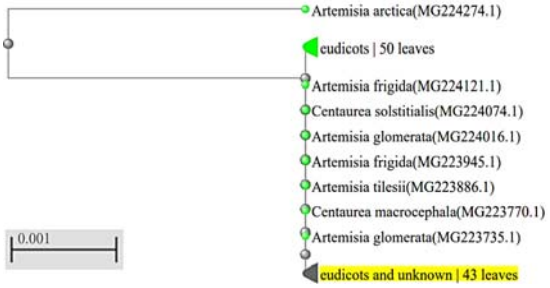

Figure 2. Nucleotide sequences of rbcL for Artemisia L. samples

Note: marked in yellow is the cluster containing the studied samples.

When comparing the nucleotide sequences of matK gene of 286-bp in the first and third samples, the sequences proved to be identical. The second sample in matK sequence contained deoxycytidine and deoxyadenosine in positions 265 and 266 respectively (Figure 3).

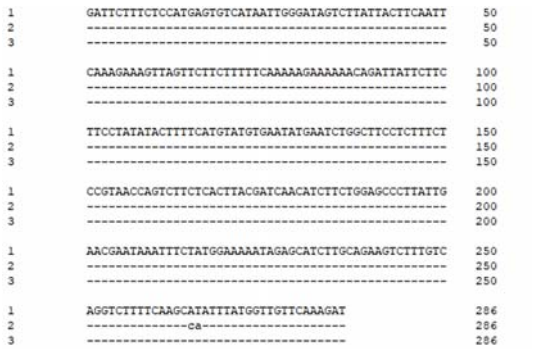

Figure 3. Comparison of nucleotide sequences of matK for three samples of Artemisia L.

Based on the analysis of matK nucleotide sequences of the studied samples, it was possible to plot the phylogenetic tree with a genetic distance relative to the known species of the international genetic database NCBI (Figure 4).

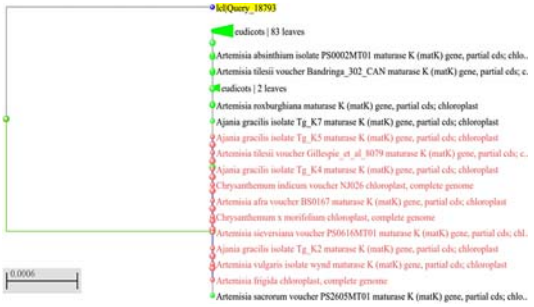

Figure 4. Nucleotide sequences of matK for Artemisia L. samples.

Note: marked in yellow are the studied samples.

The outcome of the phylogenetic analysis proves that the studied samples form monophyletic groups with different species of *Artemisia L*. genus.

At the outcome of the analysis of three samples of trnH-psbA sequence of 255-bp. no variations were recorded. The sequences of three samples are identical (Figure 5).

Figure 5. Comparison of nucleotide sequences trnH-psbA for three samples of Artemisia L.

Phylogenetic analysis of the studied samples using trnH-psbA sequence versus the known sequences from NCBI database revealed the genetic distance between different *Artemisia L*. species (Figure 6). Genetic distance (GD) is a measure of genetic divergence between species, or between populations within one species.

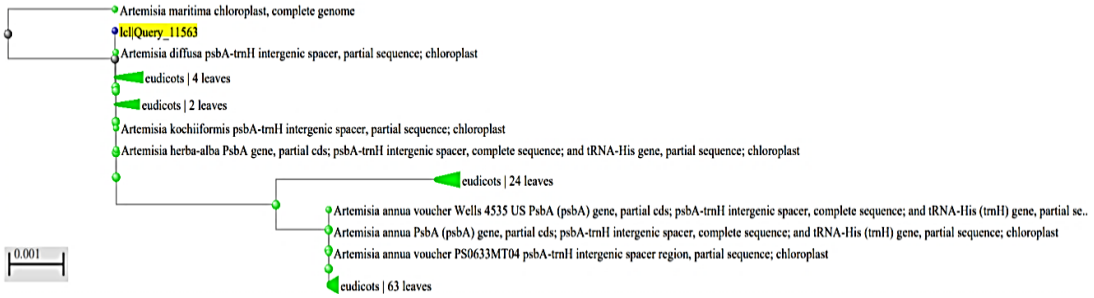

Figure 6. Nucleotide sequences of trnH-psbA for Artemisia L. samples.

Note: marked in yellow are the studied samples.

There exists a multitude of parameters worth taking into account when measuring genetic distances. In the simplest scenario, the genetic distance between two populations of the same species can be determined based on the difference in frequencies of occurrence of a particular feature.

The genetic distance between samples was determined by cross-linking nucleotide sequences of three genetic markers for each sample. The genetic distance reached 0.9% between the second and first, as well as between the second and third samples. The distance between the first and third samples was equal to zero.

The identification results for the studied samples using matK and rbcL markers from the international BOLD database (BARCODE OF LIFE DATA SYSTEM) for DNA barcoding revealed that the greatest overlap for the studied samples was with *Artemisia annua L* species. (Figure 7).

The study evaluated the population structure, genetic diversity, and relationships of *Artemisia annua* germplasm across Iran using 18 IRAP primers. RTN-based markers are being newly used, and are yet to be exploited fully. This approach can provide comprehensive information about the level of genetic diversity and population structure of *Artemisia annua* which could be useful for conservation and management of *Artemisia annua* germplasm genetic resource base. Single IRAP primer Tnt1.OL16 revealed more distinguished contribution in defining the genetic diversity of the studied taxon's or species and their segregation. These results revealed that the genetic diversity of the Iranian Sweet *Artemisia annua* is insufficient, and that makes it uniformly a distinct population for extraction of artemisinin (Valizadeh et al., 2021).

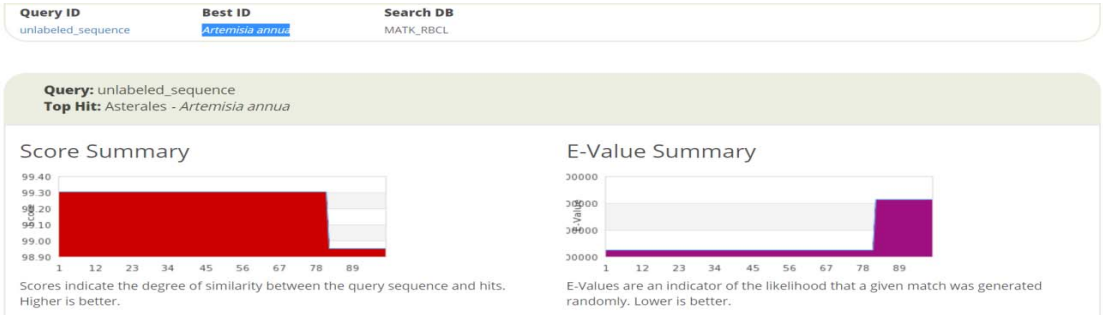

Figure 7. Identification of the studied samples in BOLD database.

Based on DNA barcoding results, the samples of Artemisia karatavica Krasch. & Abolin ex Poljakov, Artemisia cina Berg ex Poljak. и Artemisia porrecta Krasch. ex Poljakov proved the greatest affinity with Artemisia annua species. The identification of the results in DNA barcoding database demonstrated the greatest overlap with Artemisia annua species for three samples.

Upon purification of the genomic DNA, its quality analysis was measured by a spectrophotometer (NanoDrop 2000C) and by electrophoresis with 1% agarose gel in a triple track sequence (Figure 8).

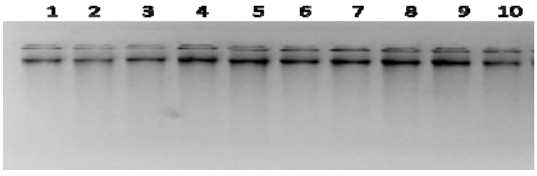

Figure 8. Electrophoresis of genomic DNA with 1% agarose gel

(Tracks 1 to 3 correspond to sample 1; tracks 4 to 6 to sample 2; tracks 7 to 10 to sample 3).

In order to prove absence of inhibitors in DNA preparations purified in accordance with the modified protocol (Sukhareva and Kuluev, 2018), a pilot PCR analysis was undertaken using oligonucleotides specific to 18S gene of the ribosomal RNA. The electrophoretogram of the products of PCR analysis of the ribosomal DNA of artemisia prove absence of reaction inhibitors in the resulting DNA preparations (Figure 9).

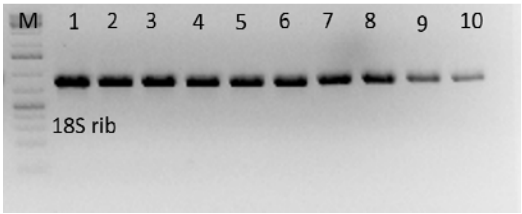

Figure 9. Electrophoretogram of PCR product from 18S gene

(Tracks 1 to 10 correspond to PCR products of 18S gene fragment of the ribosomal RNA for the studied samples; M is DNA marker of GeneRuler^{TM} 1kb).

The following stage of the sequenced products analysis of involved primers 274 and 275 complementary to different microsatellites (Figures 10-12).

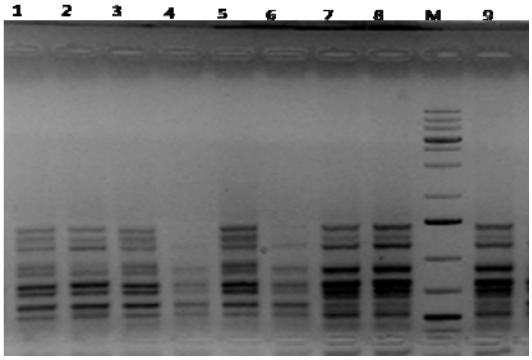

Figure 10. Electrophoretogram of amplification products when using primer 274

(Tracks 1 to 3 correspond to sample 1; tracks 4 to 6 correspond to sample 2; tracks 7 to 9 correspond to sample 3; M is DNA marker of GeneRuler^{TM} 1kb).

The results of analysis proved that all artemisia samples are genetically related under primer 274.

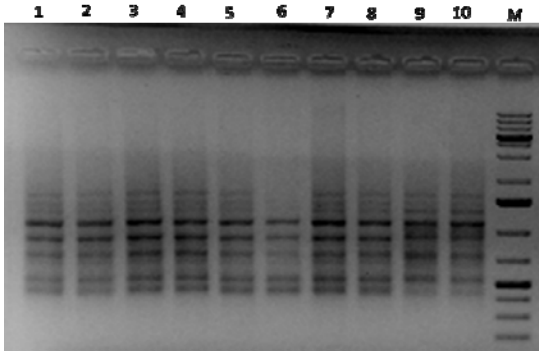

Figure 11. Electrophoretogram of amplification products when using primer 275

(Tracks 1 to 3 correspond to sample 1; tracks 4 to 6 to sample 2; tracks 7 to 10 to sample 3; M is DNA marker of GeneRuler^{TM} 1kb).

The analysis of amplification results proved that the studied artemisia samples were not genetically different under primers 274 and 275. PCR analysis revealed no genetic variability among the studied samples.

4. Discussion

This research study was based on the classical approach to molecular and genetic passportization of resource plant species which includes the stages as follows: selection of effective methods to analyse polymorphism of DNA, collection of material, determination of effective primers, molecular and genetic analysis using PCR, identification of DNA markers, monomorphic and polymorphic DNA fragments, compilation of a molecular genetic formula, barcode and genetic passport (Dilshad et al., 2015). The last three stages, i.e. compilation of a molecular genetic formula, barcode and genetic passport, which are often used for the certification purposes of commercial varieties of agricultural plants, were not involved into this study owing to a different scope of research tasks.

DNA barcoding based on sequencing of standard DNA fragments, especially mitochondria and chloroplasts, is widely used in molecular systematics of organisms (Kondratenko, 2015). The methods used in this study, i.e. DNA barcoding, sequencing, phylogenetic analysis and final control of the results by PCR, altogether helped to reveal polymorphism of the greater part of the genome of the plant species under study. These methods are stable, and provide clearly visible results.

DNA banks are created and integrated with the already existing herbarium collections, seed banks, and plant collections in botanical gardens (Kanukova et al., 2019). The methods described above and the results obtained at this stage of the study were carried out using three medicinal species of *Artemisia L.* of a high pharmaceutical potential and growing in the South Kazakhstan. Particularly this approach to the analysis of DNA polymorphism is considered promising for the purposes of identification of the genetic mechanisms responsible for adapting population systems in a heterogeneous environment. The content of DNA in plants is determined in more than 10 thousand species (Chen et al., 2010). In the course of this research, DNA sequences of three pharmaceutically significant resource species among the plants of Kazakhstan were sequenced for the very first time, and the absence of genetic variability among the studied samples was proved. On the grounds of comparison of the obtained results with the standards of the international base BOLD, DNA sequences of *Artemisia karatavica Krasch. & Abolin ex Poljakov, Artemisia cina Berg ex Poljak.,* and *Artemisia porrecta Krasch. ex Poljakov* displayed the greatest affinity with *Artemisia annua* species.

Relatively small size of the nuclear genome facilitates molecular studies of resource plants (Kavrakova et al., 2015). In the course of molecular genetic analysis of *Artemisia L.*, from the samples of various geographical vegetation in Southern Kazakhstan, a set of amplification products specific to 18S gene of the ribosomal RNA was obtained. The vast majority of amplified DNA fragments were polymorphic.

The number of amplicons in total sampling of plants varied depending on the primer, i.e. from 9 (primer 275) to 11 (primer 274), with their sizes not exceeding the size of 1 kb.

The level of polymorphism of ISSR markers in this species was 0.9%. The number of rare amplicons (DNA fragments with a frequency of ≤0.05) was null in the studied Artemisia L samples.

The search for rare alleles is quite important, since these rare alleles are most valuable for development of measures aimed at preservation of natural populations, and are important for passportization of populations within this species. Consequently, in the future, the assessment of genetic polymorphism of a wider range of geographically diverse plant samples of *Artemisia L.* species would be of significant practical interest, and important in terms of assessing diversity of wild plants species.

For the purposes of molecular genetic passportization, there were selected most informative ISSR primers, with the help of which effective ISSR markers for the species under study were identified and identification of the molecular markers was done (Figures 2, 4, 6). The molecular markers selected for passportization and

identification of resources for these valuable medicinal plants were visualized by way of a genetic divergency tree. When plotting it, the so-called generic, species-related or polymorphic PCR fragments of DNA were used.

Many researchers follow the protocol to purify DNA from various organs of the plant, which would not consume much time and would minimize contamination of the purified DNA (Omasheva et al., 2016; Cai et al., 2017). At that, the principal property of a molecular marker - its length - was determined using DNA marker GeneRulerTM 1kb.

This approach to describing genomes of various plants is applicable to both homogeneous plant varieties and heterogeneous natural populations. For the purposes of genetic passportization of the studied populations, the number of molecular markers involved can be further extended. Thus, numerous research papers focused on the study of genetic diversity of plants in Perm Region suggested to take 10 to 12 markers. This made it possible to passportize plants, and apply molecular genetic formula to them (Kostylev et al., 2015).

The genetic divergence tree discussed in our studies, as well as molecular genetic formula and barcode (Mezhnina and Urbanovich, 2016), enabled identification of affinity of both plant raw material and plant samples not just upto the genus and species, but also to the group of populations or one specific population. The principle advantage of the discussed method of genetic expertise is the mechanism of generalization of molecular genetic analysis data by determining the identification markers reflecting most of the genome of the studied species. The visualization of results was proposed in a generalized way by means of the genetic divergency tree, with PCR analysis of amplicons, introducing, along with conventional indicators, the description of state for every pharmaceutically significant population and introducing the specifics of its gene pool.

Currently, artemisinin and its derivatives are used in combination therapy for the treatment of malaria (Haynes R.K., 2006), as well as for the treatment of a number of oncological and viral diseases (Effert T., 2007). All these scientific undertakings prove again that further research is required for the species of Artemisia L. (Zhokhova et al., 2019; Kalko, 2015; Kondratenko, 2015; Urbanovich, 2007, Lyzhin and Savelieva, 2018; Sukhareva and Kuluev, 2018; Levin et al., 2003).

5. Conclusion

Artemisia is an economically important medicinal plant. In particular, essential oils extracted from artemisia are used in manufacturing perfumes and antiseptic agents. The species identification of artemisia ensures selection of important species and further selection activities. The search and study of various types of artemisia contributes to identification of species advantageous in view of glycosides or alkaloids content, which is crucial for pharmaceutical industry.

The World Health Organization (WHO) welcomes innovations around the world including repurposing drugs, traditional medicines and developing new therapies in the search for potential treatments for COVID-19. WHO recognizes that traditional, complementary and alternative medicine has many benefits, and Africa has a long history of traditional medicine and practitioners that play an important role in providing care to the peoples. Medicinal plants such as *Artemisia annua* are being considered as possible treatments for COVID-19 and should be tested for efficacy and adverse side effects. The Africans should use the medicines tested to the same standards as the peoples in other countries. Even if the medicinal products derived from traditional sources are natural, establishing their efficacy and safety through rigorous clinical trials is critical (World Health Organization. Africa, 2020).

Artemisinin derived from the medicinal plant *Artemisia sp.* is an effective antimalarial medicinal product. In 2015, the Nobel Prize was awarded to the findings of effectiveness of artemisinin as a potent antimalarial medication. Due to the limited market and low extraction of artemisinin, the economical way of increasing its production lies in increasing the amount of *Artemisia sp.* through various biotechnological techniques, inclusive of genetic transformation. (Bushra Hafeez Kiani, 2016).

Molecular genetic identification of artemisia species was not accomplished in Kazakhstan previously. The description of species is based on botanical properties. Molecular genetic analysis of *Artemisia L*. (rbcL, matK, trnH-psbA) displayed complete identification of *Artemisia karatavica Krasch. & Abolin ex Poljakov* and *Artemisia porrecta Krasch. ex Poljakov* samples, as well as the genetic distance between these samples and *Artemisia cina Berg ex Poljak*. being 0.9%. Comparison of DNA barcoding results with the available database proved the greatest overlap of the studied samples with *Artemisia annua L* species, which may be due to insufficient study of the species. The issues related to the origin of each plant species, because of the complexity of the process, represent quite a laborious scientific challenge. Among the most promising techniques to study genetic purity is the analysis of variability of highly polymorphic genetic systems. The large number of polymorphic markers identified in the course of decoding the genome of any particular plant species is a powerful tool to analyze the gene pool, and its main properties, dynamics and geography. Numerous studies focusing on polymorphic systems of nuclear and mitochondrial genomes led to the development of a new section of genomics - ethnogenomics. Nowadays, accumulation of data on polymorphism of autosomal microsatellite loci, microsatellites and mitochondrial genome variability in various populations worldwide is a task of paramount importance.

According to the available research finding, there is a tendency towards depletion of the gene pool among the studied populations of *Artemisia L*. Molecular analysis of highly polymorphic fragments of the genome in rare relict plant species discussed in the scientific sources represents the base to approach their molecular genetic passportization. The principle of compiling and recording a genetic formula relies on identification of DNA markers using ISSR and PCR methods to analyze DNA polymorphism. It covers most of the plant genomes, and there are few genetically studied plant species suitable for genetic passportization. The new approach to recording the data contains detailed information about the identification of molecular marker, including plant species, molecular marker type, its size, and properties of the studied part of the genome by indicating the analysis technique of DNA polymorphism and the number or sequence of primer.

Such approach to genetic passportization is of high resolution, and provides stable visual results, as well as adaptive to automation.

Data availability statement:

The data that support this study are available in the article and accompanying online supplementary material.

Conflict of Interest statement:

The authors declare no conflicts of interest

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