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Zh.Sh. Rakhymberdieva, A.N. Kaliyeva, G.D. Medeuova

Kazakh National Women's Teacher Traning University, Almaty, Kazakhstan.
E-mail: Rakhymberdieva80@mail.ru, anar.kaliyeva28@gmail.com, medeuova.galia96@gmail.com

MOLECULAR GENETIC PLANT ANALYSIS, *ARTEMISIA L.* GENUS, WITH ISSR-MARKERS

Abstract. Molecular genetic analysis of three plants of thistle family has been carried out in the scope of this paper (*Artemisia karatavica* Krasch. & Abolin ex Poljakov, *Artemisia cina* Berg ex Poljakov, *Artemisia porrecta* Krasch. ex Poljakov). The plants have been collected in Turkestan region, Shardarinsky district, 15 km north-east of the village Komsomol, and along the road in Turkestan region, Baydibeksky district, 4.5 km south-east of the village Shakpak, and in the steppes in Turkestan region, Aryssky district, 1 km north-east of the village Darmino. In this paper, we used modern methods of molecular biology in order to determine genetic relatedness.

ISSR analysis using universal primers has been conducted. *ISSR*-markers are the most common markers, and they are used for phylogenic analysis. This method is based on amplification of sequences limited by two microsatellite repeats using the primer that is complementary to the sequence of this microsatellite (4-12 repeat units). *ISSR* (region of the genome between two adjacent, oppositely oriented microsatellites) the sequence of microsatellite medullar part with some (1-3) nucleotides adjacent to the repeat tandem are used as primers. Tens of fragments of locus variety received in PCR are separated by electrophoresis and assessed for the presence or absence (due to marker dominance) of the fragments of a particular size. The main advantage of this type of markers - lack of necessity for knowledge of the sequences during primer designing.

Key words: *Artemisia L.*, genotyping, identification, ISSR-markers.

Introduction. Kazakhstan's flora is characterized by the variety of medicinal plant raw material, many species of which may be used on an industrial scale. The most common medicinal plants within the territory of the Republic of Kazakhstan include *Artemisia* L. genus - sagebrush - one of the most multi-species and complex, from a systematic viewpoint, genus of dicotyledon plants of *Asteraceae* L. *Artemisia* family includes hardy herbaceous plants and shrubs that are known for their active chemical components in essential oils. *Artemisia* genus belongs to the thistle family that includes more than 500 species spread in the areas of the northern hemisphere of the Old and the New World, Eurasia and Asia [1-3].

Artemisia L. - numerous anthodes, small, homogamous, i.e. all of the florets are androgynous, tubular, fertile, in a small, 3-8 (10), number (*Seriphidium subgenus (*Bess.*)* Rouy.), or heterogamous, heterosexual: ray florets are, pistillate, and central disk florets are bisexual; in *Artemisia Less subgenus.* All the florets, ray pistillate and bisexual leucocarpous, with half-grown subtle ovary; corollas of pistillate florets are very narrow, sometimes almost creeping and tubular, with 2-3 short teeth, almost colorless or tubular, with 2-4 teeth, colored; corollas of disk florets are tubular, campanulately amplated upwards, 5 teeth, variously colored (yellow to purple), 5 stamens on short or long filaments, anthers are linear, sharp at the apex, blunt at the base knitted into a tube, inside of which there are capillary bilobed stile, laminas of snouts are of equal or unequal length, pilose at the apex, and they are generally narrow and linear in ray pistillate florets, not pilose at the apex, stile of vestigal pistil of disc florets (*Dranculus* subgenus (Bess.) Rydb.) with very short, almost blunt, ciliated laminas; nuts are small, oblong ovoid, bare, blunt at the apex, without pappus or margin; anthodes are oblong ovoid and obloid, with a foliole spathe; primordial leaves are grass, 2-7 rows, external ones are longer but shorter than inner ones, or all of them are of equal length, with pronounced loma on the edge; anthodes are paniculate, racemous, very rare, almost spiked. Annual and perennial herbaceous plants or subshrubs with erect, ascending or more rare - lodged stems; leaves are alternate, simple, or more often - pinnatisect to different extents. Vast and polymorphous genus containing

over 500 species spread primarily in the temperate zone of Eurasia and North America, and in Kazakhstan 81 [4-6].

Nowadays, medical scientists pay more and more attention to medicinal plants and prove how each of them is beneficial for the body. In this regard, significant progress has been made and the demand for herbal medicine is growing. [7].

Great contribution to the study of sagebrush was made by W. Besser (1829, 1834, 1835) who divided genus into three sections based on the sex composition of florets in a anthode. Group of species that has only bisexual florets in the anthode was allocated by him to *Seriphidium* Bess section; species that have ray pistillate florets in the anthode, and disc ones - staminal with vestigal pistil - to *Dracunculus* Bess section; species that have ray pistillate florets in the anthode, and disc ones - bisexual, are combined into *Abrotanum* Bess section. A substantial part of sagebrushes has been described by K. Linnaeus, Weber, K.F. Ledebour, and I.M. Krasheninnikov [8].

Information about sagebrushes are also given in the scope of papers of I. Gmelin, B.Bessera, Ledsbur, I. M. Krasnoborov; "Flora of Western Siberia" (Krylov, 1949). Traditionally accepted, broad understanding of sagebrushes as a single *Artemisia* genus was approved by K. Linnaeus. Despite of the substantial volume and a colossal across-species polymorphism, the genus splits into 5 groups: *Abrotanum, Absinthium, Dracunculus, Seriphidium,* and *Tridentatae* distinguished by the whole set of features, primarily, anthode formation and sex composition of florets in them, spectrotype of biologically active substances. Many species of sagebrush are polymorphous, they have vast realm and large raw mass which determines the prospects for common use [9].

Economically important representatives of the Artemisia genus have wide application in pharmaceutics, landscape architecture and agriculture [10].

Economic character of sagebrush is of primary importance in the desert and steppe zones as a natural forage supply at autumn and winter pastures. In Kazakhstan, this includes almost *Seriphidium* subgenus. Due to content of essential oils, many species of sagebrush are used in perfume and alcoholic beverage industry. Anthodes of *A.cina* Berg. and *A.transiliensis* Poljak. contain santonine, classical helminthagogue. Almost all sagebrushes contain glucosides, and some species - alkaloids. So, *A.taurica* Willd. is considered to be a noxious plant. Testing and domestication of many sagebrushes, and their selection is quite perspective.

A study of genetic variation among 216 accessions was conducted using ISSR (Inter Simple Sequence Repeat) markers to assess the polymorphism at the species level. A total of 60 polymorphic loci were scored using four primers revealing a high level of genetic polymorphism among A. herba-alba accessions. Correlation analysis revealed no direct relation between morphological traits, geographic distance and genetic distance. Correlogram analysis showed a patchy distribution of the genetic variability of A. herba-alba accessions revealing the contribution of local ecological and geographic conditions on variability [11].

Genetic variation between A. capillaris was evaluated using random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers. RAPD and ISSR marker systems were found to be useful for the genetic diversity studies in *A. capillaris* and to identify the variation [12].

Over the past twenty years, the molecular marker field has completely transformed the meaning of conservation genetics which has emerged from a theory-based field of population biology to a full-fledged pragmatic discipline [13].

For extension and conservation of the genofond of the plants that are of medical or agricultural importance, as well as for genetic monitoring of natural populations, different methods of genetic marking are applied. RAPD analysis may serve as express method for detecting genetic polymorphism and genom marking in population studies [14].

The aim of this study is to assess the level of intraspecific differences of natural plant populations of *Artemisia karatavica* Krasch & Abolin ex Poljakov, *Artemisia cina* Berg ex Poljakov, *Artemisia porrecta* Krasch. ex Poljakov) using RAPD marking results.

Materials and methods. Three species of *Artemisia L.,* (*A. karatavica* Krasch. & Abolin ex Poljakov, *A.cina* Berg ex Poljakov, *A.porrecta* Krasch. ex Poljakov) of thistle family served as the material for the study. The plants have been collected in Turkestan region, Shardarinsky district, 15 km north-east of the village Komsomol, and along the road in Turkestan region, Baydibeksky district, 4.5 km south-east of the village Shakpak, and in the steppes in Turkestan region, Aryssky district, 1 km north-east of the village

Darmino. Test samples were collected at the end of flowering period. The aerial parts of three different species of *Artemisia L.*

DNA purification. 3 samples of each species were used for purification. Genomic DNA was purified from the aerial parts of *Artemisia L.* using CTAB [15-16] with some modifications: 100 mg of leaves were rubbed in cooled mortar in the presence of 1 mL of extraction buffer (100 mmol of Tris-HCl pH 8.0; 20 mmol of EDTA, pH 8,0; 1.4 M NaCl, 2% CTAB, PVP and 2-mercaptoethanol were added prior to the use of final concentration of 2 and 0.2%, respectively). The derived homogenate was incubated at 60°C for 30 minutes and then extracted with chloroform. 0.5 volume of 5M NaCl and 2 volumes of ethanol were added to the aqueous phase. The mixture was incubated at 4°C for 15-20 minutes and centrifuged for 15 minutes at 13,000 g. DNA residual matter was washed with 70% ethanol and dissolved in 100 µl of bidistilled water. DNA was treated with RNA. Assessment of DNA quality and amount was performed by electrophoresis in 1% agarose gel in 1x TAE buffer and upon absorption at 260 nm to 280 wavelengths using Nanodrop 2000 spectrophotometer (Thermo Scientific).

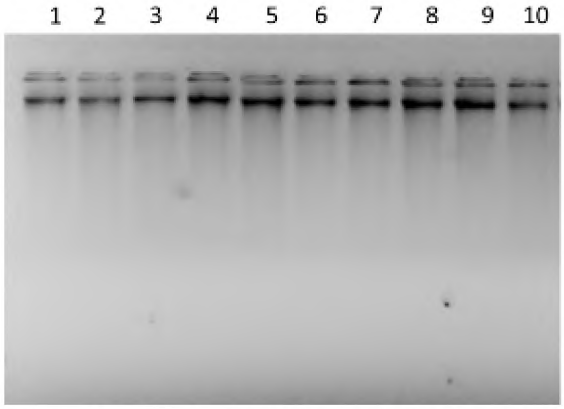
Quality control of the genomic DNA purified using PCR amplification of 18S ribosomal DNA. Quality of DNA samples was tested in PCR amplification of 18S ribosomal DNA using the following primers: f 5′-GAGAAACGGCTACCACATCCAAGG-3′;5′- rCCATGCACCACCACCCATAGAATC-3′. The expected product size was 870 bp. PCR was performed in the final volume of 25 µl containing 0.2 mmol of deoxyribonucleotide triphosphates, 0.2 µmol of each primer, 0.5 UTaqDNA polymerase (Thermo Scientific), 2.5 mmol of $MgCl_2$, and 40 ng of DNA in 1 X Taq Buffer with $(NH_4)_2SO_4$ (750 mmol of Tris-HCl, pH 8.8, at 25°C, 200 mmol $(NH_4)_2SO_4$ and 0.1% (v/v) Tween 20). Amplification: 2 min of initial denaturation at 94°C; 25 cycles at 94°C for 30 sec, annealing at 67°C for 15 seconds, and synthesis at 72°C for 15 seconds. Final elongation at 72°C for 10 min.

Selection of ISSR markers and PCR conditions. 9 *ISSR* markers were used for genotype estimation. The material for the study - 10. DNA with absorption ratio indicators at 260/280 wavelengths from 1.68 to 1.87 (Nanodrop 2000 spectrophotometer (Thermo Scientific) was used in all other experiments.

PCR was performed in 20 µl containing 2 µl of 10 x Taq buffer (750 mmol of TrisHCl, pH 8.8, 200 mmol of $(NH_4)_2$ SO_4, 0.1% Tween 20), 2.5 µl of 25 mM$MgCl_2$, 0.4 µl of 10 mmol dNTP mix, 0,8 µl of 10 mmol oligonucleotides of the marker used, 12.7 µl of sterile deionized water and 1 unit of Taq polymerase. Concentration of genomic DNA was 40-60 ng / 20 µl. Amplification was performed as per the following program: one cycle at 94°C for 2 minutes; 35 cycles consisting of the following steps: 94°C – 40 sec, 44°C–45 sec, 72°C –1,5 min; final cycle at 72°C –15 min.

Electrophoresis of amplification products resulting from ISSR-PCR, was performed in 2% agarose gel, after staining they were visualized with ethidium bromide under UV light using a BioRad gel documenting system.

Results and discussion. *ISSR analysis.* For analysis, three repeats were used for each sample, purification of genomic DNA was performed using all samples. Following purification of genomic DNA, the quality analysis of genomic DNA was performed via measurement using the spectrophotometer (NanoDrop 2000C) and gel electrophoresis in 1% agarose gel, see figure 1.

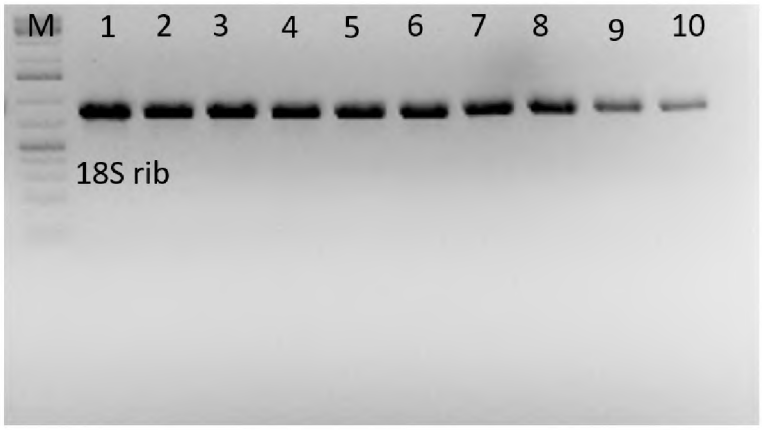


1-3 – repeats of the sample 1; 4-6 – repeats of the sample 2; 7-10 – repeats of the sample 3

Figure 1 – Electrophoresis of genomic DNA in 1% agarose gel

To verify the absence of inhibitors in the preparations of DNA purified according to modified protocol [15], test PCR using oligonucleotides specific to 18 S gene of ribosomal RNA was performed.

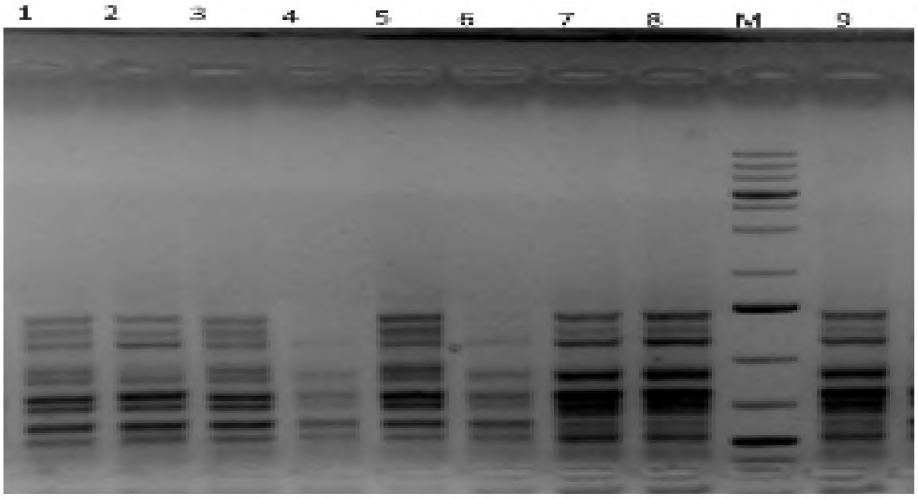
Figure 2 shows electrophoretogram of PCR products of Artemisia ribosomal DNA. Electrophoretogram of PCR products confirms the absence of reaction inhibitors in DNA preparations derived.



1-10 – PCR products of 18S gene fragment of ribosomal DNA of the analyzed samples;
M - DNA marker, GeneRuler™ 1kb (Fermentas)

Figure 2 – Electrophoretogram of PCR product of 18S RNA gene

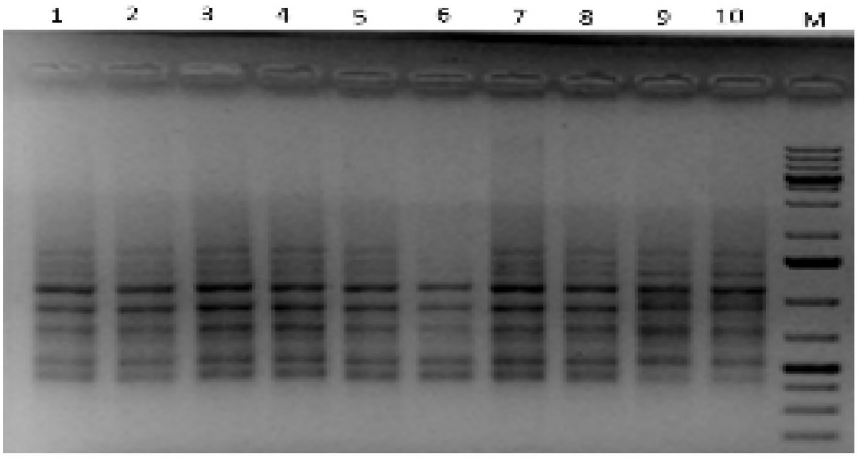
Then analysis was performed using 9 primers complementary to various microsatellites. As an example, electrophoregrams at Figure 3 and 4 show ISSR-PCR amplification results using (A)-VHV-$(GT)_7$ and (B)-$(CA)_6$-RY primers.



1-3 – repeats of the sample 1; 4-6 – repeats of the sample 2; 7-9 – repeats of the sample 3

Figure 3 – Results of amplification using VHV– $(GT)_7$ primer

Amplification using VHV– $(GT)_7$ primer for the 4th repeat of sample 3 failed. 3 repeats of sample 3 showed similar results as per the results of statistical processing of the analysis data. The analysis results show that three samples of Artemisia do not differ genetically using VHV– $(GT)_7$ primer.



1-3 – repeats of the sample 1; 4-6 – repeats of the sample 2; 7-10 – repeats of the sample 3

Figure 4 – Results of amplification using RY– $(CA)_6$ primer

Usage of the above 9 polymorphic *ISSR* primers showed no intraspecific variability. Each species has a characteristic number of discs per the primer and the total number of them.

Conclusion. Molecular genetic analysis of three plants (presumably of three various species) *Artemisia L.* has been carried out in the scope of this paper while using modern methods of molecular biology in order to determine genetic relatedness. In the scope of this paper, ISSR analysis using universal primers has been conducted. *ISSR*-markers are the most common markers, and they are used for phylogenic analysis [17-19]. This method is based on amplification of sequences limited by two microsatellite repeats using the primer that is complementary to the sequence of this microsatellite (4-12 repeat units). *ISSR* (region of the genome between two adjacent, oppositely oriented microsatellites) the sequence of microsatellite medullar part with some (1-3) nucleotides adjacent to the repeat tandem are used as primers [20-22]. Tens of fragments of locus variety received in PCR are separated by electrophoresis and assessed for the presence or absence (due to marker dominance) of the fragments of a particular size. The main advantage of this type of markers - lack of necessity for knowledge of the sequences during primer designing. It should be noted that homoplasy, non-homology of the fragments of the same size are possible due to multi-locus. Markers of this type are used to identify the genetic identity, genealogy, differentiation of clones, microclones and lines, taxonomy of closely related species [23-24].

The analysis results showed that 3 samples of Artemisia do not differ genetically using 9 *ISSR* primers. The analysis results did not show genetic variability among the study samples.

Ж.Ш. Рахимбердиева, А.Н. Калиева, Ғ.Д. Медеуова

Қазақ ұлттық қыздар педагогикалық университеті, Алматы, Қазақстан

ISSR -МАРКЕРЛЕРДІ ҚОЛДАНЫП, ARTEMISIA L. ТУЫС ӨСІМДІКТЕРІН МОЛЕКУЛАЛЫҚ-ГЕНЕТИКАЛЫҚ ТАЛДАУ

Аннотация. Мақалада күрделігүлділер тұқымдасының үш түріне (*Artemisia karatavica* Krasch. & Abolin ex Poljakov, *Artemisia cina* Berg ex Poljakov, *Artemisia porrecta* Krasch. ex Poljakov) молекулалық-генетикалық талдау жүргізілді. Өсімдіктер, оның ішінде 1-өсімдік Түркістан облысы, Шардара ауданы, Комсомол ауылынан солтүстік-шығысқа қарай 15 км жерден жиналған, 2-өсімдік Түркістан облысы, Бәйдібек ауданы, Шақпақ ауылынан оңтүстік-шығысқа қарай 4,5 км жерден, 3-өсімдік Түркістан облысы, Арыс ауданы, Дармино ауылынан солтүстік-шығысқа қарай 1 км жерден қазан, қараша айында жиналды. Жусанның экономикалық маңызы шөл және дала аймағында күзгі және қысқы жайылымдарда табиғи жемшөп қоры ретінде өте маңызды рөл атқарады. Қазақстан жусанының көп бөлігі шөл және тауда өседі. Жусан ауыл шаруашылығында, тамақ, парфюмерия және косметикалық өнеркәсіпте, медицинада қолданылады. Жусанның емдік қасиеттері ерте заманда белгілі болған. Медицинада улану, гинекологиялық және урологиялық ауруларға қолданылуы ескі кітаптарда айтылған. Көптеген түрі дәстүрлі медицинада және ветеринарияда ащы, ұстама ауруға қарсы, асқазан-ішек жолдарының ауруына

қарсы қолданылады. Жусанның *A.cina, A.glabella, A.absinthium, A.karatavica, A.annua, A.porrecta* түрлеріне қызығушылық артуда. A.cina Berg. және A. transiliensis Poljak. себеттерінде классикалық антигельминтикалық сантонин бар. Жусанның барлығында дерлік глюкозид, ал кейбір түрлерінде алкалоидтар болады. Осылайша, A. taurica Willd. улы өсімдік деп саналды. Біраз жусанды сынақтан өткізу және дақылға енгізу, сондай-ақ оларды таңдау перспективалы болып саналады.

Зерттеудің мақсаты RAPD таңбалау нәтижелерін қолдана отырып (Artemisia karatavica Krasch & Abolin ex Poljakov, Artemisia cina Berg ex Poljakov, Artemisia porrecta Krasch. ex Poljakov), табиғи өсімдік популяцияларының түрішілік айырмашылықтарының деңгейін бағалау. Жұмыста генетикалық туыстықты анықтау үшін молекулалық биологияның заманауи әдістері қолданылды.

Әмбебап праймерлерді қолдана отырып, ISSR талдауы жүргізілді. ISSR маркерлері көбірек таралған және филогенетикалық талдау жүргізу үшін қолданылады. Бұл әдіс екі микросателитті қайталаумен шектелген тізбектерді осы микросателит тізбегіне (4-12 қайталау бірлігі) толықтыратын праймермен күшейтуге негізделген. Issr (екі көршілес, қарама-қарсы бағытталған микросателлиттер арасындағы геномдық аймақ) праймерлер ретінде қайталану тандеміне іргелес бірнеше (1-3еу) нуклеотидтері бар микросателлиттің ядро бөлігінің реттілігі қолданылады. ПЦР-де алынған көптеген локустардың ондаған фрагменттері электрофорез арқылы бөлінеді және белгілі бір мөлшердегі фрагменттердің болу немесе болмау (маркерлердің үстемдігіне байланысты) жағдайы бағаланады. Маркерлердің бұл түрінің басты артықшылығы – праймерлерді жобалау кезінде біріздiлікті білу қажеттілігінің болмауы.

Талдау нәтижелері көрсеткендей, 9 ISSR праймерін қолданған кезде 3 жусан сынамасы генетикалық тұрғыдан ерекшеленбейді. Талдау зерттелген үлгілер арасында генетикалық өзгергіштік жағдайын көрсетті.

Түйін сөздер: *Artemisia L.*, генотиптеу, идентификация, ISSR-маркерлер.

Ж.Ш. Рахимбердиева, А.Н. Калиева, Ғ.Д. Медеуова

Казахский Национальный женский педагогический университет, Алматы, Казахстан

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЙ АНАЛИЗ РАСТЕНИЙ РОДА *ARTEMISIA L.* С ИСПОЛЬЗОВАНИЕМ ISSR-МАРКЕРОВ

Аннотация. В работе был проведен молекулярно-генетический анализ трех образцов (*Artemisia karatavica* Krasch. & Abolin ex Poljakov, *Artemisia cina* Berg ex Poljakov, *Artemisia porrecta* Krasch. ex Poljakov) из семейства сложноцветных. Растения собирали в Туркестанской области, Шардаринском районе, в 15 км северо-восточнее пос. Комсомол., вдоль дороги в Туркестанскую область, Байдибекский район, в 4,5 км юго-восточнее пос. Шакпак., по степям Туркестанской области, Арысского района, в 1 км северо-восточнее пос. Дармино. Хозяйственное значение полыни играет исключительно важную роль в пустынной и степной зонах в качестве естественного кормового фонда на осенних и зимних пастбищах. Казахстанская полынь в большинстве является эндемиком пустынь и гор. Полынь применяется в сельском хозяйстве, пищевой, парфюмерно-косметической промышленности, в медицине. Лечебные свойства полыни известны с древнейших времен. О применении их в медицине при отравлениях, гинекологических и урологических заболеваниях упоминается еще в старых книгах. Многие виды широко используются в народной медицине и ветеринарии при горечи, как противоглистные, противоэпилептические и при желудочно-кишечных заболеваниях. Несомненный интерес представляет полынь *A.cina, A.glabella, A.absinthium, A.karatavica, A.annua, A.porrecta.* В корзинках *A.cina* Berg. и *A.transiliensis* Poljak. находится сантонин – классическое глистогенное средство. Почти у всех полыней отмечено присутствие глюкозидов, а у некоторых видов – алкалоидов. Так, *A.taurica* Willd. считается ядовитым растением. Испытание и введение в культуру многих полыней, а также их селекция вполне перспективны.

Целью настоящего исследования является оценка уровня внутривидовых различий природных популяций растений *(Artemisia karatavica* Krasch. & Abolin ex Poljakov, *Artemisia cina* Berg ex Poljakov, *Artemisia porrecta* Krasch. ex Poljakov) с помощью результатов RAPD-маркирования. В работе использовались современные методы молекулярной биологии с целью определения генетического родства.

Метод работы. Проведен *ISSR*-анализ с использованием универсальных праймеров. *ISSR*-маркеры являются наиболее распространенными маркерами и используются для проведения филогенетического анализа. Данный метод основан на амплификации последовательностей, ограниченных двумя микросателлитными повторами с помощью праймера, комплементарного к последовательности данного микросателлита (4–12 единицам повтора). *ISSR* (область генома между двумя соседними, противоположно ориентированными микросателлитами) в качестве праймеров используются последовательность сердцевинной части микросателлита с несколькими (1-3мя) нуклеотидами, примыкающими к тандему повторностей. Десятки фрагментов множества локусов, полученных в ПЦР, разделяются электрофорезом и оцениваются на присутствие или отсутствие (вследствие доминантности маркеров) фрагментов определенного размера. Главное преимущество данного типа маркеров – отсутствие необходимости знания последовательностей при конструировании праймеров.

Результаты анализа показали, что 3 образца полыни генетически не различаются при использовании 9 *ISSR*-праймеров. Анализ не выявил генетической вариабельности среди исследуемых образцов.

Ключевые слова: *Artemisia L.*, генотипирование, идентификация, ISSR-маркеры.

Information about authors:

Rakhymberdieva Zhanar Sherahmetovna, PhD, doctoral student of KazNWTTU; Rakhymberdieva80@mail.ru, https// orcid.org//0000-0001-9752-5986;

Kaliyeva Anar Nurgaiypovna, PhD, acting associated professor KazNWTTU; anar.kaliyeva28@gmail.com, https//orcid.org//0000-0003-2429-2610;

Medeuova Galiya Dzhumahanovna, candidate of sciences (agricultural), acting associated professor KazNWTTU; medeuova.galia96@gmail.com, https//orcid.org//0000-0003-3750-4758

REFERENCES

[1] Pellicer. J. (2011) Artemisia (Asteraceae): Understanding its evolution using cytogenetic and molecular systematic tools, with emphasis on subgenus Dracunculus. /T.Garnatje, J.Valles//Transworld Research Network. №2. P. 200–221.

[2] Kubitzki K. (2007) The families and genera of vascular plants. Flowering plants. Eudicots. Asterales. / J.W. Kadereit, C.Jeffrey/ Berlin Heidelberg; Springer Verlag. Vol. 5–8. P. 358.

[3] Aкsenova L. (2008) Wormwood - bitter, healthy, beautiful. [Floriculture magazine] 6:58.

[4] Flora Of Kazakhstan (1966) IX tom.Almaty.P.76-131

[5] Boronnikova S.V. (2009). Molecular labeling and genetic certification of resource and rare plant species in order to optimize the conservation of their gene pools // Agrarian Bulletin of the Urals. T.2. p.57–59.

[6] Mahmood T, Nadia H, Nazia N, Ishrat N. Phylogenetic analysis of different *Artemisia* species based on Chloroplast gene Rps11. Archives of Biological Sciences. 2011; 63(3):661-65.

[7] Kaliyeva, A.N.,.,Dyuskalieva G.U, Kurmanbaeva M.S. (2013) *National Academy* of *Sciences* of the Republic of *Kazakhstan. NEWS*. Biology and medicine. Almaty. №1 (295). P.18-23. https://doi.org/10.32014/2018.2518-1629.00 ISSN 2518-1629 (Online), ISSN 2224-5308 (Print).

[8] Besser W.(1835)Dracunculi seu de sectione IV et ultima Artemisiarum Linnaei//Bull.Soc.Nat. Moscou.Vol.8.P.3–97.

[9] Mamatova A.S. (2018) Pharmacognostic, pharmacological study of Artemisia gmelinii wormwood and creation of phyto-substances on its basis. dissertation, Almaty.

[10] Onur Koloren,Zeynep Koloren,Seçil Eker (2016)Agriculture and Environmental Biotechnology. Molecular phylogeny of Artemisia species based on the internal transcribed spacer (ITS) of 18S-26S rDNA in Ordu Province of Turkey. .P 929-934 Received 05 Dec 2015, Accepted 09 May 2016, Published online: 31 May.2016 Download citation https://doi.org/10.1080/13102818.2016.1188674

[11] Haouari Mohsen.(2008)Study of genetic polymorphism of Artemisia herba-alba from Tunisia using ISSR markers. African journal of biotechnology. January

[12] Mohammad Reza Shafie, Sayed M. Zain Hasan, Abdullah M Hasan. (2011) RAPD and ISSR markers for comparative analysis of genetic diversity in wormwood capillary (Artemisia capillaris) from Negeri Sembilan, Malaysia. Journal of medicinal plant research 5 September

[13] Maryam Sarwat,Gowher Nabi,Sandip Das &Prem Shankar. Molecular markers in medicinal plant biotechnology: past and present. Srivastava P 74-92 | Received 21 Aug 2010, Accepted 01 Jan 2011, Published online: 08 Jun 2011 Download citation https://doi.org/10.3109/07388551.2011.551872

[14] Kaliyeva, A.N.,Dyuskalieva, G.U.,Newsome, A. (2015) Study of polymorphism of different populations of agrimonia asiatica juz. and agrimonia pilosa ldb., common in South-East Kazakhstan. Ldb. Experimental Biology, [S.l.], v. 61, n. 2, p. 111-116, apr.. ISSN 2617-7498.

[15] Aubakirova K., Omasheva M., Ryabushkina N., Tazhibaev T., Kampitova G., Galiakparov N. Evaluation of five protocols for DNA extraction from leaves of *Malus sieversii, Vitis vinifera* and *Armeniaca vulgaris* // Genetics and Molecular Research. 2014. Vol. 13 (1). P. 1278-1287.

[16] Doyle J.J. and Doyle JL. Isolation of plant DNA from fresh tissue // *Focus*. 1990. Vol.12.P.13-15.

[17] Bhatia R., Singh K.P., Jhang T., Sharma T.R. Assessment of clonal fidelity of micropropagated gerberaplants by ISSR markers Scientia Horticulturae. 2009. V.119. P. 208–211.

[18] Blair MW, Panaud O, McCouch SR (1999) Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (Oryza sativa L.). Theor Appl Genet 98:780–792.

[19] Ammiraju JSS, Dholakia BB, Santra DK et al. (2001) Identification of inter simple sequece repeat (ISSR) markers associated with seed size in wheat. Theor Appl Genet 102:726–732.

[20] Leroy XJ, Leon K (2000) A rapid method for detection of plant genomic instability using unanchored-microsatellite primers. Plant Mol Biol Rep 18 (2000) 283a–283 g.

[21] Wang HZ, Wu ZX, Lu JJ et al (2009) Molecular diversity and relationships among Cymbidium goeringii cultivars based on inter-simple sequence repeat (ISSR) markers. Genetica 136(3):391–399.

[22] Gupta S.K., Souframanien J, Gopalakrishna T (2008) Construction of a genetic linkage map of black gram, Vigna mungo (L.) Hepper, based on molecular markers and comparative studies. Genome 51:628–637.

[23] Anderson GR, Brenner BM, Swede H et al. (2001) Intrachromosomal genomic instability in human sporadic colorectal cancer measured by genome-wide allelotyping and inter- (simple sequence repeat) PCR. Cancer Res 61:8274–8283.

[24] Argade N.C., Tamhankar S.A., Karibasappa G.S., Patil G.S., Rao V.S. DNA profiling and assessment of genetic relationships among important seedless grape (Vitis vinifera) varieties in India using ISSR markers// J. Plant Biochemistry& Biotechnology. 2009. Vol. 18(1). P. 45-51.