УДК 58.088

GENETIC DIVERSITY OF *RHODIOLA* sp. IN ALTAI AS ASSESSED BY INTRON POLYMORPHISM MARKERS

TagimanovaD.[1], KhapilinaO.[1], AmenovA.[1], DanilovaA.[2], Kalendar R.[1]

[1]National Center for Biotechnology
Korgalzhyn hwy, 13/5Astana, Kazakhstan
[2]Altai Botanical Garden, Ridder, Kazakhstan
tagds@mail.ru

ABSTRACT

Roseroot (*Rhodiola rosea* L.) is a widely used medicinal herb in Russia, Scandinavia, and China. We used intron polymorphism markers for several genes families with high levels of polymorphism to investigate 32 accessions. In addition, we collected plants from 2 separate natural populations in the Altai region (West Altai and South Altai) of Kazakhstan. Universal PCR primer pairs were based on sequenced genes of related *Rhodiola*species. The intron polymorphism markers were used to assess genetic diversityusing the GenAlex 6.5 program. The discriminatory potential of the selected markers was sufficientto determine the intrapopulation variability of *Rhodiola*. Observed heterozygosity at the loci averaged 0.235, compared to the expected 0.249. A dendrogram based on genetic distances was calculated from the results and confirmed that the two populations were genetically diverse. An analysis of molecular variance indicated that species level genetic diversity was relatively high (p = 70%) and an analysis using Shannon's index showed that within and between genetic diversity in roseroot wasapproximately equal. Nei's genetic distance and unweighted pair-group method with arithmetic averages cluster analysis showed that the two populations formed three major clusters. Understanding the genetic structure of *R.rosea* L. will improve the conservation and management of this endangered species.

Keywords: Genetic diversity, intron-length polymorphism, *Rhodiola rosea*, roseroot

---

INTRODUCTION

Roseroot (*Rhodiola rosea L.*) (*Crassulaceae*) is used extensively in Russia, Scandinavia and China as medicinal herb. Siberian people used *R. rosea* for centuries to deal with the cold climate and stressful life. Rhodiola species were used as adaptogens and have been widely accepted as an herb that can reinforce human resistance to stress or fatigue and promote longevity. Chinese people referred to *Rhodiola* species as Hongjingtian and regularly used it as adaptogens, hemostatics, and tonics in conventional Tibetan medicines for thousands of years. *R. rosea L*. type species is a popular conventional medicinal plant in east Europe and Asia, and is known for exciting nervous system, fighting depression, improving work performance, decreasing fatigue, and preventing high-altitude sickness. Therefore, many species of this genus are critically endangered in Asia due to excessive and indiscriminate exploitation. In spite of their wide use and medicinal significance, the identification of closely related species of *Rhodiola* is very hard because of their morphological similarity.

The underground part of *Rhodiola*has about one hundred forty components. The herb is spread throughout the Altai, Northern Mongolia, Sayan mountains. The species occurs in the Alpine belt of the Republic of Kazakhstan in the altitude limit of 1800-2300 m above sea stage at the ridges of the Kazakh Altai. The different growth conditions caused the formation of different forms. *Rhodiola* plants strongly vary both in phytochemical and morphological indices. Geographical and genetic isolation of *Rhodiola* populations led to high morphological differentiation in species, and thus requires study of this species using latest molecular genetic markers [1, 2].

To study genetic diversity, we used molecular markers of polymorphic genes that control biochemical and physiological processes in plant. Mutations can be located in the coding sequence - exon and cause change in the amino acid composition of the proteins, as well as in the regulatory elements, thereby affecting gene transcription.

Introns containing functional elements, such as amplifiers and suppressors, regulate alternative splicing, trans-splicing and other regulatory processes. They are the most variable regions of genes and have higher level of polymorphism than exons, since selective pressure is higher in exons. This can be used to study phylogenetic relationships and population diversity [3-5]

Exon-primed intron-crossing' (EPIC-PCR) (Palumbi and Baker 1994) is based on development of primers that are selected to anneal to highly conserved regions of the exons and the technique is highly favored in genetic study of plants and animals. For instance, this technique was used to study conserved regions within eukaryotic 18S and 28S ribosomal genes and prokaryotic 16S and 23S ribosomal genes, for amplification of variable intergenic regions known as internal transcribed spacers (ITS), containing 5.8S ribosomal gene[6].

EPIC has a number of advantages in the evolution study of populations. Firstly, universal primers of EPIC, complementary to exon regions can be applied across a broad taxonomic range. Secondly, homology of EPIC-amplified sequences can be easily defined by comparing either their exon or intron portion depending on the genetic distance between the taxons. Lastly, exonic and intronic fragments can help in the simultaneous studies of genetic variety at intraspecific and interspecific levels [7-10].An additional benefit of EPIC is that it avoids development of species-by-species development of DNA markers.

Previously, researches were focused on the study of population structure and genetic diversity of cultivated populations from different producing regions of China. There were no studies focusing on locality-level identification of *R. rosea L*.

In this research, our aim was to study genetic relationships amongst *Rhodiola sp*. growing in different locations of Altai, by using intron polymorphism markers of several genes.

To conduct genetic analysis of *Rhodiolasp.* polymorphism following gene families were studied: eukaryotic translation initiation factor 4e, superoxide dismutase, H-ATPase and auxin response factors. These gene families were selected due to their key roles in regulating physiological processes and adapting plants to stressful environmental factors These genes should be able to detect the high adaptability of *Rhodiola* plants to significant amplitude of temperatures during the day and high solar radiation. Population's adaptability depends on the magnitude of the genetic polymorphism of the genes that regulate the physiological processes controlling response to stress conditions.

MATERIALS AND METHODS

PlantmaterialsandDNAisolation

Atotalof27samplesof*Rhodiola sp.*werecollectedfromthreegeographicallydistinctpopulationsintheterritoryofKazakhstan'sAltai.

TotalgenomicDNAwasextractedfromherbariumspecimensorgreenleavesusingacidCTABbufferfollowingestablishedprotocols(http://primerdigital.com/dna.html)withRNAseAtreatment.TheDNAsampleswerediluted in 1×TE bufferandtheDNAqualitywascheckedelectrophoreticallyandspectrophotometricallywithaNanodrop(ThermoFisher ScientificInc.).DNAqualitywasdeterminedbyagarosegelelectrophoresis.ThepreparedDNAsampleswerestoredat−20°Cforfurtheranalysis.

DesignofEPICprimers

PCRprimersweredesignedusinggenomicsequencesforgenefamilies:H-ATPase(ATPase,H+/K+exchanging,alphapolypeptide)andARF6(AuxinResponseFactor 6)retrievedfromtheNCBIdatabase(http://www.ncbi.nlm.nih.gov/nuccore/)andEnsemblePlants(http://plants.ensembl.org) (table 1).

DNAmultiplesequencealignmentsofthegenesequencesidentifiedregionsofDNAconservationwithinexons,aswellasvariabilitywithinintronicregions.Thesequencealignmentwasusedinconjunctionwiththeprogram,Multain[11]andMuscle(http://www.ebi.ac.uk/Tools/msa/muscle/).

Specificandconservativeprimerdesignwasperformedusing[12-14].Itshouldbenotedthattheexonicregionschosenforprimerannealingshowhighlevelsofconservationnotonlyfor*Rhodiola*sp.,butalsoformoredicotsplantspecies,thusdemonstratingtheflexibilityofEPICforplantmolecularstudies.

Table 1. Nucleotide sequences of primers used in this study

ID	Sequence	Tm, °C	CG (%)	Primer location
H-ATPase (ATPase, H+/K+ exchanging, alpha polypeptide) (XM_013610133, 382bp)				
5182	TGTAAAGAGACTCCAGGAT	48.2	29.9	1716→1734
5183	AGGAGATGGCTTTACTCT	48.0	31.9	2080←2097
ARF6 (Auxin Response Factor 6) (XM_011009313)				
5175	AYTTYCCACARGGYCACAGTGARCA	60.7	50.0	1065→1089
5176	TCACAYTGGCGNTCAGTNAAGGTT	59.2	47.9	1922→1945
5177	GTCATCTGNGCRTANACTTCATCNGTCTC	60.9	48.3	1199←1227
5178	GAAGATRTGYCTRAAYTTCCATTCRTTAYCATG	58.4	36.4	1451←1483

5179	GTCAACAAATACAAGCTGCCAGCCTGATCT	62.7	46.7	3353←3382

PCR amplification

The PCR was performed in a 20 μl reaction mixture containing 20 ng DNA, 1x Phire buffer, 0.3μM each primer, 0.2 mM each dNTP and 0.2 U Phire Hot Start II DNA Polymerase (Thermo Fisher Scientific). The amplification was performed in the T100 Thermal Cycler (Bio-Rad). The PCR reaction program consisted of 1 cycle at 98ºC, 2 min; 30 cycles of 98ºC for 10 secs, 60ºC for 20 secs, 72ºC for 2 min; 72ºC for 5 min.

Each primer or primers combinations was tested in PCR reactions using a genomic DNA mixture composed of equal amounts from all the accessions. The PCR products were separated by electrophoresis at 70 V for 8 hours in a 1.3% agarose gel (RESolute Wide Range, BIOzym) with 1×TBE electrophoresis buffer. Gels were stained with EtBr and scanned using a Molecular Imager PharosFX™ Plus System (Bio-Rad) with a resolution of 50 μm.

Intron markers analyses

From the PCR profiles, all distinct bands were scored as present (1) or absent (0) at each band position for each primer in the 32 samples. Each PCR band was treated as a single locus. The presence or absence of a fragment of a given length was recorded in binary code. The sets that contained missing values were removed from the raw scored data sets. The gels were scored for the presence and absence of bands totaling 62 polymorphic bands for the samples. Based on the primary data, the level of genetic diversity [15] was determined using the Arlequin software. The primary genetic data were bootstrapped with SEQBOOT, after which the pairwise genetic distances were calculated using GENDIST (http://www.bablokb.de/gendist/), both programs are from the PHYLIP software package (http://evolution.gs.washington.edu/phylip.html). The ability of intron markers to reveal genetic relationships among all *R. rosea* L. accessions was evaluated phylogenetically by Neighbor-Joining (NJ) [16]. An algorithm was constructed using PAUP software. Support for the tree was determined by performing one thousand bootstrap operations on the data set generated by distance analysis. To study the partition of intron markers genetic variation to among-and within-population variance components, an analysis of molecular variance (AMOVA) was conducted with the program GenAlex 6.5 [17] and FAMD programs [18]. The number of permutations was set at 999 for AMOVA for a test of significance of the genetic distance among groups.

RESULTS AND DISCUSSION

PCR amplification of the genes of the plasma membrane family H-ATPase (Nydrogen potassium ATPase) and the family of transcription factors of the auxin response 6 (ARF6) Rhodiola sp. by the EPIC method

The search for markers revealing polymorphism in introns is an urgent task when studying genetic and geographical diversity and identifying population variability. Various marker systems are used to detect genetic polymorphism, the most commonly used are RAPD, ISSR and AFLP, SSR methods [19-22]. However, works on the use of markers detecting polymorphism in introns are not many. Intron regions of genes have a high level of polymorphism in comparison with exons and this allows using their polymorphism to reveal phylogenetic relationships and population diversity. Studies on the genetic diversity of natural populations of *Rhodiola sp*.have not been carried out although the distribution range of this species is quite wide: significant populations of *Rhodiola* have been identified in Altai, East Kazakhstan, and in the foothills of Altai.

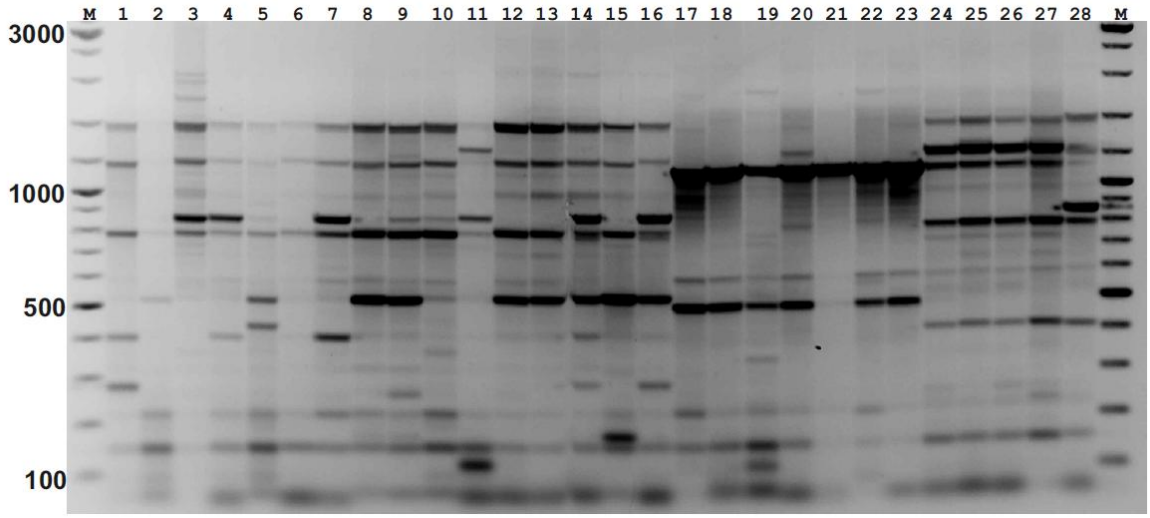
The design of PCR primers for the study of plasma membrane genes and transcription factor 6 of the auxin response was carried out on the basis of the complete nucleotide sequences of the H-ATPase and ARFs genes obtained from the Ensemble Plants database (http://plants.ensembl.org). Based on the obtained data, primer design was carried out using the FastPCRsoftware and "universal" EPIC primers were designed which were used to identify polymorphic alleles of the plasma membrane genes and detect the genes of the transcription factors of the auxin response.

PCR with using the designed primers provided the appearance of the corresponding products of amplification of plasma membrane genome regions and transcription factors of auxin response in the explored representatives of the Kazakhstani populations of *Rhodiola*. PCR primers to the H-ATPase and ARF6 genes revealed specific amplification products in all of the studied *Rhodiola* species.

During amplification with primers to H-ATPase genes, PCR products measuring approximately 200-1500 bp were obtained, which corresponds to the expected results (figure 1) The PCR fragments generated with the primers to the gene of the transcription factors of the auxin response of ARF6 had the expected size of 400-1000 bp, which also indicates the specificity of these primers.

The amplification profile data obtained using the primers was evaluated using fingerprinting techniques. The level of detectable polymorphism for the H-ATPase gene using primer pairs 5182 and 5183 ranged from 45 to 67%. A high level of polymorphism of the H-ATPase gene illustrates a high adaptive ability of Rhodiola

plants, representing Kazakhstani populations. The variety of alleles of this gene, represented in the populations of Kazakhstan, conditioned by the existence of *Rhodiolarosea*under extreme conditions.



1-16– samples of plants of the species *Rhodiolarosea* (14-SA1, 16-SA2), 17-23– samples of plants of the species *Rhodiolaalgida*, 24-28– samples of plants of the species *Rhodiolaquadrifida*, M –Thermo Scientific GeneRuler DNA Ladder Mix (100-10,000 bp)

Fig. 1. Results of DNA amplification of rodiola populations with primers 5182-5183 to the H-ATPase gene

The polymorphism level was 67% when using the combination of primers 5176 and 5177 to the gene families of the transcription factors of the auxin response (ARF6). Comparative analysis of the amplification spectra with the above primers for the gene families of the plasma membrane of H-ATPase and the gene family of the auxin response of ARFs revealed that *Rhodiolaalgida*and *Rhodiolaquadrafita* species, living under favorable water-temperature conditions on soils with a high humus content (high humidity soils, temperature regime) are distinguished by a smaller variety of alleles of these genes in comparison with samples of *Rhodiolarosea* plants.

Allelic variants and frequency of their occurrence are specific for each population of *Rhodiola*. Analysis of allelic diversity within the *Rhodiolarosea* species revealed that SA-1 and SA-2 populations from the southern Altai have the greatest variability of alleles compared to the populations of the western Altai. We assume that this explains the possibility of the existence of individuals of this population at an altitude of 2140-2300 m above the sea level, in conditions of extreme habitat.

To determine the discriminatory potential, the developed main criteria for genetic variability, genetic distances and similarities in Neu, an analysis of the molecular dispersion of AMOVA was carried out.An analysis of the genetic diversity of the *Rhodiola* populations was carried out using the GenAlEX program. Parameters of genetic diversity are presented in table 2.

Table 2. The main indicators of the genetic diversity of *Rhodiola*populations

Rhodiola species	Na	Ne	I	He	uHE	PIC	%, polymorphism
Rhodiolarosea	1,6	1,481	0,412	0,277	0,285	0,8244	80
Rhodiolaaldiga	1,4	1,265	0,267	0,166	0,179	0,8689	60
Rhodiolaquadrafita	1,2	1,501	0,374	0,263	0,283	0,9014	67
Total	1,4	1,416	0,351	0,235	0,249	0,8649	68
Na – observed number of alleles; Ne – effective number of alleles; I – Shannon's Information index; He– Expected Heterozygosity, uHE– Unbiased Expected Heterozygosity, P – percentage of polymorphic loci							

Comparative analysis of populations showed that each of the studied populations of *Rhodiola* is characterized by some degree of uniqueness in the number of alleles. Analysis of the genetic variability of the Kazakh populations of *Rhodiola* showed that the number of alleles per locus varied from 1.2 in the *Rhodiolaquadrafita* population to 1.6 in the *Rhodiolarosea population*.

The heterozygosity index is the most common measure of genetic variability in populations. The *Rhodiolarosea* population had the highest observed heterozygosity He (0.277), while in the

*Rhodiolaaldiga*population was 0.166.The values of the expected heterozygosity of uHE (estimation of the level of genetic variability in the population) ranged from 0.179 in plants of the *Rhodiolaaldiga*population to 0.285 in the *Rhodiolarosea* population.

The level of genetic variability between the populations studied was determined by calculating the genetic distances of Neu (table 3).

Table 3. The matrix of genetic distances, constructed by the method of genetic distance by Nei

Populations	*Rhodiolaquadrafita*	*Rhodiolaaldiga*	*Rhodiolarosea*
Rhodiolaquadrafita	0,000		
Rhodiolaaldiga	0,098	0,000	
Rhodiolarosea	0,053	0,120	0,000

The lowest genetic distance was found by the populations of *Rhodiolaaldiga* and *Rhodiolarosea* (0.120), the largest - between the *Rhodiolaquadrafita*and *Rhodiolaaldiga* population (0,098).

To assess the interpopulation diversity of *Rhodiola,* the Shannon Information Index was used. The Shannon diversity index expresses the complexity of the population structure and makes it possible to estimate the wealth of the population under study, that is, to determine the number of species and the uniformity of their distribution.

The greatest values of polymorphism and Shannon index (I) were observed in the *Rhodiolarosea*population – 0,412, which lives in extreme natural conditions. Analysis of genetic diversity revealed that the *Rhodiolaalgida* population living in a favorable water-temperature regime is less diverse compared to samples of *Rhodiolarosea* plants growing under more severe conditions.

A dendrogram constructed on the basis of data was divided into three clusters(figure 2).The dendrogram constructed from the results of cluster analysis breaks up the population into 3 main clusters, with the maximum genetic distance being observed between the populations of *Rhodiolaquadrafita* and the *Rhodiolaaldiga* population, which is confirmed by an estimate of the genetic distances of Ney.

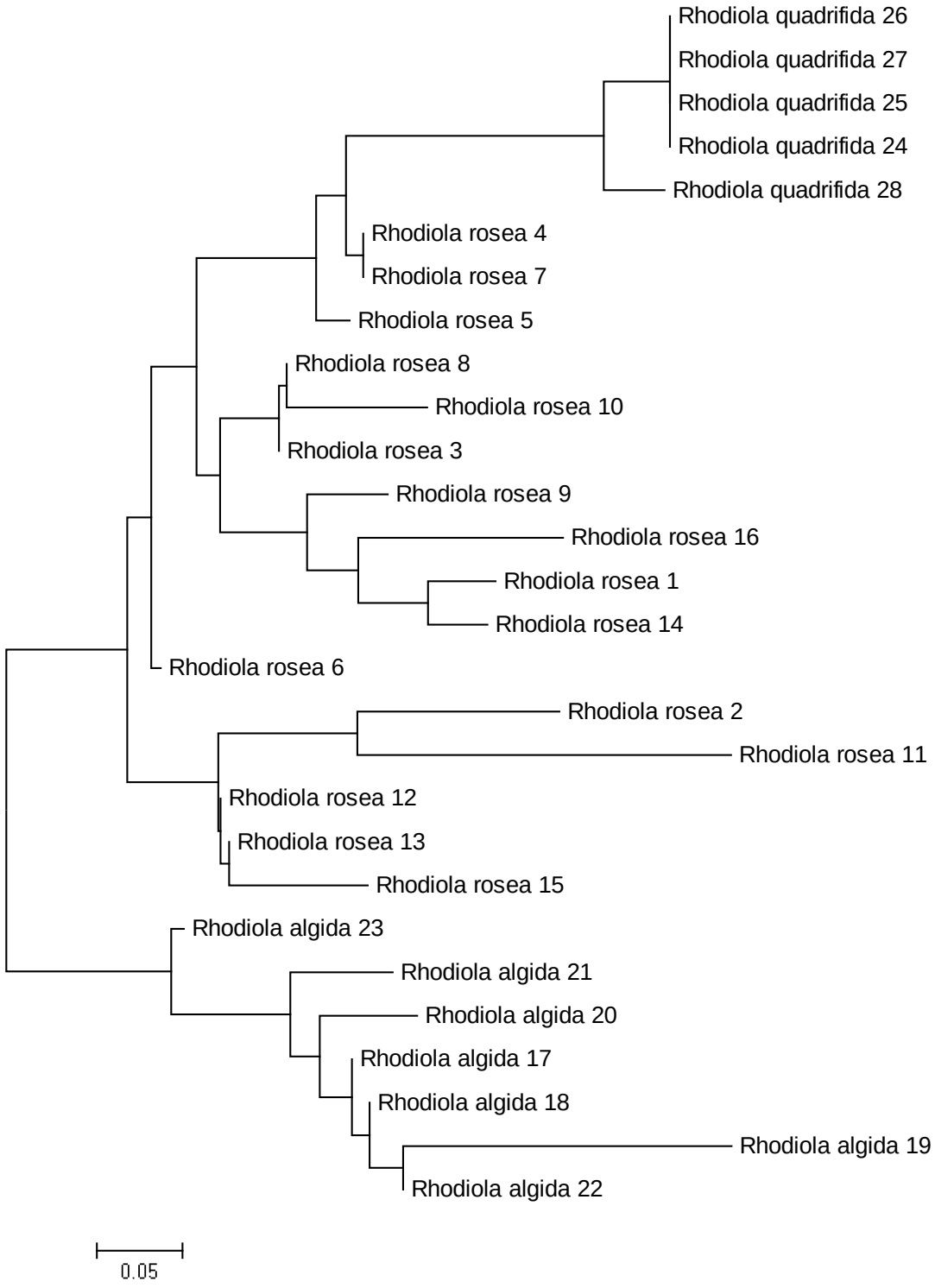


Fig. 2. Neighbor-joining dendrogram of showing the genetic relationships among 26 accessions of *Rhodiola*based on H-ATPaseintronic markers analysis

AMOVA (molecular dispersion assay) analysis was performed (table 4) to assess intra- and interspecific variability. Advantages and differences AMOVA for the analysis of genetic data from the classical analysis of variance (ANOVA) are that in the analysis of molecular dispersion, different evolutionary models can be used without modifying the basic structure of the analysis. Analysis of the molecular variability of AMOVA demonstrated significant differences within populations (70%) and slightly less between populations (30%).

Table 4. The results of the analysis of molecular variability (AMOVA) of *Rhodiola* populations

Source	df	SS	MS	Est. Var.	%
Among Pops	2	19,875	9,938	0,849	30
Within Pops	29	56,500	1,948	1,948	70
Total	31	76,375		2,798	100

Analysis of the genetic diversity of the Kazakhstan *Rhodiola* populations using the primers developed to us for the H-ATPase and ARFs gene showed that the level of differentiation between the studied populations was rather high, the most part of the identified genetic diversity is in the interspecies component (70%), which can be explained by the range of distribution of the individuals within populations, the presence in the population of individuals of different ages, the specific nature of the location of populations within the habitat and the mosaic distribution suitable for the growth areas.

The carried out analysis of population diversity shows that conservative PCR primers we developed allow to obtain unique, well reproducible spectra of amplification products convenient for studying the interspecies and interpopulation polymorphism of *Rhodiola* and confirms our assumption that these gene families may be used as a new type of molecular genetic markers.

CONCLUSION

The results of the studies showed that the EPIC (exon-primed intron-crossing) PCR amplification method, in which primers complementary to the terminal regions of the gene exons, can be used to search for new types of highly informative markers. The primers developed for the gene family of the plasma membrane of H-ATPase and the family of the auxin response of ARF6 are universal for the detection of alleles of these genes in various *Rhodiola* species. Variable regions of intron genes can be used as a new type of molecular markers, due to high efficiency for interpopulation clustering, in studying genetic diversity and searching for new taxonomic units.

Acknowledgements

The work was supported by the Ministry of Education and Science of the Republic of Kazakhstan under the project 4869/GF4 "Research of the genetic and geographical diversity of the natural populations of *Rhodiolarosea* L. in Kazakhstan by sequencing the non-coding part of the genome (non-transcribed spacers and introns of genes) on 2015-2017 (no. GR0115RK01775).

REFERENCES

1. Alm T. Ethiopian of Rhodiola rosea (Crassulaceae) in Norway. Applied Biostatistics, 2010, Inc:321-344.
2. Poczai P., Hyvonen J. Nuclear ribosomal spacer regions in plant phylogenetics: problems and prospects. *Mol Biol Rep*, 2010, vol. 37 (4), pp. 1897-1912. doi:10.1007/s11033-009-9630-3.
3. Rose A.B., Beliakoff J.A. Intron-Mediated Enhancement of Gene Expression Independent of Unique Intron Sequences and Splicing. *Plant Physiology*, 2000,vol. 122 (2), pp. 535-542.
4. Jeon J-S. et al. Tissue-Preferential Expression of a Rice α-Tubulin Gene, OsTubA1, Mediated by the First Intron. *Plant Physiology*, 2000,vol. 123 (3), pp. 1005-1014.
5. Holland J.B. et al. Polymorphism of PCR-based markers targeting exons, introns, promoter regions, and SSRs in maize and introns and repeat sequences in oat. *Genome*, 2001, vol. 44 (6), pp. 1065-1076.
6. Gardes M., Bruns T.D. ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. *Mol Ecol*, 1993,vol. 2 (2), pp. 113-118.
7. Bierne N. et al. Screening for intron-length polymorphisms in penaeid shrimps using exon-primed intron-crossing (EPIC)-PCR. *Molecular Ecology*, 2000,vol. 9 (2), pp. 233-235. doi:10.1046/j.1365-294x.2000.00842.x.
8. Lessa E.P. Rapid surveying of DNA sequence variation in natural populations. *Mol Biol Evol*, 1992, vol. 9 (2), pp. 323-330.
9. Van Camp W et al. Enhancement of oxidative stress tolerance in transgenic tobacco plants overproducing Fe-superoxide dismutase in chloroplasts. *Plant Physiology*, 1996, vol. 112 (4), pp. 1703-1714.
10. Morsomme P., Boutry M. The plant plasma membrane H(+)-ATPase: structure, function and regulation. *Biochim Biophys Acta*, 2000,vol. 1465 (1-2), pp. 1-16. doi:10.1016/S0005-2736(00)00128-0.
11. Corpet F. Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Research*, 1988,vol. 16 (22), pp. 10881-10890.

12. Kalendar R. et al. FastPCR: An in silico tool for fast primer and probe design and advanced sequence analysis. *Genomics.*,2017, doi:10.1016/j.ygeno.2017.05.005.

13. Kalendar R. et al. In Silico PCR Tools for a Fast Primer, Probe, and Advanced Searching. *Methods Mol Biol,* 2017, vol. 1620, pp. 1-31. doi:10.1007/978-1-4939-7060-5_1.

14. Kalendar R. et al. Introduction on Using the FastPCR Software and the Related Java Web Tools for PCR and Oligonucleotide Assembly and Analysis. *Methods Mol Biol.*,2017, vol. 1620, pp. 33-64. doi:10.1007/978-1-4939-7060-5_2.

15. Nei M. Analysis of Gene Diversity in Subdivided Populations. *Proceedings of the National Academy of Sciences of the United States of America*,1973,vol.70, (12 Pt 1-2), pp. 3321-3323.

16. Tamura K., Stecher G., Peterson D. et al. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol.*, 2013,vol.30, no. 12, pp. 2725-2729.

17. Peakall R., Smouse P.E. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*,2012, vol. 28 (19), pp. 2537-2539. doi:10.1093/bioinformatics/bts460.

18. Schluter P.M., Harris S.A. Analysis of multilocus fingerprinting data sets containing missing data. *Molecular Ecology Notes*, 2006,vol. 6 (2), pp. 569-572. doi:10.1111/j.1471-8286.2006.01225.x.

19. Arif I.A. et al. A Brief Review of Molecular Techniques to Assess Plant Diversity. *International Journal of Molecular Sciences*,2010, vol. 11 (5), pp. 2079-2096. doi:10.3390/ijms11052079.

20. Costa R. et al. Comparison of RAPD, ISSR, and AFLP Molecular Markers to Reveal and Classify Orchardgrass (Dactylis glomerata L.) Germplasm Variations. *PLoS ONE*, 2016,vol. 11 (4), pp. e0152972. doi:10.1371/journal.pone.0152972.

21. Nilkanta H. et al. ISSR Marker Based Population Genetic Study of Melocanna baccifera (Roxb.) Kurz: A Commercially Important Bamboo of Manipur, North-East India. *Scientifica*,2017, pp. 3757238. doi:10.1155/2017/3757238.

22. Shehata A.I. et al. Application of simple sequence repeat (SSR) markers for molecular diversity and heterozygosity analysis in maize inbred lines. *Saudi Journal of Biological Sciences*,2009, vol. 16 (2), pp. 57-62. doi:10.1016/j.sjbs.2009.10.001.

АЛТАЙДАҒЫ АЛТЫН ТАМЫРДЫҢ (RHODIOLA SP.) ГЕНЕТИКАЛЫҚ ТҮРЛІЛІГІН ИНТРОН ПОЛИМОРФИЗМІН АНЫҚТАЙТЫН МАРКЕРЛЕР НЕГІЗІНДЕ БАҒАЛАУ

Тагиманова Д.С.[1], Хапилина О.Н., Аменов А.А.[1], Данилова А.Н.[2], Календарь Р.Н.[1]

[1]Ұлттық биотехнология орталығы,
Қорғалжын тас жолы, 13/5, Астана, Қазақстан
[2]Altai Botanical Garden, Ridder, Kazakhstan
tagds@mail.ru

ТҮЙІН

Roseroot (Rhodiolarosea L.) **Қытай, Скандинавия, Ресейде кең қолданылатын дәрілік өсімдіктердің бірі болып табылады. Бірнеше гендер тұқымдастары үшін интрон полиморфизмін анықтайтын маркерлермен ПТР амплификациясы көмегімен біз 32 өсімдік үлгісін зерттедік. Өсімдіктер Алтай территориясында кездесетін (Батыс Алтай, Оңтүстік Алтай) 3 түрлі табиғи популяциялардан алынды. Біз *Rhodiola sp. Dicots*. қатысты секвенирленген гендердің белгілі бір аймақтарына бағытталған әмбебап ПТР праймерлерін әзірледік. Әзірленген интрон полиморфизмін анықтайтын маркерлер қызғылт родиоланың генетикалық түрлілігін анықтау үшін пайдаланылды. Генетикалық түрлілікті анықтау бойынша талдау жұмысы GenAlex 6.5 бағдарламасы арқылы жүргізілді. Нәтижесінде, әзірленген маркерлердің дискриминационды потенциалы семізот популяцияларының түрішілік өзгерістерін анықтау үшін едәуір ақпаратты болатыны анықталды. Бақыланатын гетерозиготтылық орташа 0.235, ал күтілетін 0.249 құрады. Алынған зерттеу нәтижелері бойынша құрылған дендрограмма популяциялар арасында айырмашылықтардың барын дәлелдейді, неғұрлым олар бір-бірінен алыс болса, соғұрлым олардың арасындағы генетикалық дистанция жоғары болады.**

Молекулярлық дисперсияны талдауы (AMOVA) генетикалық түрлілік деңгейінің салыстырмалы түрде жоғары болғанын (p=70%), ал Шеннон индексі қызғылт семізоттың популяциялар арасындағы және популяциялар ішіндегі генетикалық түрліліктің біршама

айырмашылықтары бар екенін көрсетті. Генетикалық арақашықтықтарды Ней бойынша талдау мен кластерлі талдау нәтижелері (UPGMA) қызғылт семізоттың қазақстандық популяцияларының 3 негізгі кластерге бөлінетінін көрсетті. *R. Rosea L.* популяциясының генетикалық құрылымын зерттеу мен түсіну осы түрді сақтап қалуға септігін тигізеді.

Негізгі сөздер: генетикалық түрлілік, интронды полиморфизм, *Rhodiola rosea, roseroot.*