Genetic Diversity Within and Among Populations of Roseroot (Rhodiola rosea L.) Based on Molecular Markers

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Abstract

Rhodiola rosea L. is a perennial adaptogenic medicinal plant found in cool climate of the northern hemisphere. The species is very diverse both in terms of morphological characteristics and in the content of the pharmacologically active substances. The genetic diversity of four geographically distant roseroot populations was studied with ISSR and SSR markers. Using 7 ISSR primers 64 DNA fragments were generated and 85.94% of those were found to be polymorphic, indicating high genetic variability at the species level (gene diversity = 0.33, Shannon index = 0.48). Lower level of diversity was detected at the population level (Shannon-index ranged from 0.2173 to 0.2696). Only four out of the eight SSR markers used were informative during this study. The primer pairs for these four SSR markers produced 25 fragments with an average of 6.25 putative alleles per locus. Observed heterozygosity ranged from 0.4 to 1.0, whereas expected heterozygosity ranged from 0.47 to 0.84. Cluster analysis based on both markers revealed the same groups, individuals clustered according to their geographic origin. The Southern-Uralian population was the most genetically isolated. ITS analysis was used for the determination whether these Southern-Uralian individuals belong to the same species.

Keywords: ISSR, ITS, molecular markers, Rhodiola rosea, SSR

Introduction

Rhodiola rosea L., commonly known as golden root or roseroot is a traditional adaptogenic medicinal plant. Scandinavian, Eastern-European and Asian people have used it for centuries as general immune-stimulant. Roseroot belongs to the family Crassulaceae. It is a herbaceous plant with thick rhizome, which contains pharmacologically important secondary metabolites (Brown et al., 2002).

Rhodiola rosea displays a circumpolar distribution in the higher latitudes and elevations of the Northern hemisphere mainly in Asia and Europe (Brown et al., 2002; Furmanowa et al., 1995). According to Hegi (1963), its distribution in Europe extends from Iceland and the British Isles across Scandinavia as far south as the Pyrenees, the Alps, the Carpathian Mountains and other mountainous Balkan regions. Roseroot is highly variable both in phytochemical (Kurkin et al., 1988, Wiedenfeld et al., 2007) and in morphological aspects (Asdal et al. 2006; Ohba 1981, 1989).

According to Flora Europaea Rhodiola rosea has several synonyms like Sedum rhodiola DC., Sedum rosea (L.) Scop., Rhodiola arctica Boriss., Rhodiola iremelica Boriss., Rhodiola crenulata Simonk., Sedum scopolii Simonk. Rhodiola iremelica Boriss. is described in Flora U.S.S.R as an endemic species of Southern-Ural (1939). Analysis of the ITS (internal transcribed spacer) region is widely used in taxonomy because it is easy to amplify and has high degree of variation even between closely related species. Mayazu and Ohba (2004) have performed a large scale analysis of the Cassulaceae family, but no information is available about R. iremelica.

For genetic diversity studies mostly inter simple sequence repeats (ISSR) have been applied in the genus Rhodiola. Xia et al. (2005) studied the genetic variation within and among populations of Rhodiola alba and later (Xia et al., 2007) of Rhodiola chrysanthemifolia native to the Tibetan Plateau. Lei et al. (2006) explored the genetic variation in Rhodiola crenulata from the Hengduan Mountains. Finnish Rhodiola rosea populations were analysed by György et al. (2009). Yanbaev et al. (2007) studied Rhodiola iremelica populations in the Southern-Urals with isoenzymes. Elameen et al. (2008) investigated using AFLP (Amplified Fragment Length Polymorphisms) technique the genetic diversity in a Norwegian germplasm collection. Meng et al. (2007) used AFLP for analysing intra-specific genetic variation in the genus Rhodiola and the same method was used by Wang et al. (2009) for studying the genetic diversity of Rhodiola rosea populations from Tianshan Mountain. Recently Kozyrenko et al. (2011) analysed the genetic structure of Rhodiola rosea accessions mostly of Russian origin using ISSR technique.

In 2009 Zini et al. (2009) published eight microsatellite sequences (simple sequence repeats, SSR) of Rhodiola
extracted from the frozen leaves according to a CTAB-based protocol (Pirttilä et al., 2001). DNA concentration and quality was assessed using NanoDrop spectrophotometer (BioScience, Hungary) and visualized on 1% agarose gel.

PCR amplification of SSR and ISSR markers

PCR was performed in 25 μl reaction volume containing 20-80 ng DNA, 10×PCR reaction buffer, 2.5 mM MgCl₂, 2 mM dNTP mix, 2.5 μmol of each 5’ and 3’ end primers, 1 unit of Taq DNA polymerase (Fermentas, Szeged, Hungary) and sterile distilled water. Eight SSR primer pairs, designed specifically for roseroot by Zini et al. (2009) were used for the DNA amplification. The forward primers were fluorescently labelled (FAM). Seven ISSR primers from the UBC primer set #9 developed at the University of British Columbia, (Canada) (BC807, BC809, BC840, BC841, BC857, BC885 and BC888) were chosen based on preliminary experiments. PCR was carried out in a PTC 200 thermocycler (MJ Research, Budapest, Hungary) using touchdown strategy as described in the paper of Zini et al. (2009) in the case of SSR markers. For the amplification of ISSR fragments the following program was used: initial denaturation at 94°C for 4 min; followed by 40 cycles of 94°C for 60 s, 49°C for 90 s, 72°C for 90 s; and a final extension at 72°C for 7 min.

The PCR products were electrophoresed on a 1% (w/v) ethidium bromide-stained agarose gel (SeaKem LE Agarose, Lonza, Rockland, ME USA) in 1xTBE buffer with xylene cyanol loading buffer to verify the occurrence of the amplification. For the ISSR analysis PCR products were separated for 2 h at 120 V. Amplified fragments were scored visually for presence (1) or absence (0) and the result were summarised in MS Excel table.

Materials and methods

Plant material and DNA preparation

_Rhodiola rosea_ plants were provided by the Institute of Biology, Komi Science Centre, Ural Division, Russian Academy of Sciences, Syktyvkar, Russia. Roseroot plants were collected in the Northern-Ural Mountains, Southern-Ural Mountains, in Novaya Zemlya and in the Altai Mountain (Fig. 1). The collected plants were cultivated in the experimental field of Syktyvkar University. Due to difficulties of transporting plant material only 5 plants were included in the study from each population, all together 20 individuals. The plant material was frozen in liquid nitrogen and was stored in -80°C until used. DNA was extracted from the frozen leaves according to a CTAB-based protocol (Pirttilä et al., 2001). DNA concentration and quality was assessed using NanoDrop spectrophotometer (BioScience, Hungary) and visualized on 1% agarose gel.

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Fig. 1. Map showing the approximate locations of the four examined rosroot populations
The amplified SSR fragments were separated on 8% polyacrylamide gel at 80 W (55°C) for 4 h, and stained with a simple silver staining method (Bassam and Gresshoff, 2007). Amplified fragments were scored visually. To determine the exact size of different fragments, 3 from each detected fragment sizes were run in an automated sequencer ABI PRISM 3100 Genetic Analyzer (Applied BioSystems, Budapest, Hungary). Band scoring was analysed using Peak Scanner software 1.0 (Applied BioSystems, 2006).

Genetic relatedness among genotypes was studied by UPGMA (Unweighted Pair Group Method with Arithmetic averages) cluster analysis using Poppgene version 1.32 (1997). Also Poppgene was used to estimate expected ($H_e$), observed ($H_o$) heterozygosity, Nei’s (1978) gene diversity and Shannon’s Information Index (I) for co-dominant marker data (SSR). For dominant marker data (ISSR), Poppgene was used to estimate number of polymorphic bands, percentage of polymorphic bands, Nei’s gene diversity (h) and Shannon’s Information Index (I). For all individuals Hamming distance was calculated with PASSaGE 2 (Rosenberg and Anderson, 2011) in case of both marker method. The distance matrices were compared with Mantel test also performed with PASSaGE 2.

**PCR amplification of ITS regions**

The internal transcribed spacer region includes the two internal transcribed spacers (ITS1, ITS2) and the 5.8S gene of the 18S/26S nuclear ribosomal DNA (rDNA) repeats. This region was amplified using the primers ITS-LEU (Baum et al., 1998) and ITS4 (White et al., 1990). PCR mix was the same as described earlier. The PCR program started at 94°C for 4 min, followed by 30 cycles of 94°C for 30 sec, 50°C for 1 min and 72°C for 1 min, followed by an additional 7 min extension at 72°C. The PCR products were separated on a 1% (w/v) ethidium bromide-stained agarose gel in 1×TBE buffer with xylencyanol loading buffer to verify the occurrence of the amplification. Fragment lengths were estimated by comparison with the 1-kb DNA ladder (Promega, Madison, USA).

The gel-purified PCR products (EZ-10 Spin Column DNA, Gel Extraction Kit, Bio Basic INC, Biocenter, Hungary) were directly used in the sequencing reaction. Sequencing used the BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Warrington, UK) and was performed in an automated sequencer ABI PRISM 3100 Genetic Analyzer (Applied BioSystems, Budapest, Hungary). Both strands were sequenced and the same primers (ITS-LEU and ITS4) were used as in the PCR.

DNA sequences were compared using BLASTN at NCBI and ClustalW program (Thompson et al., 1994), and the aligned sequences were edited with BioEdit v.7.0.9.0.

**Results**

**SSR markers**

Amplification was successful with 7 out of the 8 available roseroot SSR primers. The numbers of alleles per locus amplified in course of the study are presented in Tab. 1. Primers for marker RRE4 in most samples failed to amplify genomic DNA. Primer pair for marker RRF4 amplified mostly only one allele per locus indicating homozigosity for that allele or the presence of null alleles. The primers for RRE9 and RRF3 amplified monomorphic fragments for the tested plants (146 and 155; 133 and 143 bp respectively). Therefore only 4 (RRC10, RRD6, RRE2, RRE3) out of the 8 markers were informative during the study. The primer pairs for these four SSR markers produced 25 fragments. The number of alleles per locus ranged from 2 (RRE9 and RRF3) to 8 (RRC10). Sizes ranged from 103 (RRF4) to 185 bp (RRE2) (Tab. 2).

Genetic diversity parameters are presented in Tab. 2. Observed heterozygosity ($H_o$) ranged from 0.4 to 1.0, whereas expected heterozygosity ($H_e$) (genetic diversity) ranged from 0.47 at RRD6 for Altai to 0.84 at RRC10 for Altai population.

Genetic relationships among the studied populations of various origins are shown in Fig. 2. According to this dendrogram the S-Uralian population forms the most distinct group from the others.

| Locus name | No. of alleles obtained in this study | Expected size range, based on Zini et al. 2009 (in bp) | Obtained allele size (in bp) |
|------------|--------------------------------------|-----------------------------------------------------|-----------------------------|
| RRC10      | 7                                    | 146-164                                             | 140-158                     |
| RRD6       | 6                                    | 168-186                                             | 166-182                     |
| RRE2       | 8                                    | 161-182                                             | 155-185                     |
| RRE3       | 4                                    | 173-183                                             | 173-183                     |
| RRE4       | 2 (-)                                | 146-149                                             | 146-149                     |
| RRE9       | 2                                    | 143-161                                             | 146-155                     |
| RRF3       | 2                                    | 121-137                                             | 133-143                     |
| RRF4       | 2 (-)                                | 103-118                                             | 103-115                     |
The selected 7 primers generated 64 bands, corresponding to an average of 9.14 bands per primer. An example for ISSR gel is presented in Fig. 3. The number of population specific bands (either being specifically present or specifically missing) ranged from 1 to 7. The least variable band pattern was generated with primer BC857 (1 population-specific band out of 9), while the most variable band pattern was generated with primer BC840 (6 population-specific band out of 8). At the population level the Shannon-index was 0.48 and the percentage of polymorphic loci (PPL) was 85.94%. The comparison of the value of the Shannon-index and PPL in the 4 habitats is shown in Tab. 3. As indicated by these parameters, the highest level of variability occurred in New Land population (PPL =46.88%; I = 0.2696), whereas the lowest level in the S-Uralian population (PPL = 37.5%; I = 0.2173). Genetic relationships among the studied individuals of various origins are shown in Fig. 4. Similarly to the dendrogram shown in Fig. 2, the S-Uralian population formed the most distinct group from the other populations.

**Comparison of the variability based on the two marker methods**

Mantel test (Z=84.59) was performed for the comparison of the variability data gained with the two different marker system. The correlation was found to be 0.24 (p=0.001), the Mantel test was highly significant. Based on this result the two marker methods found the same individuals to be close or to be distant. This confirms the two dendrograms (Fig. 2 and 4) being very similar.

**Amplification and sequencing of the ITS region**

The S-Uralian roseroot population clustered to a separate group in both dendrograms drawn based on the SSR and ISSR data (Fig. 2 and 3). Sequencing of the ITS region of all individuals from this population and also 2-3 individuals from the others was undertaken to clarify if the S-Uralian population belongs to *Rhodiola rosea* or possibly to *Rhodiola iremeltica* (Yanbaev et al., 2007). The ITS-LEU and ITS4 primers amplified a 690 bp fragment from all samples. Fig. 4 shows the alignment of these sequences.
sequences to ITS sequence from other *Rhodiola* species (*R. rosea* GQ374198.1, *R. yunnanensis* AB088602.1, *R. wallychiana* AB088607.1, *R. macrocarpa* AB088590.1, *R. heterodonta* AB088596.1, *R. fastigiata* AB088594.1 and *R. chrysanthemifolia* AB088606.1) available in the NCBI genbank. The ITS sequences of the present study are almost identical as seen in Fig. 5.

**Discussion**

The aim of the present study was to compare the SSR and ISSR methods for the analysis of the genetic diversity of *Rhodiola rosea*. Both SSR and ISSR markers revealed genetic diversity within the examined populations of roseroot.

Genetic variability within the populations detected with the ISSR method was somewhat higher than in the study of Kozyrenko *et al.* (2011) where eight primers were used. Shannon index ranged between 0.22-0.27 while in the study of Kozyrenko *et al.* (2011) it ranged between 0.16-0.26. The genetic diversity observed among the populations was much higher in the present study. Shannon index was 0.48 while in the study of Kozyrenko *et al.* (2011) it was just 0.29. This big alteration can be the result of the fact that the examined populations in this study are located at much diverse latitude, while Kozyrenko *et al.* (2011) took samples from more similar latitude, rather than the difference in the used markers, since half of the primers used are the same in the two studies. Earlier two Finnish roseroot populations were compared with five primers (György *et al.*, 2011) and Shannon-indexes of 0.3129 and 0.2316 were calculated within the two populations, while 0.3385 among the populations. Generally all these Shannon index values are similar to those of *R. crenulata* from the Hengduan Mountains (0.168-0.325, 12 primers), (Lei *et al.*, 2006) and somewhat higher than of *R. alisa* (0.0729-0.2235, 13 primers), (Xia *et al.*, 2005) and *R. chrysanthemifolia* (0.0833-0.2415, 13 primers), (Xia *et al.*, 2007) in the Tibetian Plateau.

Zini *et al.* (2009) developed the eight SSR markers available for *Rhodiola rosea*. The genetic diversity of two Italian roseroot populations was examined as validation of these markers. Kylin (2010) used four out of these SSR markers to ITS sequence from other *Rhodiola* species.

![Fig. 4. Dendrogram of the 20 roseroot samples of 4 populations assayed in this study generated by UPGMA cluster analysis based on the similarity matrix obtained using Nei’s genetic distance based on ISSR data (Nei, 1978)](image_url)
Fig. 5. Alignment of ITS sequences of 3 individuals from N-Uralian population (1/1, 1/3, 1/4), 5 individuals from S-Uralian population (2/1, 2/2, 2/3, 2/4, 2/5), 2 individuals from Altaian population (3/1, 3/3), 3 individuals from New Land population and further *Rhodiola* ITS sequences found in the NCBI genbank: *R. rosea*, *R. yunnanensis*, *R. wallichiana*, *R. macrocarpa*, *R. heterodonta*, *R. fastigiata* and *R. chrysanthemifolia*. 
markers for exploring genetic diversity in the Swedish *Rhodiola rosea* collection (NordGen).

As it can be seen from Tab. 4, RRC10, RRD6 and RRE2 loci showed the highest polymorphism. RRE4, RRE9, RRF3 and RRF4 are either monomorphic (with two alleles) or many times even failed amplification. Higher number of alleles detected in this study indicates higher genetic diversity in the studied populations compared to the study of Zini et al. (2009) and Kylin (2010). Observed heterozygosity (Ho) ranged from 0.4 to 1.0, whereas expected heterozygosity (He) (genetic diversity) ranged from 0.4667 at RRD6 for Altai to 0.8444 at RRC10 for Altai. Lowest and highest observed heterozygosity for the same loci analysed by Zini et al. (2009) ranged from 0.091 at RRE3 to 0.760 at RRF3, and lowest value for expected heterozygosity was achieved at RRE3 (0.165) and highest value was at RRC10 (0.661). In the study of Kylin (2010) observed heterozygosity ranged from 0.0 to RRE9 to 1.0 at RRC10, while expected heterozygosity ranged from 0.198 at RRE3 to 0.733 at RRC10. Both expected and observed heterozygosity were higher in all four populations than reported previously for the same primer set, which indicates higher genetic variation in the studied roseroot populations.

The highest genetic diversity was found in the Novaya Zemlya population, while the lowest in the S-Uralian population both with the used SSR and ISSR markers (Tab. 3 and 4). The Novaya Zemlya population is the northernmost population examined so far. The optimal environmental circumstances may favour the maintenance of high genetic variability.

The S-Uralian population according to Yanbaev et al. (2007) is an endemic plant (*R. iremelica*). According to our results these plants form a clearly separated cluster on the dendrograms based both on SSR and ISSR markers. ITS sequence analysis was performed. Ishmuratova (2004) and Yanbaev et al. (2007) has written about these plants as *R. iremelica*. Borissova (1939) described *R. iremelica* in Flora USSR as new species. The difference between *R. iremelica* and *R. rosea* are the shape of the leaves and the size of the seeds. As mentioned earlier *R. rosea* displays high morphological variability. *Flora Europaea* states that *R. iremelica* is only a synonym for *R. rosea* (http://rbge-web2.rbge.org.uk/FE/fe.html). Mayuzumi and Ohba (2004) examined the phylogenetic position of Eastern-Asian *Sedoideae* and Gontcharova et al. (2006) has studied members of the *Sedoideae* subfamily based on ITS region. The ITS region sequences of different *Rhodiola* species gained in their studies were used in the alignment together with the sequences of the present study. Unfortunately no sequence data is available for *R. iremelica*. In those positions where *Rhodiola* species differ no alteration can be observed among the ITS sequences of the individuals from the four examined populations, which supports the S-Uralian population being *Rhodiola rosea* or a subspecies of it. Further studies are needed to clarify this issue.

In conclusion, using ISSR markers and SSR markers recently developed for roseroot we were able to assess genetic diversity of roseroot populations of different geographical origin. Both marker systems revealed similar results. However according to our results only four out of the eight SSR primers are feasible (RRC10, RRD6, RRE2, RRF3). Developing more roseroot specific SSR markers would be needed for more accurate studies.

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**References**

Asdal A, Galambosi B, Olsson K, Wedelsbäck Bladh K, Porvaldsdóttir E (2006). Spice-and Medicinal Plants in the Nordic and Baltic Countries. Conservation of Genetic Resources 94-104.

Bassam BJ, Gresshoff PM (2007). Silver staining DNA in polyacrylamide gels. Nature Protocols 2(11):2649-2654.

Baum DA, Small RL, Wendel JF (1998). Biogeography and floral evolution of baobabs (Adansonia, Bombacaceae) as inferred from multiple data sets. Systematic Biology 47(2):181-207.

Borissova AG (1939). The family *Crasulaceae*, p. 472. In: KomarovVL (Ed.). Flora of USSR, vol. 9. Academiae Scientiarum URSS, Moscow, Leningrad.

Brown RP, Gerbarg PL, Ramazanov Z (2002). *Rhodiola rosea*, a phytomedical overview. Herbal Gram 56:40-52.

Elameen A, Klemdal SS, Dragland S, Fjellheim S, Rognli OA (2008). Genetic diversity in a germplasm collection of roseroot (*Rhodiola rosea*) in Norway studied by AFLP. Biochemical Systematics and Ecology 36(9):706-715.

Furmanowa M, Oledzka H, Michal'ska M, Sokolnicka I, Radomska D (1995). *Rhodiola rosea* L. (Rosroot): In vitro regeneration and the biological acivity of roots. Biotechnology in Agriculture and Forestry Vol. 33, Medicinal and Aromatic Plants VIII:412-426.

Gontcharova SB, Artyukova EV, Gontcharov AA (2006). Phylogenetic relationships among members of the subfamily *Sedoideae* (Crasulaceae) inferred from the ITS region sequences of nuclear rDNA. Russan Journal of Genetics 42(6):654-661.

György Z, Derzső E, Galambosi B (2009). Finnországi *Rhodiola rosea* populációk diverzitásának vizsgálata ISSR markerekkel. Lippay János - Ormos Imre - Vas Károly Tudományos Úlészak, 28-30 October, 2009, Budapest, Hungary.

György Z, Derzső E, Galambosi B, Pedryc A (2011). Genetic diversity of Finnish *Rhodiola rosea* populations based on SSR and ISSR analysis. Acta Horticulturae 955:197-202.
Pirttilä AM, Hirsikorpi M, Kämäräinen T, Jaakola L, Hohtola A (2001). DNA isolation methods for medicinal and aromatic plants. Plant Molecular Biology Reporter 19:273a-f.

Rosenberg MS, Anderson CD (2011). PASSaGE: Pattern Analysis, Spatial Statistics and Geographic Exegesis. Version 2. Methods in Ecology & Evolution 2(3):229-232.

Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acids Res 22:4673-4680.

Wang Q, Ruan X, Jiang H, Meng Q, Wang L (2009). Genetic diversity of different geographical populations of Rhodiola rosea based on AFLP markers. China Journal of Chinese Materia Medica 34:2279.

White TJ, Birns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic. PCR protocols: A guide to methods and applications. Academic Press, San Diego.

Wiedenfeld H, Dumaa M, Malinowski M, Furmanowa M, Narantuya S (2007). Phytochemical and analytical studies of extracts from Rhodiola rosea and Rhodiola quadrifida. Pharmazie 62(4):308-11.

Xia T, Shilong C, Shegyun C, Defang Z, Dejun Z, Qingbo G, Xuejun G (2007). ISSR analysis of genetic diversity of the Qinghai-Tibet Plateau endemic Rhodiola chrysanthemifolia (Crassulaceae). Biochemical Systematics and Ecology 35:209-214.

Zini E, Clamer M, Passerotti S, Vender C, Vendramin GG, Komjanc M (2009). Eight novel microsatellite DNA markers in Rhodiola rosea L. Conservation Genetics 10(5):1397-1399.