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Genetic diversity of golden root (*Rhodiola rosea* L.) in northern Norway based on recently developed SSR markers

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Abstract: Roseroot (*Rhodiola rosea* L.), an adaptogenic herb, has received increased attention in recent years. The genetic diversity of roseroot was studied with simple sequence repeat (SSR) markers, which have not been widely used so far. Plants were collected in Finnmark County, Norway, from 10 habitats. Eight recently developed microsatellite (SSR) markers were used to assess genetic diversity. However, only 4 SSR markers were informative during this study. The primer pairs for these 4 SSR markers produced 20 fragments with an average of 5 putative alleles per locus. Observed heterozygosity was 1.0 at each locus, whereas expected heterozygosity ranged from 0.60 to 0.65. The generated unrooted dendrogram based on genetic distances calculated from the results confirms that genetic diversity exists between the populations; the more distant they are from each other, the higher the genetic difference is.

Key words: *Rhodiola rosea*, molecular markers, simple sequence repeat, genetic diversity

1. Introduction

Rhodiola rosea, commonly known as golden root or roseroot, is a traditional adaptogenic medicinal plant. Scandinavian, East European, and Asian peoples have used it for centuries as a general immune system stimulant. Roseroot belongs to the family Crassulaceae. It is a herbaceous plant with a 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. 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 (Ohba, 1981, 1989; Asdal et al., 2006) aspects. Nowadays, several commercially available products exist based on extracts of the rhizome of roseroot, the raw material of which mostly comes from harvesting wild populations. A key to its successful cultivation is the stable high-value cultivars achieved through breeding. Establishing a successful breeding program starts with the assessment and evaluation of the natural populations.

In 2006, approximately 200 plants from 10 geographic regions distributed along the coast of Finnmark County in northern Norway were collected (Fjellidal et al., 2010). One mixed root sample (including several individuals) from each habitat was analyzed for glycoside content. The results showed large geographical variations in the content of the studied metabolites. The total content of rosavin varied between 0.067% and 2.7%, with a mean value of 1.54% for the 10 studied regions.

Studies concerning the genetic diversity of roseroot have been conducted with different methods. Elameen et al. (2008) investigated the genetic diversity of a Norwegian germplasm collection by amplified fragment length polymorphism (AFLP). Finnish *Rhodiola rosea* populations were analyzed by György et al. (2012) using intersimple sequence repeat (ISSR) markers. In 2009, Zini et al. published 8 microsatellite sequences (simple sequence repeats; SSRs) and flanking primer pairs. These primers were tested on 2 *Rhodiola rosea* populations from the Trentino Alps. Four of these primers were also used by Kylin (2010) for evaluating the genetic diversity of roseroot plants collected in Sweden, Greenland, and the Faroe Islands. Recently, Kozynenko et al. (2011) analyzed the genetic structure of *Rhodiola rosea* of mostly Russian origin using ISSR polymorphisms. The use of the co-dominant markers (SSRs) is preferred over the dominant

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markers (ISSRs, AFLP). In studying natural populations, co-dominant markers could provide data on the population structure as well as genetic diversity.

The aim of the present work was to characterize genetic diversity among roseroot individuals from habitats in northern Norway using the recently developed SSR markers, and to examine whether these genetic markers are able to evince the major differences among the populations or individuals that were earlier detected in the course of studying the chemical composition of the investigated plant material.

2. Materials and methods

2.1. Plant material

Rhodiola rosea plants were collected in Finnmark County, northern Norway, in 10 habitats along the coast (Figure 1). The collected plants were further cultivated in the experimental field of Bioforsk, Svanhovd. From each habitat, 5–6 plants were included in the study; altogether, 58 plants were used. The plant material was frozen in liquid nitrogen and stored at -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 (BioScience, Hungary) on 1% agarose gel.

2.2. PCR amplification of SSR loci

PCR was performed in a 25- μL reaction volume containing 20–80 ng DNA, 1X PCR reaction buffer (75 mM Tris-HCl, 20 mM $(\text{NH}_4)_2\text{SO}_4$, 2.5 mM MgCl_2 , 0.02 mM dNTP

mix, 2.5 μmol each of 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 labeled (FAM). PCR was carried out in a PTC 200 thermocycler (MJ Research, Budapest, Hungary) using the touchdown strategy, as described by Zini et al. (2009): initial step at 95°C for 5 min, followed by 5 cycles at 95°C for 30 s, $65\text{--}60^{\circ}\text{C}$ (-1°C every cycle) and 72°C for 1 min, and 25 cycles at 95°C for 30 s, 60°C for 1 min, and 72°C for 1 min.

The PCR products were applied on a 1% (w/v) ethidium bromide-stained agarose gel in 1X TBE buffer with xylene cyanol loading buffer to verify the occurrence of the amplification. The amplified SSR fragments were run in an automated sequencer ABIPRISM 3100 Genetic Analyzer (Applied Biosystems, Budapest, Hungary). Band scoring was analyzed using Peak Scanner software, version 1.0 (Applied Biosystems).

2.3. Data analysis

Genetic relatedness among genotypes was studied by unweighted pair group method with arithmetic averages (UPGMA) cluster analysis using POPGENE, version 1.32 (Yeh et al., 1999). POPGENE was also used to estimate expected (H_E) and observed (H_O) heterozygosity, Nei's genetic distance, and Shannon's information index (I) for co-dominant data markers (SSR).

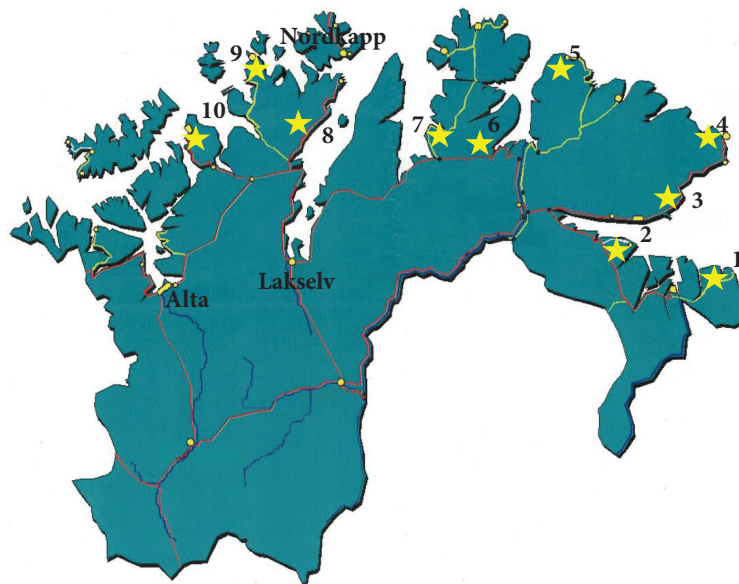


Figure 1. Map showing the 10 locations of the examined roseroot populations.

3. Results

Using 8 SSR primer pairs, analysis of the genetic diversity of 58 roseroot plants from the coast of northern Norway was conducted.

Amplification was successful with 5 out of the 8 available roseroot-specific SSR primers. The number of alleles per locus amplified in the course of the study ranged from 2 (RRE9) to 6 (RRE2). For comparison, the number of alleles obtained by Zini et al. (2009) and Kylin (2010) are also presented in Table 1. The sizes of alleles for SSR loci were within the expected range based on published data (Zini et al., 2009). Sizes ranged from 121 (RRF3) to 182 (RRD6) (Table 2). Primers for markers RRE3, RRE4, and RRF4 in most samples failed to amplify genomic DNA. The primer pair for RRE9 amplified the same 2 fragments for all of the tested plants (146 and 155). Therefore, only 4

(RRC10, RRD6, RRE2, RRF3) out of the 8 markers were informative during the study. The primer pairs for these 4 SSR markers produced 20 fragments with an average of 5 putative alleles per locus.

Genetic diversity parameters are presented in Table 3. Observed heterozygosity (H_o) was 1.0 in each locus, whereas expected heterozygosity (H_e ; genetic diversity) ranged from 0.60 at RRF3 to 0.65 at RRE2. The Shannon index, expected heterozygosity, and Nei's genetic distance calculated for the habitats ranged from 0.83 to 1.13, 0.59 to 0.69, and 0.55 to 0.63, respectively; the lowest values are for habitats 1 and 5, while the highest values are for habitat 10 (Table 4).

In Table 5, Nei's genetic identity of the individuals of the 10 habitats is shown. The highest value is 0.99, which was calculated for individuals from habitats 5 and 3,

Table 1. Comparison of the obtained allele numbers at the examined loci found by Zini et al. (2009), Kylin (2010), and the present study.

Locus name	No. of alleles found by Zini et al. (2009)	No. of alleles found by Kylin (2010)	No. of alleles obtained in this study
RRC10	4	4	5
RRD6	5	4	4
RRE2	5	-	6
RRE3	3	2	-
RRE4	2	-	-
RRE9	3	2	2
RRF3	3	-	5
RRF4	3	-	-

Table 2. Expected size range of the examined loci based on Zini et al. (2009) and the obtained size range in this study.

Locus name	Expected size range	Obtained allele sizes
RRC10	146–164	148–158 (148, 150, 154, 158)
RRD6	168–186	170–182 (170, 172, 178, 182)
RRE2	161–182	158–176 (158, 164, 152, 155, 167, 176)
RRE9	143–161	146–155 (146, 155)
RRF3	121–137	121–133 (121, 123, 125, 127, 133)

Table 3. Genetic parameters for the northern Norwegian roseroot population based on 4 SSR loci.

Locus	H _O	H _e	Nei	Ave. het.	I
RRC10	1.0	0.65	0.64	0.58	1.17
RRD6	1.0	0.64	0.63	0.56	1.10
RRE2	1.0	0.65	0.65	0.59	1.23
RRF3	1.0	0.60	0.60	0.58	1.08
Mean	1.0	0.64	0.63	0.58	1.15
St. dev.	0.0	0.02	0.02	0.01	0.07

Table 4. Genetic parameters for the 10 northern Norwegian roseroot habitats based on SSR markers.

Habitat	I	H _e	Nei
1	0.83	0.59	0.55
2	0.86	0.60	0.55
3	0.89	0.61	0.56
4	0.96	0.66	0.59
5	0.83	0.59	0.55
6	0.95	0.66	0.59
7	0.97	0.65	0.58
8	0.99	0.64	0.59
9	1.01	0.65	0.60
10	1.13	0.69	0.64

Table 5. Nei's genetic identity of the roseroot individuals of the 10 northern Norwegian geographic regions.

Population	1	2	3	4	5	6	7	8	9	10
1	X	0.98	0.98	0.93	0.97	0.88	0.79	0.74	0.79	0.81
2		X	0.97	0.94	0.97	0.88	0.77	0.70	0.74	0.86
3			X	0.96	0.99	0.93	0.87	0.81	0.83	0.81
4				X	0.98	0.96	0.91	0.85	0.82	0.84
5					X	0.94	0.87	0.79	0.80	0.81
6						X	0.96	0.89	0.88	0.85
7							X	0.95	0.90	0.75
8								X	0.88	0.71
9									X	0.71
10										X

while the lowest value is 0.70, which was calculated for individuals from habitats 8 and 2. It is very easy to see from Table 5 that the closer the habitats are located to each other, the higher the calculated value for identity is. The most distinct habitat is the habitat 10, which is actually located on an island. On the other hand, we have to state that even the lowest value (0.70) is rather high.

Genetic relationships among the 10 studied habitats were calculated from SSR data, and the UPGMA-based dendrograms obtained are shown in Figure 2. According to the unrooted dendrogram based on the SSR data, the habitats formed 5 groups. Habitats 10 and 9 form a distinct group. The 3 other groups include habitats 7 and 8, habitats 4 and 6, and habitats 1, 2, 3, and 5.

4. Discussion

The aim of the present study was to estimate the genetic diversity of *Rhodiola rosea* with SSR markers in 10 habitats in northern Norway. SSR markers revealed a relatively low level of genetic variation in the studied habitats (average H_e : 0.64).

Nevertheless, results of chemical analysis of these 10 habitats showed large differences. Salidroside content varied between 0.46% to 2.61%, and the content of total rosavins varied between 0.67% to 2.7% among the 10 habitats. Both the lowest salidroside level and the lowest level of rosavins were detected in habitat 10 (Fjeldall et al.,

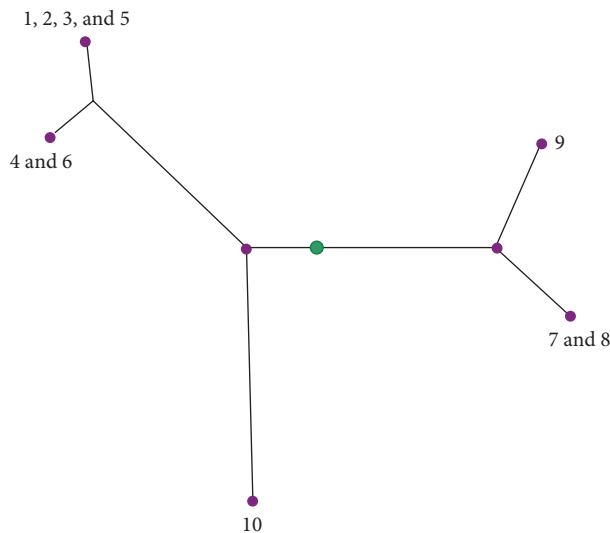


Figure 2. Unrooted dendrogram of the 10 geographically different roseroot populations assayed in this study, generated by UPGMA cluster analysis based on the similarity matrix obtained using Nei's genetic distance based on SSR data (Nei, 1978).

2010), which is the most distinct habitat according to the results of the present study.

Zini et al. (2009) developed the 8 SSR markers available for *Rhodiola rosea*. The genetic diversity of 2 Italian roseroot populations was examined as validation of these markers. Kylin (2010) used 4 of these SSR markers for exploring genetic diversity in the Swedish *Rhodiola rosea* collection (NordGen). As can be seen in Table 1, RRC10, RRD6, RRE2, and RRF3 loci showed the highest polymorphism. The others were either monomorphic (with 2 alleles) or failed amplification. The slightly higher number of alleles detected in this study indicates slightly higher genetic diversity in the studied populations compared to the studies of Zini et al. (2009) and Kylin (2010). Observed heterozygosity (H_o) was 1.0 in all loci, since each individual was heterozygous in these loci. Expected heterozygosity (H_e ; genetic diversity) ranged from 0.60 at RRF3 to 0.65 at RRE2. Lowest and highest observed heterozygosity for the same loci analyzed by Zini et al. (2009) ranged from 0.09 at RRE3 to 0.76 at RRF3; the lowest value for expected heterozygosity was achieved at RRE3 (0.17) and the highest value was at RRC10 (0.7). In the study of Kylin (2010), observed heterozygosity ranged from 0.0 at RRE9 to 1.0 at RRC10, while expected heterozygosity ranged from 0.2 at RRE3 to 0.7 at RRC10. Both expected and observed heterozygosity were higher in the 10 habitats than reported previously for the same primer set, which indicates higher genetic variation in the studied roseroot populations.

In conclusion, using SSR markers recently developed for roseroot, we were able to assess genetic diversity of roseroot populations of 10 habitats from northern Norway, although according to our results only 4 out of the 8 SSR primers are feasible (RRC10, RRD6, RRE2, RRF2). In some cases, a relatively low number of markers can be satisfactory for revealing differences, as in the case of Turkish wheat landraces where 7 SSR markers proved to be enough (Sönmezoglu et al., 2012). However, developing more roseroot-specific SSR markers would be needed for more accurate studies.

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