Flora 206 (2011) 691–696

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.de/flora

Analysis of the genetic structure of *Rhodiola rosea* (Crassulaceae) using inter-simple sequence repeat (ISSR) polymorphisms

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ARTICLE INFO

Article history:
Received 5 September 2010
Accepted 12 December 2010

Keywords:
Infraspecific genetic structure
ISSR
Rhodiola rosea

ABSTRACT

The genetic diversity and differentiation of eleven *R. rosea* populations from different parts of its wide area of occurrence were studied by ISSR markers. Using eight primers, 252 DNA fragments were generated, and 243 of those DNA fragments were found to be polymorphic, indicating a high genetic variability at the species level (P = 96.4%, h = 0.176, SI = 0.291). Relatively low levels of diversity were determined at the population level (P 30.6–59.1%, h 0.088–0.165, SI 0.137–0.257). AMOVA analysis revealed that the majority of the genetic variation was within populations (65.42%), and the variance among populations was 34.58%. Cluster analysis revealed two groups of *R. rosea* populations; these groups likely represent distinct evolutionary lines in the species, which are different in genetic structure, evolutionary history and chorological migration routes.

Introduction

Rhodiola rosea L. (golden root or roseroot; Crassulaceae) is a popular medicinal plant that has an adaptogenic effect (Brown et al., 2002; Gregory and Kelly, 2001; Panossian et al., 2010; Spasov et al., 2000). The species was first described by Carl v. Linné (Linnaeus, 1753); however, his description was not linked to a type specimen and was typified later by Ohba (1993). The habitat of the type specimen is not known with certainty. According to the herbarium label, the species "occurs in mountains of Lapland, Austria, Switzerland, [and] Britain". Thus, typification of *R. rosea* was unclear from the first description and remains so until now. It is broadly acknowledged that the species is highly polymorphic, and confident differentiation of *R. rosea* from related taxa is problematic due to a variety of phenotypic features in members of the section *Rhodiola* appears superfluous. Numerous ecological and/or geographical races of *R. rosea* are recognised as distinct species (e.g., *R. sachalinensis* Boriss., *R. arctica* Boriss., *R. iremelica* Boriss. and some others) by some authors, and others regard them as infraspecific taxa or even synonyms (Ohba, 1981, 2005). Ohba (1981) listed ca. 50 synonyms attributed to *R. rosea*, which fact clearly reflects the complexity of the species taxonomy.

R. rosea has a broad circumboreal distribution (Ohba, 1989); it occurs in alpine habitats of the boreal zone of Eurasia and Appalachia, as well as in most parts of the Arctic, the Far Eastern coasts of Eurasia and North-Atlantic coast of N. America (Clausen, 1975; Ohba, 1989). The current geographic range of *R. rosea* (Fig. 1) mostly spans historically glaciated areas with multiple Pleistocene refugia.

In Russia, this species is common in the Southern Siberian mountains (Saratikov, 1974). Most botanists agree that *R. rosea* originated in this area and then migrated along the mountain ranges latitudinally and northwards to the Arctic (Polozij et al., 1985). In the Russian Far East, *R. rosea* and/or its subspecies occur in Primorye, Sakhalin Island, and the Amur region. Its occurrences are also particularly common and abundant in Kamchatka (Borissova, 1939; Frolov and Poletaeva, 1998; Kozevnikov, 1988; Krasnov et al., 1979). According to preliminary estimates, the stock of *R. rosea* in Kamchatka is comparable with that in Altai Mts.

Despite a wide distribution, the species needs to be protected; over-harvesting has caused *R. rosea* populations to decline and even become extinct in some areas. Aridification and climate warming observed in recent years also negatively affects the species and reduces its populations, even those that are not affected directly by humans. Due to these factors, *R. rosea* has been enlisted in the Red Data Book of Russia and many regional Red Data Books.

Although *R. rosea* is an economically and pharmaceutically important species, very little information on its affinity with other species, genetic diversity and population structure exists (Elameen et al., 2008; Gontcharova et al., 2006; Mayuzumi and Ohba, 2004; Uvarova et al., 2009). The purpose of our study was to analyse the genetic variability of *R. rosea* and to identify its population

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doi:10.1016/j.flora.2010.12.002

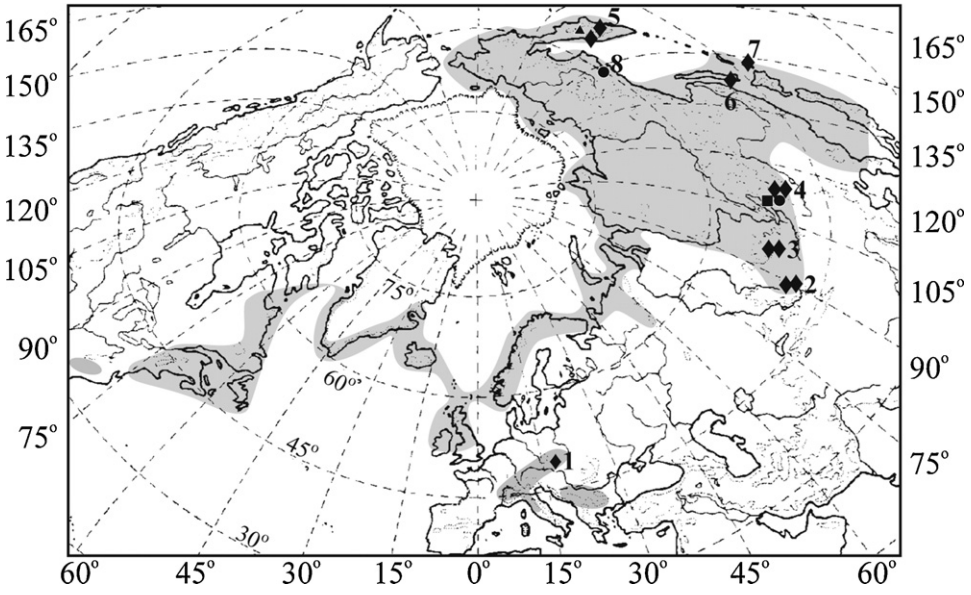

Fig. 1. A map showing distribution range of *Rhodiola rosea* (grey shadow) and location of the populations sampled for this study. ♦ – *Rhodiola rosea*; ▲ – *R. integrifolia*; • – *R. stephanii*; ■ – *R. pinnatifida*. (1) Poland; (2) Altai Mts.; (3) Sajan Mts.; (4) Zabaikal'e; (5) Kamchatka; (6) Sakhalin; (7) Iturup; (8) Khabarovsk.

structure on the major portion of its wide distribution area from Europe to the Far East, using inter-simple sequence repeat (ISSR) markers. ISSR markers are a multilocus system of a dominant type. ISSR analysis is a sensitive method with some advantages over other genetic tools and often provides sufficient information for studying the genetic diversity in plant populations (Ayres and Strong, 2001; Godwin et al., 1997; Goulao et al., 2001; Wolfe, 2005; Zietkiewicz et al., 1994).

Material and methods

One hundred forty seven plants of *Rhodiola rosea* were collected from eleven populations (Fig. 1). In addition, one population of *R. pinnatifida* Boriss. (15 specimens), two populations of *R. stephanii* (Cham.) Trautv. et C.A. Meyer (31 specimens) and one population of *R. integrifolia* Raf. (13 specimens; Table 1) were sampled for comparison. Altogether, 206 DNA samples were used for this study. Voucher specimens (VLA; 1–3 plants per population) are deposited at the Herbarium of Institute of Biology and Soil Science, Vladivostok. All specimens used for DNA extraction were stored at −70 °C in the IBSS.

Total genomic DNA was isolated from 150 to 200 mg of silica-dried leaves following the protocol established by Isabel et al. (1993). DNA quality and quantity was determined by electrophoresis on a 1.0% agarose gel in TBE buffer and visualised with ethidium bromide using lambda phage DNA (Sigma, USA) as a standard.

PCR amplification of the total genomic DNA with ISSR primers was carried out on a UNO II 48 thermocycler (Biometra, Germany) using 25 μl of reaction mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.0 mM $MgCl_2$, 0.001% gelatine, 0.250 mM of each dNTP, 0.2–0.4 mM primer, 30–50 ng of template DNA and 0.8 U *Taq* polymerase. The thermocycler program was 5 min at 94 °C, followed by 40 cycles of 45 s at 94 °C, 45 s annealing at 48 or 58 °C (Table 2) and 45 s extension at 72 °C, and a final 5 min extension at 72 °C. The PCR products were separated on a 1.4% agarose gel in TBE buffer and visualized with ethidium bromide. Molecular weights were estimated using lambda DNA/*Eco*RI + *Hin*dIII markers (Fermentas, Lithuania).

Table 1
Geographical location and sample size (*n*) of *Rhodiola rosea, R. pinnatifida, R. stephanii* and *R. integrifolia* populations.

Population	Location	*n*
Rhodiola rosea		
Altai-1	Russia, Altai Republic, Ulagan Dist., upper current of Jarlinri stream, 2500 m asl	12
Altai-2f	Russia, Altai Republic, Kosh-Agach Distr., Southern Chujski Mt. Range, Lake Karakul vicinity, 2700 m asl	13
Sayan-1	Russia, Krasnojarsk Territory, Western Sajan Mts., Egarki Nature Park, Mt. Tushkanchik	16
Sayan-2	Russia, Krasnojarsk Territory, Western Sajan Mts., Egarki Nature Park, vicinity of Lake Circus	15
Sakhalin	Russia, Sakhalin Territory, Holmsk Distr., vicinity of town Chehow, sea cliffs	11
Iturup	Russia, Sakhalin Territory, Kurilsky Distr., Iturup Island, vicinity of Burevestnic settlement	16
Kamchatka-1	Russia, Kamchatski Kray, Ust-Kamchatsky Distr., 1000 m asl	12
Kamchatka-2	Russia, Kamchatski Kray, Bistrinski Distr., Esso settlement vicinity, Sredinni Mt. Range, 1000 m asl	12
Zabaikal'e-1	Russia, Zabaikalski Kray, Kirinsky Distr., Sokhondo Nature Reserve, Tcagan-Ula Mt., 2200 m asl	15
Zabaikal'e-2	Russia, Zabaikalski Kray, Kirinsky Distr., Sokhondo Nature Reserve, Bolshoj Sokhondo Mt., 2300 m asl	11
Poland	Poland, Tatrzanski Park Narodowy, a ridge between Kondracka Kopa Mt. and Kondracka Przelcz Pass, 1700 m asl	14
Rhodiola stephanii		
Khabarovsk	Russia, Khabarovski Kray, Okhotsky Distr., Okhotsk town vicinity, Laptinskie Mts., Gadatelni stream valley	17
Zabaikal'e-1	Russia, Zabaikalski Kray, Kirinsky Distr., Sokhondo Nature Reserve, Lake Bukukunskoe shore, 2090 m asl	14
Rhodiola pinnatifida		
Zabaikal'e-2	Russia, Zabaikalski Kray, Kirinsky Distr., Sokhondo Nature Reserve, Bukukun stream upper current, 1700 m asl	15
Rhodiola integrifolia		
Kamchatka	Russia, Kamchatski Kray, Ust-Kamchatsky Distr., Sopka Ploskaja volcano, 1000 m asl	13