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Morphological and AFLP-Based Genetic Diversity in *Rosa platyacantha* Population in Eastern Tianshan Mountains of Northwestern China

YANG Shuhua, GUO Ning, and GE Hong *

National Center for Flowers Improvement, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, 100081 Beijing, China

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Abstract

Morphological and AFLP-based genetic analyses of six natural populations of *Rosa platyacantha* in the Eastern Tianshan Mountains of Xinjiang in Northwestern China were performed. Nested analysis of variance models (ANOVA) showed that there were significant differences ($P > 0.05$) in morphological traits among and within the populations of *R. platyacantha*. The phenotypic variation coefficient (*CV*) of the traits varied from 9.78% to 37.71%, confirming the abundant phenotypic variations in the species. The average value of the phenotypic differentiation coefficient (V_{st}) was 27.50%, indicating a lower phenotypic diversity among populations than within populations. Principal component analysis (PCA) showed that the two decisive traits of ANH and TAW contributed to the proportion of 97.19% in the 1st principal component (PC), suggesting that the phenotypic variations of the traits primarily originated from the achene characters. The percentages of polymorphic loci (*PPL*), Nei's genetic diversity index (*h*) and Shannon information index (*I*) were highly similar among populations, with average values of 63.96%, 0.2361 and 0.3506, respectively. Moreover, the gene differentiation (G_{st}) and estimated value of gene flow (N_m) were 8.10% and 5.6766, respectively, indicating very low genetic diversity among populations. Clustering analysis based on morphological traits and AFLP markers showed that the populations were mainly grouped with similar altitudes and geographic distances, respectively. The Mantel test revealed that there was no significant correlation between the phenotypic and genetic diversity among populations, implying a possible influence of environments on the genotypes of *R. platyacantha*.

Keywords: *Rosa platyacantha*; phenotype; genetic diversity; morphology; AFLP

1. Introduction

The genus *Rosa*, which is composed of over 150 species, is widely distributed in all kinds of geographical locations and environments in the northern hemisphere (Rehder, 1940). This genus is one of the most important ornamental crops, and more than 25 000 registered cultivars exist among modern roses (Gudin, 2000). However, only 10–15 species participated in the evolution of the most cultivars, leaving vast untapped genetic resources (Chen, 2001). These wild rose species are quite ornamental themselves and contain desirable traits that breeders can introgress into modern cultivars.

Northwest China is typically characterized by large annual temperature fluctuations and a lack of precipitation. Two wild species, *R. laxa* and *R. beggeriana*, originating from Xinjiang in Northwestern China have been utilized for cold resistance breeding of roses (Bryson and Buck, 1979; Huang and Ge, 1989). However, there are more than 10 endemic rose species in Xinjiang. *R. platyacantha* is another species that is widely distributed in the Tianshan Mountains of Xinjiang at altitudes of 800 to 2 000 m (Gu and Robertson, 2003). This species is proposed to be an excellent wild rose germplasm for introgression into the cultivars because of its golden petals and potential for cold resistance (Ma and Chen, 1990).

Analysis of the genetic diversity between or within different populations, species, and individuals is essential to germplasm evaluation. In recent decades, many studies have been conducted to evaluate the genetic diversity of rose species. The phenotypic and genetic variations of the accessions and cultivars of *R. damascena* have been continuously studied in Bulgaria and Iran using morphological traits, essential oil contents and molecular markers, and the results comprehensively reflected the

* Corresponding author. Tel.: +86 10 82109542.
E-mail address: gehong@caas.cn

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Table 1 Information regarding six *R. platyacantha* populations in Eastern Tianshan Mountains of Xinjiang

Population	Sample number	Collection place	Habitat	Latitude (N°)	Longitude (E°)	Altitude/m
P1	30	Dayou, Jimusaer County	Hillside with shrubs	43°53’	89°03’	1 170
P2	30	Banjiegou, Qitai County	Farmland side	43°41’	89°45’	1 370
P3	30	Baiyanggou, Urumqi County	Hillside with shrubs	43°32’	87°19’	1 580
P4	30	Banjiegou, Qitai County	Valley with shrubs	43°38’	89°45’	1 650
P5	30	Rose Village, Barkol County	Hillside with shrubs	43°34’	92°58’	1 850
P6	30	Tianchi Lake, Fukang City	Lake side with shrubs	43°52’	88°08’	1 920

genetic diversity of *R. damascene* (Rusanov et al., 2005; Babaei et al., 2007; Tabaei-Aghdaei et al., 2007; Kiani et al., 2008, 2010). As a unique simple-leaf species in *Rosa* L., AFLP-based analysis indicated that the genotypes of 128 accessions of *R. persica* from Iran were highly variable and genetically distinct from their origins (Basaki et al., 2009). Investigations of six wild rose species in southwestern China based on their morphological traits and/or SSR markers revealed very different population genetic diversities among species (Zhou et al., 2009, 2011, 2012; Qiu et al., 2010, 2011, 2013; Shao et al., 2010; Li et al., 2013). The morphological, cytological and molecular differentiations of *R. laxa* and *R. beggeriana* were also investigated in natural populations in Xinjiang, China (Guo et al., 2011; Yang et al., 2013, 2014; Li et al., 2014), and higher genetic diversities were observed in the populations of *R. laxa* than *R. beggeriana*. However, there is no published data available regarding the genetic diversity of *R. platyacantha* in the original niches of Northwestern China.

In the present study, we analyzed morphological and molecular variations in *R. platyacantha* populations using morphological traits and AFLP markers to assess genetic diversity of *R. platyacantha* in the Eastern Tianshan Mountains of Xinjiang, China.

2. Materials and methods

2.1. Population selection and material collection

Six natural populations of *R. platyacantha* were investigated from different altitudes in the Eastern Tianshan Mountain of Xinjiang, China. The geographic location and ecological factors were recorded for each research site (Table 1). Thirty individual plants of each population were selected at random. All sampled individuals were separated by at least 10 m to prevent resampling of the same clone. The morphological measurements for the hips, leaves, and inflorescences were conducted in October 2008 and June 2009, respectively. Leaves were collected and stored in liquid nitrogen for DNA extraction.

2.2. Measurement of morphological traits

The leaf traits were measured on the 4th or 5th healthy compound leaves from the top of the current-year branches. Flower and fruit traits were measured at random during the full-blooming stage and the mature stage, respectively. Eleven traits of leaves, flowers and fruit were measured: the length of compound leaf (LCL), the length and width of leaflets (LL and WL), the ratio of the length and width of leaflets (RLWL), the length of the peduncle (LP), flower diameter (FD), the length and width of the hips (LH and WH), the ratio of the length and width of the hips (RLWH), achene numbers per hip (ANH), and thousand-achene weight (TAW). All traits were measured with five replicates per plant except for TAW.

2.3. DNA extraction and AFLP analysis

Total genomic DNA was extracted from the leaflets of each plant by a cetyltrimethylammonium bromide (CTAB) based method (Doyle and Doyle, 1990). The primer pairs for *Eco*RI and *Mse*I were designed and eight pairs of primers were applied for AFLP analysis using a modified version of the procedure described by Vos et al. (1995). PCR products were electrophoresed on 8% (w/v) non-denaturing polyacrylamide gels. The electrophoresed gels were silver-stained to visualize the DNA bands, after which they were recorded using a gel image analysis system (BioSpectrum Imaging System, UVP, Upland, CA, USA).

2.4. Parameter calculation and statistics

All morphological traits except TAW were analyzed by nested ANOVA as described by Li et al. (2002). The phenotypic differentiation coefficient (V_{st}) and phenotypic variation coefficient (CV) of the traits were calculated according to Ge et al. (1988). Duncan’s multiple comparison was conducted for the test of significant differences of morphological traits. Principal component analysis (PCA) and clustering analysis were applied to all morphological traits. A dendrogram was drawn using the unweighted pair-group method of the arithmetic averages (UPGMA).

Polymorphic AFLP bands were scored and the values were used to compile a binary data matrix by GENESCAN analysis. The percentage of polymorphic loci (PPL), Nei’s gene diversity index (h), Shannon’s information index (I), gene differentiation (G_{st}) and the estimated value of gene flow (N_m) were calculated using PopGen3.2 (Yeh et al., 1999). UPGMA cluster analysis of pairwise Nei’s unbiased genetic distances was conducted and displayed as a dendrogram using TFPGA.

Pearson’s correlation analysis for the average CV of morphological traits and the parameters of PPL, h and I was conducted among populations. Mantel test between the matrix of genetic distances based on morphological traits and AFLP markers was further conducted using the software NTsys 2.11a (Mantel, 1967).

3. Results

3.1. Phenotypic differentiation based on morphological traits

Nested ANOVA revealed the significant differences ($P < 0.05$) in all morphological traits (expect TAW) among and within populations (Table 2). Significant differences ($P < 0.05$) were only