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Genetic diversity in *Vicia amoena* (Fabaceae) germplasm resource in China using SRAP and ISSR markers

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ABSTRACT

The genetic diversity in 17 germplasms of *Vicia amoena* L. from north China was analyzed using SRAP and ISSR markers. Three hundred and sixty-eight (94.11%) polymorphic bands (211 and 157 obtained from 20 pairs of SRAP and ISSR primers, respectively) were scored. Although SRAP was more effective than ISSR markers with higher PIC, RP and larger variation range of genetic distance, both the markers were useful for assessing *V. amoena* genetic diversity. Cluster analysis showed that the 17 germplasms were clustered into 5 groups. The results of principal coordinate analysis supported UPGMA clustering. The germplasms from source areas where the annual average temperature ranged from −1.0 to 5 °C exhibited the highest level of genetic diversity with the highest PPI, I and H. These results have important implications in genome mapping, breeding purposes, and germplasm conservation.

1. Introduction

Vicia amoena L. is a perennial herbaceous plant that is widely distributed in north China. It belongs to the *Cracca* subgenus of the *Vicia* genus of Leguminosae. The subgenus *Cracca* shows a rather heterogenous assemblage with a wide variation in many characters (Kupicha, 1976). Because of its properties of large biomass, high nutrition, long-term utilization, and strong trampling endurance, *V. amoena* is pasturable and suitable for large-area cultivation (Dong and Yu, 1994; Cheng and Jia, 2000). The species has over 17% crude protein content (Cho et al., 1998), which is similar to that of *Medicago sativa*; however, *V. amoena* can survive in cold areas where *M. sativa* cannot grow. The whole plant of *V. amoena* is commonly used as a source of traditional Chinese crude drugs. According to the report of Zhang et al. (1999), *V. amoena* is the botanical origin of most commercial Tougucao (Chinese crude drug) germplasms. Hence, identification of the chemical components of this species has become essential (Kamo et al., 2008; Yang et al., 2010).

Empirical studies in ecology and evolution often depend on accurate assessment of genetic diversity to address questions regarding genetic relatedness among individuals to determine the population structure (Mueller and LaReesa Wolfenbarger, 1999). This information can provide predictive estimates of genetic variation within a species, thus facilitating breeding

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material selection (Qi et al., 2008; Wang et al., 2012). Unlike morphological and biochemical markers, molecular markers are not prone to environmental influence. Therefore, they are considered as very powerful tools for genotype characterization and genetic diversity estimation (Cheng and Huang, 2009; Thimmappaiah et al., 2009). Of the different molecular markers, sequence-related amplified polymorphism (SRAP) and inter-simple sequence repeat (ISSR) have been widely used to assess species genetic diversity and relationships (Ariss and Vandemark, 2007; Lin et al., 2009; Uysal et al., 2010) because of their cost effectiveness, simple operation without requiring sequencing information (Williams et al., 1990), and requirement of very little starting DNA template (Zietkiewicz et al., 1994). Although some studies have reported the phylogenetic relationships of *Vicia* by using chromosomal landmarks (Li et al., 2001), morphological data (Leht, 2009), and electrophoretic patterns of seed proteins (Potokina et al., 2003), there is no report on the application of ISSR or SRAP markers in genetic diversity assessment of *V. amoena*. The objective of this study is to assess genetic diversity in 17 *V. amoena* germplasms collected from 17 different regions of north China by using SRAP and ISSR molecular markers.

2. Materials and methods

2.1. Plant material and DNA extraction

The 17 germplasms were divided into 3 groups (G1–G3) on the basis of the annual averaged temperature of the sources (Table 1). They were maintained in the greenhouse of China Agricultural University. Genomic DNA was extracted from fresh leaves by using Tiangen plant genomic DNA purification kit. Purified total DNA was quantified using 0.8% agarose gel, with known amount of uncut lambda DNA (Tiangen Biotech Co. Ltd., Beijing, China) as standard.

2.2. SRAP amplification

Twenty combinations of SRAP primers, as described in Table 2, were selected from the 99 primer combinations (Li and Quiros, 2001) previously used for the analysis of genetic diversity in *V. amoena*. These simple sequence repeats were synthesized and obtained from Sangon (Sangon Biotech Co., Ltd., Shanghai, China). Individual SRAP-polymerase chain reactions (PCRs) were carried out in a 25 μL reaction volume containing 30 ng DNA, 2.0 mM $MgCl_2$, 2.0 μM of each primer, 200 μM dNTPs, 1.5 U Taq DNA polymerase, and 2 μL of 10 × PCR reaction buffer (Mg^{2+} free). All amplifications were performed using a TC-512 PCR thermocycler (TECHNE, UK). The cycling conditions were as follows: initial denaturing at 94 °C for 4 min, followed by 5 cycles of 94 °C for 60 s, 37 °C for 45 s, and 72 °C for 60 s; additional 35 cycles of 94 °C for 60 s, 50 °C for 45 s, and 72 °C for 60 s; and a final elongation at 72 °C for 7 min. The PCR products of SRAP markers were resolved by electrophoresis on 8.0% polyacrylamide gels.

2.3. ISSR amplification

Twenty ISSR primers, as shown in Table 3, were selected from 85 original ISSR primers according to the public ISSR primers screened by the Wolfe Lab of University of British Columbia in Canada. Individual ISSR PCRs were carried out in a 20 μL reaction volume containing 20 ng DNA, 2.5 mM $MgCl_2$, 0.4 μM primers, 300 μM dNTPs, 0.5 U Taq DNA polymerase, and 2 μL of 10 × PCR reaction buffer (Mg^{2+} free). All amplifications were performed using a TC-512 PCR thermocycler with initial denaturing at 94 °C for 5 min, followed by 40 cycles of 94 °C for 30 s, a specific temperature for particular primer (Table 3) for

Table 1
Details of the germplasm sources of *V. amoena*.

Annual average temperature of the source (code)	No.	Source	Longitude (E)	Latitude (N)
°C < −1.0(G1)	2	Genhe city, Inner Mongolia	121.51	50.80
	3	Yakeshi city, Inner Mongolia	120.74	49.29
	9	Hulunbuir city, Inner Mongolia	119.73	49.25
5>°C > −1.0(G2)	6	Wudai mountain, Shanxi	113.70	39.21
	7	Liangcheng county, Inner Mongolia	112.49	40.74
	13	Motenlin, Aershan city, Inner Mongolia	120.01	48.79
	14	Baihua mountain, Bingjing	115.62	39.87
	15	Changling county, Jilin	123.91	44.23
	20	Goukou village, Boketu town, Inner Mongolia	121.96	48.65
	22	Boketu town, Inner Mongolia	121.90	48.76
°C > 5(G3)	4	Ling mountain, Bingjing	115.33	40.03
	5	The roadside of Ling mountain, Bingjing	115.33	40.03
	8	Hohhot city, Inner Mongolia	111.76	40.80
	10	Guojiashan village, Shanxi	112.01	36.37
	11	Qinyuan county, shanxi	112.33	36.50
	12	Zhangjiayao village, Shanxi	110.98	34.93
	16	Saiqin Tara, Chifeng city, Inner Mongolia	119.09	43.00