



Analysis of the genetic diversity of garlic (*Allium sativum* L.) germplasm by SRAP



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ABSTRACT

Cluster analysis and principal component analysis were used to investigate the genetic diversity of 40 garlic germplasms analyzed with 23 sequence-related amplified polymorphism (SRAP) primer combinations. A total of 130 polymorphic loci were detected among these germplasms, with an average of 5.65 polymorphic loci per SRAP primer combination. The percentage of polymorphic loci was 69.1%, whereas the mean effective number of alleles, the mean Nei's gene diversity, and the mean Shannon's information index were 1.4446, 0.2788, and 0.4365, respectively. Cluster analysis revealed that the 40 germplasms could be divided into 3 groups. The results of principal component analysis were consistent with those of unweighted pair-group method with arithmetic averages (UPGMA) clustering analysis. The Shannon-Weaver information index ranged from 0.2419 to 0.4202, indicating that the garlic germplasms had high genetic diversity.

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1. Introduction

Garlic (*Allium sativum* L.) is an important vegetable that has its origin in central Asia. Garlic, first cultivated 4000 years ago, has become one of the most popular worldwide, both as a medicine and a vegetable. Garlic is vegetatively propagated in production. However, it was discovered that garlic displays a wide range of variation under various ecological conditions, and some germplasms have adapted to specific environments through artificial and natural selection (Lu et al., 2001). The agricultural traits of garlic germplasms have normally shown wide variations in characteristics such as bulb weight, coat layer, leaf length, growth habit, and stress resistance (Fan et al., 1997; Lu et al., 2001; Volk and Stern, 2009). Assessment of germplasm resources is necessary for their effective use (Kamenetsky, 2007). However, few reports have assessed the germplasm resources of garlic in China, and even fewer have used molecular methods for assessment. The current classification of garlic focuses mainly on its morphology and biological characteristics as determined by numerical taxonomy (Buso et al., 2008; Volk et al., 2004). However, the classification result of using this method is always affected by the environment and the size of the population. Therefore, it does not accurately reflect the real situation regarding garlic germplasm resources in China (Wang et al., 2012), and a uniform standard for the classification of garlic is, therefore, unavailable (Pappu et al., 2008). In addition, the movement of plant germplasms among geographic areas disorders nomenclature and backgrounds. In other words, a classification system based on morphology and biological characteristics is not indicative of cultivation and breeding.

China is the chief producer of garlic in the world, and garlic produced by this country has formed abundant ecotypes through artificial and natural selection over a long period. In addition, these ecotypes have adapted well to specific ecological environments, and germplasm generally exhibit obvious regional adaptability (Waterer and Schmitz, 1994). The garlic

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germplasm must be evaluated and identified to use these resources effectively, which is both necessary and urgent for extending the genetic bases of the germplasms (Pappu et al., 2008; Tsukazaki et al., 2010).

To date, few reports have classified the genetic diversity of garlic using sequence-related amplified polymorphism (SRAP) molecular markers. Herein, we report the genetic diversity of garlic germplasms from a wide range of geographical distributions in China on the basis of an analysis of SRAP molecular markers.

2. Materials and methods

2.1. Plant material

The 40 germplasms was used in this study were chosen from the garlic germplasms at Northwest A & F University, College of Horticulture, China. Of these, 37 originated from different provinces of China (Hubei, Sichuan, Shaanxi, Shandong, Shanghai, Jiangsu, Yunnan, Guizhou, Zhejiang, and Taiwan), 1 germplasm originated from Ethiopia, 1 germplasm originated from Thailand, and 1 germplasm originated from Korea. The number, name and source of collection are listed in Table 1.

These germplasms were planted in the garlic germplasm nursery of the Horticulture College, Northwest A&F University, from September 2009 to June 2010. Row spacing was 20 cm and the plant spacing was 8 cm. The depth of the seed furrow was 5 cm.

2.2. DNA extraction

Genomic DNA was extracted from young leaves (from at least 15 plants) and sampled at the two- and three-leaf stages by using cetyltrimethylammonium bromide procedure (Chen et al., 2006). DNA was quantified using a NanoDrop 2000™ and agarose gel electrophoresis. The DNA concentration was adjusted to 30 ng/μL.

2.3. SRAP amplification

Twenty-three SRAP primers were used (Table 2) (Li and Quiros, 2001). The SRAP reaction mixture contained 20 ng DNA, 2.0 mM MgCl₂, 5 pmol each forward and reverse primers, 0.2 mM dNTP, 1× PCR buffer, and 0.5 U Taq DNA polymerase (Takara, Japan) in a total volume of 12.5 μL. SRAP PCR was performed as follows: 8 cycles of 5 min at 94 °C, 30 s at 94 °C, 45 s at 36 °C, and 90 s at 72 °C; 38 cycles of 30 s at 94 °C, 45 s at 47 °C, and 90 s at 72 °C; and a final extension of 7 min at 72 °C followed by storage at 4 °C.

PCR products were separated via polyacrylamide gel electrophoresis on 6% denaturing gel in 1× TBE buffer at 350 constant volts for 3 h, visualized with silver staining, and photographed with a Panasonic DMC-T27 camera.

2.4. Data analysis

Each polymorphic band was independently identified by two researchers, and the distinct condensed fragments were counted. For SRAP analyses, the polymorphic bands were scored as being present (1) or absent (0) on the gels. A similarity matrix was generated according to simple matching coefficients. The similarity coefficient and genetic distance were analyzed according to the method described by Nei (1972). The polymorphic information from the SRAP primers was

Table 1

The numbers, cultivar names, and source of collection of the 40 major garlic germplasms.

No.	Cultivar	Source of collection	No.	Cultivar	Source of collection
1	Xiangfan ershui early	Xiangfan, Hubei	21	No. 21	Yangling, Shaanxi
2	Wenjiang seven star red	Wenjiang, Sichuan	22	No. 22	Yangling, Shaanxi
3	Peng county early	Peng county, Sichuan	23	Xuzhou white	Jiangsu
4	Peng county mid-maturity	Peng county, Sichuan	24	Sichuan purple	Sichuan
5	Jiading No.2	Jiading, Shanghai	25	Ethiopia white	Ethiopia
6	Putuo	Yang county, Shaanxi	26	Hanzhong purple	Yangling, Shaanxi
7	Baihe early	Baihe, Shaanxi	27	Sichuan red	Sicuan
8	Thailand qingmai	Qingmai, Thailand	28	Hanzhong red	Caijiapo, Shaanxi
9	Long county early	Long county, Shaanxi	29	Japan white	Japan
10	Xingping white	Xingping, Shaanxi	30	Fenggan white	Fenggan
11	Gailiang	Yangling, Shaanxi	31	No. 31	Pucheng, Shaanxi
12	Cangshan	Cangshan, Shandong	32	No. 32	Taiwan
13	Caijiapo seven leaves	Qishan, Shaanxi	33	No. 33	Guizhou
14	Yao county red	Yao country, Shanxi	34	No. 34	Guizhou
15	Xiangfan red	Xiangfan, Hubei	35	No. 35	Guizhou
16	Peng county late	Peng county, Sichuan	36	No. 36	Guizhou
17	Taican white	Taican, Jiangsu	37	No. 37	Guizhou
18	Russian garlic	Tai'an, Shandong	38	Korea solyeng	Zhejiang
19	Luliang garlic	Luliang, Yunnan	39	Ningqiang mountain garlic	Ningqiang, Shaanxi
20	Bijie garlic	Bijie, Guizhou	40	No. 40	Jeju Island, Korea

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