Scientia Horticulturae 132 (2011) 50–58

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Analysis of genetic diversity in Japanese apricot (*Prunus mume* Sieb. et Zucc.) based on REMAP and IRAP molecular markers

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ARTICLE INFO

Article history:
Received 4 June 2011
Received in revised form 7 October 2011
Accepted 11 October 2011

Keywords:
Prunus mume
Genetic diversity
REMAP
IRAP

ABSTRACT

Japanese apricot (mei) originated in China, and was divided into two types: fruiting mei and flowering mei, and fruiting mei can be divided by their pericarp into green mei, red mei and white mei. 84 cultivars (43 fruiting mei and 41 flowering mei) were used in this study. To identify their genetic relationships, genetic diversity analysis using REMAP with IRAP techniques was performed. The results showed that the observed number of alleles was 1.9955 in Japanese apricot populations, the effective number of alleles was 1.4887, Nei's gene diversity was 0.2910, and Shannon's information index was 0.4465. Eighty-four Japanese apricot varieties could be divided into 18 groups at the similarity coefficient of 0.743, and 4 groups at the similarity coefficient of 0.705 with the UPGMA cluster method. Fruiting mei and flowering mei clustered into different groups which showed that the complexity of inheritance and the extent of evolution were different. PopGen32 analysis suggested that Japanese fruiting mei would be introduced from Zhejiang province in China and Japanese flowering mei from Jiangsu province in China.

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1. Introduction

Japanese apricot (*Prunus mume* Sieb. et Zucc.), native to China, belongs to the Rosaceae family, genus *Prunus* L., called mei in China (Chu, 1999), and is famous for its ornamental appreciation and producing healthy food. It is mostly distributed in 10 provinces and areas of China mainland, including Yunnan, Guangdong, Zhejiang, Jiangsu, Guangxi, Fujian, etc., and in Japan, Korea and Vietnam. According to different purposes, Japanese apricot is divided into two types: fruiting mei and flowering mei. The fruits of fruiting mei are mostly used for food production, disease treatment (Chu, 1999; Chuda et al., 1999), preventing cancer cell proliferation (Jenog et al., 2006), and so on. Japanese apricot blooms in the last ten days of February to March (Chen, 1989). The earliest blooming plant, Japanese apricot, especially flowering mei, is also cultivated as an ornamental plant. Japanese apricot contains more than 500 varieties including 197 fruiting mei (Chu, 1999) and 323 flowering mei varieties (Chen, 2001), and new Japanese apricot varieties will be registered in succession.

To explore the genetic diversity of these numerous Japanese apricot varieties and their complicated sources, molecular marker technology was used for kinship studies; for example, AFLP, RAPD and SSR analyses have been done on the core germplasm structure (Gao et al., 2003) and kinship (Shimada et al., 1994; Hagen et al., 2002; Gao and Shen, 2004; Fang et al., 2005) of fruiting mei and AFLP analysis has been performed on the flowering mei's kinship and identification of cultivars (Ming and Zhang, 2004; Lv et al., 2006; Zhang et al., 2004; Yang et al., 2004, 2007; Ming et al., 2005). Although studies on the kinship of the entirety of combined fruiting and flowering mei are few, there is a bifurcation in the kinship between fruiting mei and flowering mei. The binary classification method (Chen and Bao, 1992) on the entirety of the fruiting and flowering mei showed that the flowering mei was a branch of the fruiting mei. However, SSR analysis (Hayashi et al., 2008) indicated that there is no distinct genetic difference between flowering and fruiting mei, and Japanese flowering and fruiting mei clustered with Chinese mei.

REMAP (retrotransposon-microsatellite amplified polymorphism) is a relatively new retrotransposon-based marker system, developed by Kalendar to detect the polymorphisms of marked points in the genome. In REMAP, primers are designed in the conserved sequence region (extended orientation outside of the retrotransposon) and anchored SSR primers are located in the long terminal repeat (LTR). In PCR, the LTR primers anneal to the LTR region of retrotransposons to amplify the retrotransposon segment and its nearest SSR segment, followed by detecting the polymorphism between the retrotransposon and the simple sequence repeat. Because no restriction enzyme ligestion and subsequent

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doi:10.1016/j.scienta.2011.10.005

ligation is needed, this is an easy operation and, for rich polymorphisms, REMAP detection efficiency is relatively high (Kalendar et al., 1999). REMAP was recently used in germplasm identification of orange (Bréto et al., 2001; Guo et al., 2005) and apple (Baumel et al., 2002; Kristiina et al., 2006; Xiao and Xu, 2006; Jia, 2008), fingerprint identification of between and within barley species and the genetic map of barley (Provan et al., 1999; Manninen et al., 2000), the genetic diversity assessment of rice (Boyko et al., 2002; Chadha and Gopalakrishna, 2005,2007; Branco et al., 2007), wheat (Georgi et al., 2010), banana (Teo et al., 2005; Nair et al., 2005), cotton (Hafeze et al., 2006), olive (Natali et al., 2007) and orange (Manosh et al., 2010), etc., and has also been used for assisted selection in oat breeding.

IRAP (inter-retrotransposon amplified polymorphism) was first developed by Kalendar to detect retrotransposon insertional polymorphisms within the genome. Its principle is to design special LTR primers (Kalendar et al., 1999; Provan et al., 1999) based on LTRs in the conserved region of the retrotransposon which can anneal to the LTR retrotransposon corresponding domain during PCR to amplify the segment between two retroelements. Because of the random nature of most retrotransposon insertion points and orientation in the genome and stable insertion sides, the fact that the primers designed from LTRs in the retrotransposon are used to perform PCR amplification is reflected in the polymorphisms resulting from different retrotransposon insertion styles. At present, IRAP has been used in many areas, for example, in germplasm identification of barley (Kalendar et al., 1999; Bradley et al., 2010), flue-cured tobacco (Xiao and Xu, 2006), barley (Manninen et al., 2000), orange (Bréto et al., 2001) and grape (Onofrio et al., 2010); gene mapping and genetic mapping of the structure of flue-cured tobacco (Xiao and Xu, 2006), barley (Manninen et al., 2000), orange (Bréto et al., 2001; Guo et al., 2005), wheat (Boyko et al., 2002) and sunflower (Vukich et al., 2009), the genetic diversity assessment of barley (Kalendar et al., 1999;), banana (Nair et al., 2005), rice (Branco et al., 2007), medicinal plants (Boronnikova and Kalendar, 2010), orange (Manosh et al., 2010) and wheat (Georgi et al., 2010), somatic cell clone variance detection of barley and IRAP-SCAR mark identification of mushroom, etc.

There has been no report on the application of REMAP and IRAP marker methods in Japanese apricot. In recent years, with the gradual increased understanding of Japanese apricot's health benefits and Japanese apricot's cultural importance, it is necessary to further study and utilise Japanese apricot sources, especially the entirety of the fruiting and flowering mei. This study aimed to explore the genetic diversity based on the REMAP and IRAP techniques and to provide a technique platform for further study on Japanese apricot's genetic variance and kinship.

2. Materials and methods

2.1. Plant materials

A total of 84 Japanese apricot varieties (in which No. 1–43 are fruiting mei and No. 44–84 are flowering mei) from the National Japanese apricot germplasm pool of Nanjing Agricultural University were used as test materials (Table 1).

2.2. Template preparation and primers

Total genomic DNA was extracted from the young leaves of 84 Japanese apricot cultivars with a modified CTAB method based on the method for extracting total DNA from fruiting mei used by Wang (Wang et al., 2006), Hasan (Hasan et al., 2008) and Gui (Gui et al., 2008). Before adding 3.0% β-mercaptoethanol, 1 mL of lotion was added twice. The lotion consisted of 0.1 M Tris–HCl, 0.35 M sorbic alcohol, 0.5 M EDTA (pH 8.0) and 100 g/L PEG 20000. The CTAB method with RNase after extraction was used to extract DNA.

Twenty-four LTR primers for REMAP were derived from the cloned LTR sequence of Japanese apricot in our laboratory (Wang, 2010; Table 2). Due to good generality of EST-SSR marker on the variety (Shangguan et al., 2010; Chai et al., 2010), designed by Primer 3 software (Brant, 2008; Chen, 2009; Table 2), the SSR primers, derived from EST of apricot (Shangguan et al., 2011) and mei. Combination primers with clear visible brand in PAGE gels were screened from each of 24 LTR primers combined with 29 SSR primers respectively optimized by REMAP system.

2.3. PCR reaction conditions and program

An optimal reaction conditions suitable for REMAP analysis in the Japanese apricot populations was established (25 μL total reaction buffer), comprising 40 ng of template DNA, 1.5 μL of 2.5 mM $MgCl_2$, 2.0 μL of 2.5 mM dNTPs, 2.5 μL of 10× PCR buffer, 1.5 U of *Taq*DNA polymerase (Dalian Baoshengwu company), 1.0 μL of 10 μM LTR primer and 1.0 μL of 10 μM SSR primer. The PCR profile consisted of an initial denaturation for 4 min at 94 °C followed by 36 cycles of 1 min at 94 °C, 1 min at 48 °C to 50 °C, 1 min 30 s at 72 °C, and a final extension of 10 min at 72 °C. The annealing temperature of the PCR program was determined by the primer T_m value.

IRAP-PCR amplification was performed in a 25 μL reaction, including 30 ng of template DNA, 2.0 μL of 2.5 mM $MgCl_2$, 2.5 μL of 2.5 mM dNTPs, 2.5 μL of 10× buffer, 1.0 U of *Taq* DNA polymerase and 1.0 μL of 10 μM LTR primer. Amplification for IRAP analysis in the Japanese apricot populations was devised as flows: initial denaturation at 94 °C for 4 min; 45 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 2 min; and a final extension at 72 °C for 10 min.

2.4. Product detection

REMAP and IRAP amplified PCR products were detected in non-denaturated PAGE gels. Of these, REMAP-PCR products were detected after electrophoresis for 2 hours at the invariable power of 20 W in a 5% PAGE non-denatured gel, IRAP-PCR products were detected after 2.5–3.0 h of electrophoresis at the invariable power of 20 W in a 6% PAGE non-denatured gel.

2.5. Statistical analysis

Statistics were performed on the REMAP and IRAP profiles, all clearly detectable polymorphic and monomorphic bands were scored for the analysis, and band presence as score 1 and band absence as score 0 in each sample. Analysis referred to the Lu (Lu et al., 2010) method, and used PopGen32 software, assuming allele frequency in line with Hardy–Weinberg equilibrium, to calculate Nei's gene diversity (He), Shannon information index (I), cultivar group total gene diversity (Ht), gene diversity within a population (Hs), gene flow (Nm), the between populations genetic differentiation coefficient (Gst) and between-group genetic distance (GD). NTSYS-pc (Numerical Taxonomy System, version 2.11) software was used to calculate the SM similarity coefficient, and UPGMA was used for clustering analysis. Statistical analysis was performed according to 84 varieties of the Japanese apricot source origin.

3. Results

3.1. Screening of primers for REMAP and IRAP

There were five LTR primers and four SSR primers available for REMAP amplification with more legible and polymorphic amplified bands (Fig. 1).

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