S-genotyping in Japanese Plum by PCR and Capillary Gel Electrophoresis Detection

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Abstract

In this work a PCR S-genotyping method using capillary electrophoresis detection was assayed in Japanese plum. Sweet cherry primers designed for S-RNase and SFB intron length polymorphism detection by capillary electrophoresis were assayed in Japanese plum cultivars. Amplification of both genes was successful and amplified sizes were correlated with Japanese plum S-alleles. The S-RNase genotype of 58 Japanese plum type cultivars previously determined by other methods was confirmed using this technology and the SFB alleles of these cultivars were also determined. Allele sizes of both genes are reported for 13 different S-alleles found in Japanese plum and will allow efficient S-genotype characterization in the species.

INTRODUCTION

Japanese plum (*Prunus salicina* Lindl.) type cultivars exhibit gametophytic self-incompatibility and hence cross-compatible pollinator trees have to be interplanted in most cultivars to ensure fruit set. There are several techniques available to determine cross-compatibility among different cultivars. Cross pollinations in the field and/or in the laboratory followed by pollen tube growth observation or the estimation of fruit set can be used. PCR-based-typing is also often used for *S*-genotype determination and to establish cross-compatibility and Incompatibility Groups. First *S-RNases* in Japanese plum were cloned by Yamane et al., (1999), identifying alleles Sa and Sb. Since then, other authors have identified the S- genotype of a great number of Japanese plum type cultivars using molecular techniques. Today 36 S-alleles have been described in cultivated Japanese plum; 19 have been labelled using letters (Sa to Ss) and further 17 have been labelled using numbers (S_7 , S_8 , S_{10} , S_{11} and S_{15} to S_{27}) (Beppu et al., 2002, 2003; Sapir et al., 2004; Halász et al., 2007; Guerra et al., 2009).

In this work, a PCR S-genotyping method using capillary electrophoresis detection was assayed, and the S-RNase genotype of 58 Japanese plum type cultivars previously determined by other methods was confirmed using this methodology.

MATERIALS AND METHODS

Fifty-eight Japanese plum-type cultivars of known S-genotype were analyzed (Table 1). Genomic DNA from these cultivars was isolated from young leaves and used for S-allele typing by PCR amplification (Guerra et al., 2009) of the S-locus genes S-

RNase and *SFB* using sweet cherry (*Prunus avium* L.) primers PaConsIF-PaConsIR2 (Sonnelveld et al., 2003, 2006) and Fbox5'A-FboxIntronR (Vaughan et al., 2006), respectively. Forward primers were fluorescently labelled and PCR products were detected by capillary electrophoresis using a genetic analyzer (ABI PRISM 310). Size calling of the fragments was carried out with (Peak ScannerTM Software 1.0, Applied Biosystems) and the size standard GeneScan TM 500 Liz ® (Applied Biosystems).

Initially a set of cultivars that have 14 different *S*-alleles were analysed to establish the fragment size of each *S*-allele in each gene. Subsequently and using the *S*-allele sizes identified, the *S*-genotype of the rest of cultivars was determined. Finally, to validate the methodology, the *S*-genotype obtained by this method was compared to that previously determined for the same cultivars by other methods.

RESULTS AND DISCUSSION

The *S*-genotype of 58 Japanese plum type cultivars was determined using capillary electrophoresis detection of *S*-locus genes PCR fragment amplification (Table 1). Twenty different *S*-genotypes were identified and the cultivars were included in 14 Incompatibility Groups.

The *S-RNase* and *SFB* primers PaConsIF-PaConsIR2 (Sonneveld et al., 2006) and Fbox5'A- FboxIntronR (Vaughan et al., 2006) efficiently amplified two fragments, each primer pair, in each cultivar, except 'Joana Red', initially analyzed. Thus, *S*-allele sizes by capillary electrophoresis detection using these primers were established for 13 *S*-alleles: *Sa-Sh*, *Sk* and *So-Sr*. The *SFB* sizes detected for some allele fragments had the same or very similar size. For example, *S*-alleles *Sb* and *Sr* (185 bps), *Sk* and *Sp* (184 bps), *Sd* and *So* (171 bps) or *Sg* and *Sq* (190 bps) had the same size. Similarly the *S-RNase* sizes of *Sa* and *Sh* was 388 bps. However, the combination of both gene sizes allowed the unequivocal differentiation of the 13 alleles identified.

The sizes detected for each allele were used to determine *S*-genotype of all the cultivars analysed (Table 1). The *SRNase* genotype of these 58 cultivars described previously (Beppu et al., 2002, 2003; Sapir et al., 2004; Guerra et al., 2009) was confirmed using this methodology, and the *SFB* genotype was determined for first time. As expected both genes had the same alleles in all the cultivars, and two *S*-alleles were identified in all the cultivars with the exception of 'Joana Red', in which only one *S*-allele was detected (Table 1).

The reliability of this detection method is higher than using agarose electrophoresis because it allows a better differentiation of S-alleles with similar sizes. Using this methodology, other Japanese plum type cultivars can be efficiently S-genotyped.

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Tables

Table 1. Incompatibility Groups of 58 Japanese plum cultivars determined using primers PaConsIF-PaConsIR2 and Fbox5'A-FboxIntronR, for *S-RNase* and *SFB* gene respectively, detected by PCR and capillary electrophoresis.

Incompatibility Group	S-genotype	Cultivars
I	SaSb	Red Beaut, 606, Sordum
II	SbSc	Black Beaut, Black Amber, Fortune, Golden Globe, Golden Plum, Green Sun, Laroda, October Sun, TC Sun, Zanzi Sun
III	SbSf	Frontier
IV	SbSh	Eldorado, Freedom, Friar, Hiromi Red, Larry Ann, Nubiana, Owen T, Queen Ann, Songria 10
IX	SfSg	Golden Japan
VII	ScSh	Angeleno, Gaia, Queen Rosa, Ruby Crunch
VIII	SeSh	Black Diamond, Black Gold, Black Late, Earliqueen, John W, Laettitia, Showtime, Souvenir
X	ShSk	Howard Sun, Songold
XI	ScSe	Autum Giant, Black Splendor, Casselman, Royal Garner, Royal Zee, Santa Rosa
XII	SbSe	Pioneer, Saphire
XIII	SeSf	Black Star, Morris, Primetime
XIV	SaSc	Crisom Glo
XVI	SfSk	Kelsey
0		Songria 15 (SaSh), Ambra (SbSo), Oishiwasesumomo (ScSd), Simka (SeSk), October Red (ShSp), Mitard (SfSq), Joana Red (Sr-)