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

M. E. Guerra and M. López-Corrales
Departamento de Hortofruticultura.
Centro de Investigación Agraria 'Finca La Orden-Valdesequera'. A-V. Km 374, Guadajira, 06187 Badajoz
Spain.

J. Rodrigo and A. Wünsch
Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA).
Av. Montañana 930, 50059 Zaragoza,
Spain

Keywords: *Prunus salicina* Lindl., *S*-allele, *SFB*, *S-RNase*, gametophytic self-incompatibility

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 Scanner™ Software 1.0, Applied Biosystems) and the size standard GeneScan ™ 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.

ACKNOWLEDGMENTS

This work was supported by Spanish Ministry of Science and Innovation (MICINN) - European Regional Development Fund (ERDF), European Union (research grants AGL2009-12621-C02-02 and INIA-RTA2009-00144-00-00) and Gobierno de Aragón (Grupo de Excelencia A-43). We gratefully acknowledge Agroseguro S.A., Grupo ALM, J.L. Espada [Centro de Técnicas Agrarias (CTA-DGA)], Manuel Alonso [Cooperativa de regantes de Extremadura (CREX)], Ignasi Iglesias [Institut de Recerca I Tecnología Agroalimentàries (IRTA-Generalitat de Catalunya)], Viveros Atanasio Naranjo, Viveros Provedo, Dr. R. Tao (Kyoto Universisty, Japan), Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) and USDA-ARS-National

Clonal Germplasm Repository (Davis, California) for providing plant material used in this study. M.E. Guerra was supported by a Spanish Ministry of Science and Education (MEC) INIA pre-doctoral fellowship.

Literature cited

Beppu, K., Yamane, H., Yaegaki, H., Yamaguchi, M., Kataoka, I. and Tao, R. 2002. Diversity of *S*-RNase genes and *S*-haplotypes in Japanese plum (*Prunus salicina* Lindl.). J. Hort. Sci. Biotechol. 77:658-664.

Beppu, K., Takemoto, Y., Yamane, H., Yaegaki, H., Yamaguchi, M., Kataoka, I. and Tao, R. 2003. Determination of *S*-haplotypes in Japanese plum (*Prunus salicina* Lindl.) cultivars by PCR and cross-pollination tests. J. Hort. Sci. Biotechol. 78:315-318.

Guerra, M. E., Rodrigo, J., López-Corrales, M. and Wünsch, A. 2009. *S-Rnase* genotyping and incompatibility group assignment by PCR and pollination experiments in Japanese plum. Plant Breeding. 128:304-311.

Halász, J., Hegedus, A., Szabo, Z., Nyeki, J. and Pedryc, A. 2007. DNA-Based *S*-genotyping of Japanese plum and pluot cultivars to clarify incompatibility relationships. Hortscience. 42:46-50.

Sapir, G., Stern, R. A., Eisikowitch, D. and Goldway, M. 2004. Cloning of four new Japanese plum *S*-alleles and determination of the compatibility between cultivars by PCR analysis. J. Hort. Sci. Biotechol. 79: 223-227.

Sonneveld, T., Tobutt, K.R. and Robbins, T.P. 2003. Allele-specific PCR detection of sweet cherry self-incompatibility (*S*) alleles *S1* to *S16* using consensus and allele-specific primers. Theor Appl Genet. 107: 1059-1070.

Sonneveld, T., Robbins, T.P. and Tobutt, K.R. 2006. Improved discrimination of self-incompatibility *S-RNase* alleles in cherry and high throughput genotyping by automated sizing of first intron polymerase chain reaction products. Plant Breeding. 125:305-307.

Vaughan, S.P., Russell, K., Sargent, D.J. and Tobutt, K.R. 2006. Isolation of *S*-locus F-box alleles in *Prunus avium* and their application in a novel method to determine self-incompatibility genotype. Theor Appl Genet. 112:856-866.

Yamane, H., Tao, R. and Sugiura, A. F. 1999. Identification and cDNA cloning for *S-RNases* in self-incompatible Japanese plum (*Prunus salicina* Lindl. cv. Sordum). Plant Biothechnol. 16:389-396

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*-)