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Preliminary characterization of the self-incompatibility genotypes of European plum (*Prunus domestica* L.) cultivars

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Summary: European plum is an important fruit crop with complex, hexaploid genome of unknown origin. The characterization of the self-incompatibility (*S*) locus of 16 European plum cultivars was carried out using the PaConsI-F primer in combination with the EM-PC1consRD primer for the first intron and the EM-PC2consFD and EM-PC3consRD primers for the second intron amplification. Altogether, 18 different alleles were scored indicating high genetic diversity. These alleles were labelled using alphabetical codes from S_A to S_S. We identified 5 different alleles in 9 cultivars, 4 alleles in 5 cultivars, while 3 alleles were shown in two of the assayed cultivars. A total of 16 different *S*-genotypes were assigned, and discrimination of all plum cultivars was successful based on their unique *S*-genotypes. However, further research is required to reliably identify the *S*-alleles based on their DNA sequence and clarify complete *S*-genotypes.

Keywords: self-incompatibility, *S*-allele, European plum, *Prunus domestica* L.

Introduction

European plum is an important fruit crop. The majority of world 10.7 million tons of 'plums and sloe' (as mentioned in the Faostat database) production quantity has been harvested in Asia (68%) while Europe ranks second with a value of 21% (*Faostat*, 2012). In Hungary, a total of 43,268 t was harvested in 2012, which makes it the second most important stone fruit crop in the country after sour cherry. Considering its economical prospects and naturally occurring wild and semi-wild populations throughout the country, the genetic characterization of the species may bring advantage through the exploitation and valorization of this fruit.

Throughout the world, several plant species are regarded and their fruit is marketed as "plum". These include *P. cerasifera* Ehrh. (cherry plum), *P. cocomilia* Ten. (Italian plum), *P. simonii* Carr., *P. spinosa* L. (blackthorn or sloe), *P. insititia* L., *P. americana* Marsh. (American plum), *P. mexicana* S.Watson (Mexican plum), *P. nigra* Ait. (Canada plum, Black plum) and others (*Faust & Surányi*, 1999). The species European plum (*P. domestica*) is grown in Hungary and other Central European countries. The species is characterized by a hexaploid genome ($2n=6x=48$) (*Crane & Lawrence*, 1929). Although hypotheses have been drawn pointing to both the allo- and autopolyploid origins of the species, no doubt clarification is still not available for either of the two.

Most *Prunus* species express gametophytic self-incompatibility (GSI) where the phenotype of the pollen is determined by its own haploid genotype (*de Nettancourt*, 2001). In *Rosaceae*, GSI is controlled by the single polymorphic *S*-locus (named after the term, sterility) containing two genes, the *S-ribonuclease* (*S-RNase*) and *S-haplotype-specific F-box* (*SFB*) (*Hegedűs* et al., 2012). Both genes exhibit high polymorphism and high sequence diversity. While self-incompatibility studies are common in case of diploid plum species (*Halász* et al., 2007; *Guerra* et al., 2009, 2012), information on the *S*-genotype of European plum cultivars is rather limited (*Hegedűs & Halász*, 2006). This might be explained by the fact that self-incompatibility occurs less frequently in polyploid than diploid plums and also by the hexaploid genome that makes allele identification a complex and elusive task. First study regarding the molecular genetics of self-incompatibility in domestic plum was published by *Sutherland* et al. (2004b).

Deduced polypeptide sequences of three myrobalan and three domestic plum *S*-RNases showed over 97% identity with *S*-RN-ases from other *Prunus* species, including almond, sweet cherry, Japanese apricot and Japanese plum (*Sutherland* et al., 2008). The second intron, which is generally highly polymorphic between alleles was also remarkably well conserved within these *S*-allele pairs. Degenerate consensus primers were developed and used to amplify and sequence the corresponding *SFB* alleles. Sequence comparisons also indicated high degrees of polypeptide sequence identity between three myrobalan and the three domestic plum *SFB* alleles and the corresponding *Prunus SFB* alleles supporting the trans-specific evolution of *Prunus* allele specificities.

The applicability of six *S*-locus-specific markers previously used for other *Prunus* species were compared in the study of *Kota-Dombrovska & Lacis* (2013) for the characterization of 33 domestic plum cultivars. A range of 14–37 alleles was amplified for the markers used, the average observed heterozygosity was high. Applification of the tested primer pairs allowed discrimination of all plum cultivars by unique *S*-genotypes. Primer pairs EM-PC2consFD/ EM-PC3cons RD, PasPcons-F1/ PaC1cons-R1 and F-Box50A/ F-Box intronR provided the highest level of variability. The markers used did not distinguish plum cultivars according to self-compatible or –incompatible phenotype.

This study was aimed to obtain preliminary information regarding the self-incompatibility genotype of a range of important European plum cultivars grown in Hungary. We wanted to test the efficiency of the PCR markers developed for *S*-genotyping of diploid *Prunus* species and assess how many *S*-alleles of the hexaploid genome can be determined based on this approach.

Material and methods

Plant material

The study was performed on 16 domestic plum (*Prunus domestica* L.) cultivars (Table 1). Samples were obtained from the orchard of the Corvinus University of Budapest, Soroksár, Hungary.

Table 1. List of European plum cultivars with the *S*-genotype and their pedigree (*Surányi & Erdős*, 2006)

	Cultivar name	Provisional S-genotype	Pedigree
1.	Althann ringló	$S_FS_KS_OS_P$	unknown
2.	Bluefre	$S_HS_IS_K$	Stanley × President
3.	Elena	$S_BS_GS_IS_KS_O$	Fellenberg × Stanley
4.	Empress	$S_AS_DS_ES_HS_N$	–
5.	Haganta	$S_CS_HS_IS_JS_O$	Cacaks Beste × Valor
6.	Hanita	$S_HS_IS_OS_R$	President × Auerbacher
7.	Haroma	$S_HS_IS_O$	(Ortenauer × Stanley 34) × Hanita
8.	Jojo	$S_IS_LS_MS_O$	Ortenauer × Stanley
9.	Katinka	$S_AS_KS_LS_O$	Ortenauer × Ruth Gerstetter
10.	Presenta	$S_CS_HS_KS_MS_P$	Ortenauer × President
11.	President	$S_CS_DS_HS_IS_K$	unknown
12.	Sermina	$S_AS_FS_IS_NS_O$	old Italian cultivar
13.	Stanley	$S_HS_IS_OS_S$	Agen × Grand Duke
14.	Topend Plus	$S_CS_IS_KS_OS_S$	Cacaks Beste × Valor
15.	Topfive	$S_IS_KS_OS_R$	Cacaks Beste × Auerbacher
16.	Tophit	$S_CS_FS_HS_KS_S$	Cacak's Beste × President

DNA extraction and genomic PCR with S-RNase-specific primers

Genomic DNA was extracted from fully expanded young leaves using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). PCR was conducted according to *Sutherland* et al. (2004a) using the degenerate primers EM-PC2consFD and EM-PC3consRD for the amplification of the second intron region of the *S-RNase* gene. To amplify the first intron, the fluorescently labelled ($_{JOE}$) PaConsI-F primer (*Sonneveld* et al., 2003) was used in combination with the EM-PC1consRD primer (*Ortega* et al., 2005). PCR was carried out in a PTC 200 thermocycler (MJ Research, Budapest, Hungary) using the program described for the primers. Approximately 20–80 ng of genomic DNA were used for PCR amplification in a 25-µL reaction volume, containing 1 × PCR buffer (Sigma, Budapest, Hungary) with final concentrations of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 0.4 µM of each primer and 0.625 U of *Taq* DNA polymerase (Sigma, Budapest, Hungary).

The PCR products were separated on 2% TAE agarose gels at 100 V for 2 h and DNA bands were stained with ethidium bromide. Fragment sizes were estimated by comparison with the 1 kb + DNA ladder (Promega, Madison, Wis.). For exact size determination of *S-RNase* first intron region fragments smaller than 500 bp, the fluorescently labeled products were run in an automated sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Budapest, Hungary) using the GENOTYPER 3.7 software and GS500 LIZ size standard (Applied Biosystems, Budapest, Hungary).

Results and discussion

The characterization of the *S*-locus of 16 European plum cultivars was carried out using the PaConsI-F primer (*Sonneveld* et al., 2003) in combination with the EM-PC1consRD primer (*Ortega* et al., 2005) for the first intron and the EM-PC2consFD and EM-PC3consRD primers (*Sutherland* et al., 2004a) for the second intron amplification. Both primer pairs exhibited successful PCR amplification and yielded several amplification products for all assayed cultivars. Since domestic plum is hexaploid, a maximum of six amplification fragments can be found in one sample. Primer combination for the second intron region of the *S-RNase* gene amplified 4 to 6 bands for each of the cultivars. A total of 15 distinct bands were amplified. PCR products varied greatly in size, with bands ranging from 300 bp to 2500 bp in length. *Kota-Dombrovska & Lacis* (2013) tested six different primer combinations for *S*-genotyping of plum cultivars and the EM-PC2consFD and EM-PC3consRD primer pair was characterized by the highest number of amplified fragments per sample (4.0 in average).

To complete the *S*-genotype determination, precise first intron lengths were also measured for all cultivars using fluorescently labelled primers and automated sizing on a

capillary sequencer. Analysis of the first intron in all 16 plums resulted in 4-5 fragments in each of the assayed cultivars, their sizes ranged between 232 and 416 bp. Altogether, 18 different alleles were scored indicating high genetic diversity. Due to the lack of any sequence information of the DNA fragments, we labelled these alleles using alphabetical codes from S_A to S_S. This provisional labelling system will be modified when DNA sequence of the corresponding alleles will allow reliable identification and harmonization, based on searching on a public sequence database. We identified 5 different alleles in 9 cultivars, 4 alleles in 5 cultivars, while 3 alleles were shown in two of the assayed cultivars (Table 1). It means that only partial *S*-genotypes were established. Analysis of these two regions of the *S-RNase* gene did not allow the identification of complete *S*-genotypes because two or more copies of the same allele (homozygosity) could not be excluded based on these PCR assays. However, another explanation may be also relevant since certain alleles were not detected in some cases using DNA amplification with consensus primers due to the so-called preferential PCR amplification (*Kodad* et al., 2013). The most frequent alleles were S_I and S_O occurring in 11 cultivars, followed by S_H and S_K (9), S_C (5), S_A, S_F and S_S (3), S_D, S_L, S_M, S_N, S_P and S_R (2), while each of the S_B, S_E, S_G and S_J-alleles were found in only one cultivar. Most of the cultivars share common *S*-alleles because of the same cultivars were used as parents for crosses in many cases (*Figure 1*), since breeding programmes attention has focussed on improvement of fruit quality and on resistance or tolerance to Plum pox virus (*Hartmann & Neumuller*, 2006). A total of 16 different *S*-genotypes were assigned, so discrimination of all plum cultivars was successful based on their unique *S*-genotypes.

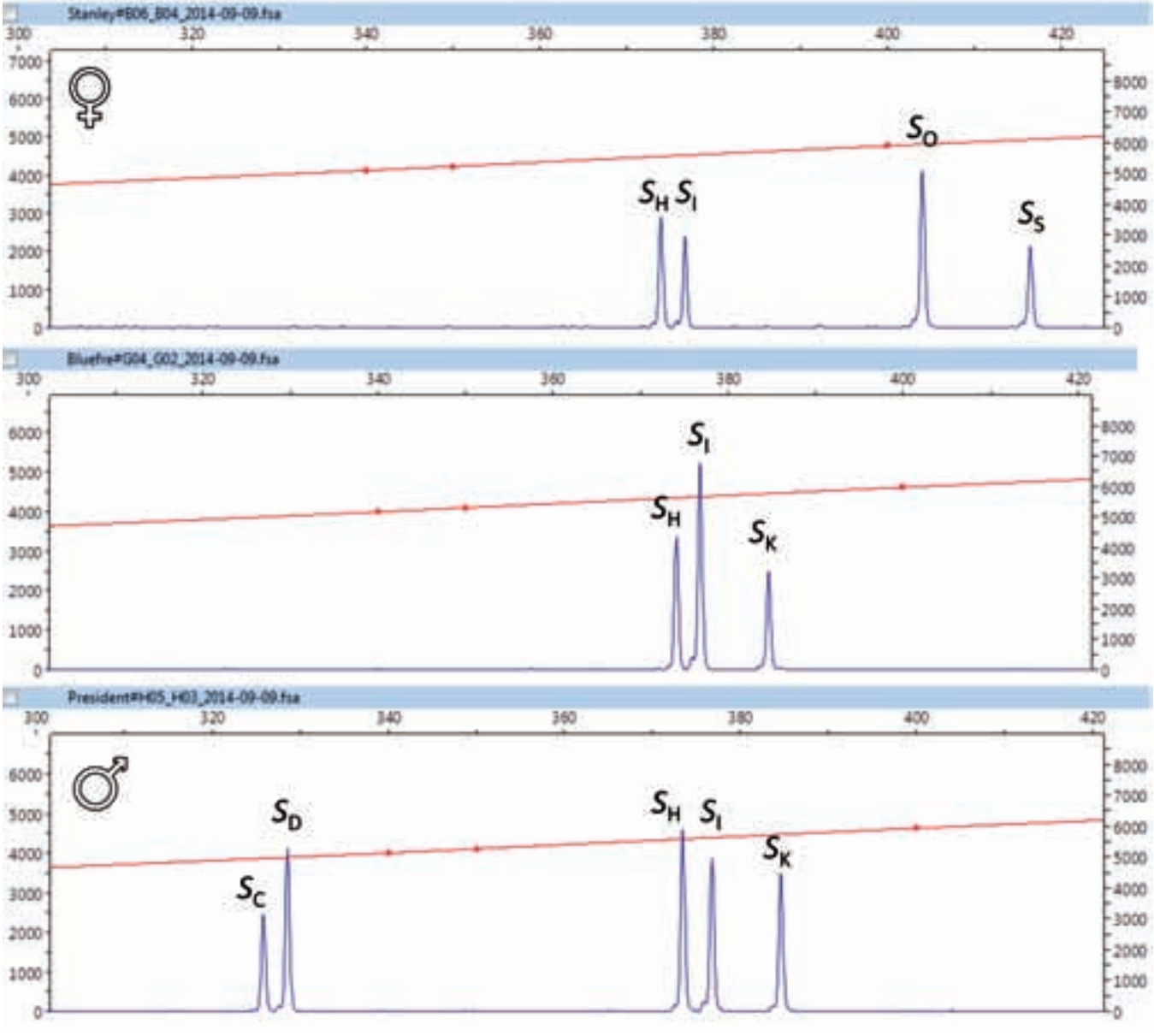

Figure 1. Chromatogram of the fluorescently labelled first intron fragments of the *S-RNase* gene indicating *S*-alleles in 3 plum cultivars. Bluefre ($S_HS_IS_K$): Stanley ($S_HS_IS_OS_S$) × President ($S_CS_DS_HS_IS_K$).

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