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S-Genotype of Japanese Pear 'Hosui'

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Summary

The *S*-genotype of 'Hosui', a self-incompatible cultivar of the Japanese pear (*Pyrus pyrifolia* Nakai), was determined by a combination of two-dimensional gel electrophoresis (2D-PAGE) and N-terminal sequence analysis for S-proteins. Four protein spots that migrated to the S-protein zone were detected and individually assigned to S_3a-, S_3b-, S_5a-, and S_5b-RNases by comparing their electrophoretic behavior with that of S_3- and S_5-RNases. Analyses of the amino acid sequences of the four proteins confirmed their assignments leading us to postulate that the *S*-genotype of 'Hosui' is S_3S_5.

Key Words: *S*-genotype, Japanese pear, self-incompatibility, S-RNase, 'Hosui'.

Introduction

Self-incompatibility is a widespread, natural mechanism that prevents inbreeding (promotes outcrossing) in flowering plants to enhance diversity and maintain preservation of species (de Nettancourt, 1977). Most rosaceous species including the Japanese pear (*Pyrus pyrifolia* Nakai) exhibit gametophytic self-incompatibility that is controlled by a single *S*-locus (de Nettancourt, 1977; Kikuchi, 1929). The *S*-locus encodes a ribonuclease (S-protein, S-RNase) that is exclusively expressed in the style and is associated with this self-incompatibility (Ishimizu et al., 1996). In this system, the growth of a pollen tube is inhibited in the style when the haploid *S*-allele of the pollen matches one of the diploid *S*-alleles of the pistil (de Nettancourt, 1977).

The Japanese pear is an important commercial fruit tree crop in Japan and breeding a new cultivar that yields disease-resistant fruits of high quality has been a challenging goal for pear breeders (Kajiura and Sato, 1990). Because of self-unfruitfulness, cross pollinaion is required to set fruits of Japanese pears. A pollen-donating cultivar must bloom earlier than a fruit-producing cultivar and must bear at least one *S*-allele different from two *S*-alleles of the fruit-producing cultivar. To this end, the identification of the *S*-genotype in a given cultivar is an initial step for the horticultural experiments, but it takes a long time because the *S*-genotype must be determined by tedious cross-pollination experiments.

In the Japanese pear, seven *S*-alleles (S_1 to S_7) have been identified in about 40 cultivars by crossing experiments (Machida, 1972; Terami et al., 1946). Using the 2D-PAGE analysis system which we established recently to identify *S*-alleles (Ishimizu et al., 1996), the S-proteins (S_1- to S_7-RNase) linked to the seven *S*-alleles were identified by their mobilities on the gel and their N-terminal sequences.

In Japan, 'Hosui' is one of the most popular cultivars, ranking with 'Nijisseiki' (S_2S_4) and 'Kosui' (S_4S_5) (Japan Fruit Growers Cooperative Association, 1996). Its genetic background, including the *S*-genotype, is still unknown. It was reported that 'Hosui' was a hybrid seedling crossed between 'Ri-14' (S_1S_2) and 'Yakumo' (S_1S_4) in 1954 and selected in 1963 (Kajiura et al., 1974). But its cross-incompatibility and skin color are not consistent with offsprings of 'Ri-14' x 'Yakumo' (Machida et al., 1982). Consequently, many attempts have been made to determine the *S*-alleles of 'Hosui' by cross-pollination trials (Hiratsuka and Okada, 1993; Terai et al., 1995), and electrophoretically (Hiratsuka and Okada, 1993). These researchers concluded that 'Hosui' was an S_3 homozygote because a) the S_3-protein in 'Hosui' extract was detected on an isoelectric focusing gel, b) pollen from 'Hosui' was rejected by 'Chojuro' (S_2S_3), and c) fruit set resulting from pollen from 'Chojuro' on the pistil of 'Hosui' was 100%. Terai et al. (1995), however, found that 'Hosui' and 'Chojuro' were cross-compatible so that they concluded that one of the *S*-alleles of 'Hosui' is S_3 because a pollen from an S_3 homozygote was rejected by 'Hosui'.

One reason why the *S*-genotype of 'Hosui' has not been determined clearly is attributed to the ambiguity of the crossing experiments, which are affected by a number of environmental and physiological factors. It is arbitrarily accepted that if a pollen source sets more than 70 % of the (pollinated) flowers, it is considered cross-compatible, whereas, if it is less than 30%, it is

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considered cross-incompatible. In contrast, the electrophoretic method that we have established (Ishimizu et al., 1996) is a rapid, reliable method for identifying the *S*-genotype of unknown cultivars because the S-proteins linked to *S*-alleles are analyzed directly and their N-terminal sequences can be determined readily.

We applied this method to ascertain the *S*-genotype of 'Hosui' and discuss the usefulness of this method for a diagnostic identification of the *S*-genotype in the Japanese pear.

Materials and Methods

Plant materials

The flower buds, at the balloon stage of Japanese pear 'Hosui', were collected in 1994 at the Tottori Horticultural Experiment Station in Daiei, Tottori. The styles with their stigmas attached were dissected from the flowers, rapidly frozen by liquid nitrogen and stored at -170°C until required.

Two-dimensional gel electrophoresis

The stylar proteins of 'Hosui' were extracted and separated by a two-dimensional gel electrophoresis using a non-equilibrium pH gradient electrophoresis (NEPHGE) as the first direction and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the the second direction as previously described (Ishimizu et al., 1996).

Amino acid sequence analyses

S-proteins (S-RNases) which were separated by two-dimensional gel electrophoresis were electroblotted onto polyvinylidene difluoride membranes and sequenced with a gas-phase protein sequencer (470A, Applied Biosystems). The detailed procedures were described previously (Ishimizu et al., 1996).

Results and Discussion

Stylar proteins of 'Hosui' were subjected to 2D-PAGE in which NEPHGE, which separates proteins

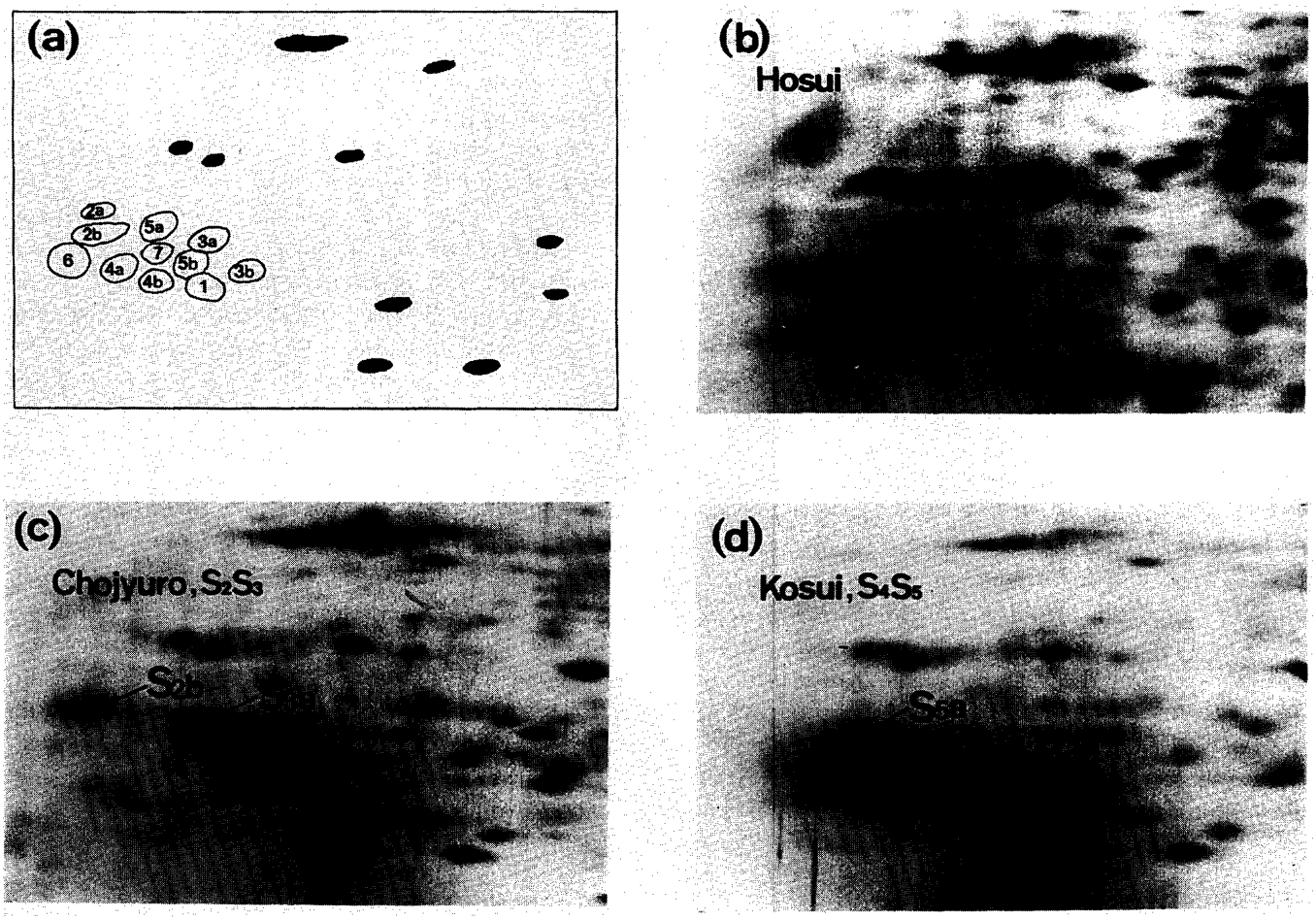

Fig. 1. (a), Previously constructed S-RNase map (Ishimizu et al. 1996). (b), S-RNases from the styles of 'Hosui' separated by 2D-PAGE to regions to which 25-42 kDa basic proteins migrated. (c), Separation of S-RNases from the styles of 'Chojuro'. (d), Separation of S-RNases from the styles of 'Kosui'. Major proteins are indicated by filled-in spots. Open circles and numbers indicate the locations of S-RNases and their corresponding *S*-alleles, respectively. Since NEPHGE was used for the 1st dimensional electrophoresis, each S-protein did not move to its accurate pI point.

with pI values of 4.5 to 10.5, and SDS-PAGE were used for the first- and second-dimensional electrophoresis, respectively. The region where seven S-RNases (S_1- to S_7-RNase) of the Japanese pear migrated was magnified (Fig.1). When protein spots in this region from 'Hosui' were compared with the S-RNase map constructed in the previous report (Ishimizu et al., 1996) (Fig.1(a) and 1(b)), proteins corresponding to S_3a-, S_3b-, S_5a- and S_5b-RNases were detected on the gel (Fig.1(b)). They were tentatively named Hosui S_3a-, Hosui S_3b-, Hosui S_5a- and Hosui S_5b-RNase, respectively. Double spots for S_3- and S_5-RNases were found for S_3-RNase from 'Chojuro' and 'Seigyoku' (Fig.1(c)) and for S_5-RNase from 'Kosui' (Fig.1(d)), respectively (Ishimizu et al., 1996), which are attributed to the heterogeneity of the sugar chain attatched to S-RNases (Ishimizu et al., 1996). Purified S-RNases from the style of 'Hosui' also bore sugar chains (unpublished data). The single spot for S_1-, S_6- and S_7-RNases (Fig.1(a)) seems to be associated with less heterogeneity in the sugar chain.

These four proteins were electroblotted onto polyvinylidene difluoride membranes and the membranes were applied to a gas-phase protein sequencer. The S_3-RNase and S_5-RNase have the same N-terminal sequence, YDYFQFTQQYxLAVxN (Ishimizu et al., 1996). Therefore, YDYFQFTQQYxLAV or YDYFQFTQQYxL (Fig.2) of the four proteins confirm their similarity if the *S*-genotype of 'Hosui' is S_3S_5. These results indicated that the *S*-genotype of 'Hosui' was S_3S_5.

		1 5 10 15
Hosui	S_3a	YDYFQFTQQYxLAV
Hosui	S_3b	YDYFQFTQQYxL
Hosui	S_5a	YDYFQFTQQYxLAV
Hosui	S_5b	YDYFQFTQQYxL
pear	S_1	YDYFQFTQQYxPAVxN
pear	S_2	ARYDYFQFTQQYQxAF
pear	S_3	YDYFQFTQQYxLAVxN
pear	S_4	FDYFQFTQQYQPAVxN
pear	S_5	YDYFQFTQQYQLAVxN
pear	S_6	YNYFQFTQQYxPAVxN
pear	S_7	YDYFQFTQQYxPAV

Fig. 2. Comparison of N-terminal amino acid sequences of S-RNases from 'Hosui' and other cultivars of the Japanese pear. Unidentified residues are denoted as "x". S_1-protein was derived from 'Hayatama' and 'Imamuraaki'; S_2-protein, from 'Hayatama' and 'Nijisseiki'; S_3-protein, from 'Chojuro' and 'Seigyoku'; S_4-protein, from 'Nijisseiki' and 'Kosui'; S_5-protein, from 'Kosui' and 'Okusankichi'; S_6-protein, from 'Imamuraaki'; and S_7-protein, from 'Okusankichi'.

According to the results of the crossing experiments, 'Hosui' is compatible with the following genotypes; S_1S_2, S_1S_4, S_1S_5, S_1S_6, S_1S_7, S_2S_2, S_2S_3, S_2S_4, S_3S_4, S_4S_5 and S_5S_7 (Sato, Y., personal communication). Furthermore, pollen from an S_3 homozygote was rejected by the pistil of 'Hosui' (Terai et al., 1995). These results do not contradict the conclusion that the *S*-genotype of 'Hosui' is S_3S_5. It has been reported that 'Hosui' is compatible with 'Tanzawa' (S_3S_5) (Tarami et al., 1946), which conflicts with our conclusion.

Because 'Hosui' is the cultivar yielding an excellent fruit, it is often used as a parent for breeding a new cultivars. The identification of the *S*-genotype of 'Hosui' makes it easy to deduce the *S*-genotype of its offspring. For example, one of the offspring of 'Hosui', 'Chikusui', was produced by crossing 'Hosui' and 'Hakko' (S_4S_5). If the *S*-genotype of 'Hosui' is S_3S_5, the *S*-genotype of 'Chikusui' is presumed to be S_3S_4 or S_4S_5, which does not contradict that 'Chikusui' is incompatible with 'Shinseiki' (S_3S_4) (Hiratsuka and Okada, 1993).

Recently, molecular biological methods for the identification of *S*-alleles have been developed using electrophoresis (Battle et al., 1995) and PCR-based techniques in *Brassica oleracea* and *Malus x domestica* (apple) (Brace et al., 1994; Janssens et al., 1995). However, these methods are not applicable for any variety in these two genera because all *S*-allele-specific proteins (or genes) have not been identified. In the case of the Japanese pear, the S-RNases corresponding to the seven *S*-alleles (S_1 to S_7) have been identified (Ishimizu et al., 1996), our electrophoretic method coupled with N-terminal sequencing is applicable for any offspring derived from parents having known *S*-alleles. Even if a cultivar bears a new *S*-allele other than these seven, its *S*-genotype can be identified as a new type, because the corresponding S-RNase will appear as a new spot on the 2D-PAGE gel and possess a new N-terminal sequence. In addition, only 50 mature flowers are enough to identify *S*-alleles by this method. Accordingly, this method will overcome the uncertainty and the lengthy time required for the conventional cross-pollination experiments and will be a powerful tool for identifying the *S*-genotype in Japanese pear cultivars.

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ニホンナシ'豊水'の*S*遺伝子型

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摘　要

ニホンナシの優れた栽培品種の一つである'豊水'の*S*遺伝子型は，長年の交配実験によっても決定されていない．ニホンナシ花柱由来の7種類の*S*遺伝子産物（S_1-RNaseからS_7-RNase）を二次元電気泳動により分離・同定する系をすでに確立したので，この系を用いて'豊水'の*S*遺伝子型の決定を試みた．

花柱タンパク質抽出液を一次元目が非平衡等電点電気泳動（NEPHGE），二次元目がSDS-ポリアクリルアミドゲル電気泳動（SDS-PAGE）からなる二次元電気泳動に供したところ，S_3a，S_3b，S_5a，S_5b-RNase（aとbは糖鎖の不均一性により分離したと考えられている）と同じ位置にそれぞれタンパク質スポットが検出された．これらのタンパク質をPVDF膜に電気転写し，気相シーケンサーにより分析したところ，4種類のタンパク質のN末端アミノ酸配列はすべて同じ（YDYFQFTQQY）で，S_3-およびS_5-RNaseのN末端アミノ酸配列と一致した（S_3-RNaseとS_5-RNaseのN末端アミノ酸配列は同じである）．以上の結果より，'豊水'の*S*遺伝子型はS_3S_5であると推測した．