



RFLP analysis reveals the genealogical relation of novel Japanese persimmon ‘Saijo’

Kenji Toyota^a, Keisuke Mochida^b, Takao Kurahashi^b, Shinya Maki^{c,*}

^a Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology, and National Institutes of Natural Sciences, 5-1 Higashiyama, Myodaiji, Okazaki, Aichi 444-8787, Japan

^b Shimane Agricultural Experiment Station, Izumo, Shimane 693-0035, Japan

^c Department of Science of Technology Innovation, Nagaoka University of Technology, Nagaoka, Niigata 940-2188, Japan

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ABSTRACT

‘Saijo’ Japanese persimmon (*Diospyros kaki* Thumb.) is one of the most popular and commercially important fruit cultivars in Japan. In 2000, origin-unknown ‘Saijo’-like cultivar was found at the Shimane farm and named as ‘M-3’. Although it has similar morphological and physiological characteristics with ‘Saijo’ cultivars, its fruit size is apparently bigger than ‘Saijo’ making them an attractive candidate as novel valuable fruit for farm household. In this study, to elucidate the genealogical relationship of ‘M-3’, we conducted the restriction fragment length polymorphism (RFLP) analysis using genomic DNA of Japanese persimmon (‘Saijo’ lines, pollinizers, and other cultivars that bloom a male flower) detected by cDNA probes involved in genes coding persimmon fruit ripening: *1-aminocyclopropane-1-carboxylate synthase 1* (ACCS-1), *ACC oxidase 1* (ACCO-1), and *polygalacturonase* (PG-9). We demonstrated that ‘M-3’ line shares several RFLPs specific to ‘Saijo’ lines, pollinizers, and other cultivars. We found that morphological features of fruit and leaf in ‘M-3’ resembled that of ‘Saijo’ and pollinizer lines. These results strongly suggest that ‘M-3’ is likely the seedlings crossbred by ‘Saijo’ and pollinizer cultivars. Our findings provide first insight of genealogical origins of valuable newly identified persimmon cultivar and an important suggestion to establish a new valuable ‘Saijo’ cultivar by cross breeding with pollinizer lines.

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1. Introduction

Persimmon belongs to the genus *Diospyros* in the family Ebenaceae and it contains more than 700 species (The Plant List, 2013). The most widespread cultivated species worldwide is *Diospyros kaki* Thumb., which is originated in Eastern Asia. Japanese persimmon is one of the most popular and commercially important fruit species in Japan, where there are more than 1000 cultivars (Agricultural Research Station, 1912). Japanese persimmon ‘Saijo’, a pollination constant astringent type cultivar, has been cultivated in western Japan, especially in the Chugoku region, and has won high praise due to its sweet taste and flavor. The ‘Saijo’ cultivar has so far been classified into six lines (Iwami, Izu mo, Kute, Hinomisaki, type-A, and type-B) based on morphological characteristics such as fruit shape, leaf shape and scale of stalk end (Kono et al., 1980). In addition to morphological features, fruit ripening period shows apparently different among these lines; the Kute, Hinomisaki and

type-B lines are grouped into the early-maturing type, whereas the Iwami and Iwami lines are categorized into late-maturing type (Kono et al., 1980).

New ‘Saijo’-like cultivar was found at the Shimane farm (Japan) and named as ‘M-3’. Its morphological and physiological characteristics are well similar to other ‘Saijo’ lines and its fruit size and weight is apparently larger and heavier than ‘Saijo’ lines. Although ‘M-3’ (novel ‘Saijo’-like line) is an ideally suitable for commercial late-maturing type cultivar as a candidate of the new specialized product, elucidation of the enigma of genealogical origin of this line including seedling or bud mutation is difficult based on only comparing their morphological features. To overcome this limitation, restriction fragment length polymorphisms (RFLPs) have been used as genetic markers to reveal the various *Diospyros* species (Nakamura and Kobayashi, 1994). Previously, we established an efficient RFLP method for discrimination among Japanese persimmon cultivars including ‘Saijo’ lines (Maki et al., 2001).

In this study, to clarify the genealogical relationship of ‘M-3’ line, we performed RFLP analysis using the genomic DNAs of eleven lines of the cultivar ‘Saijo’, four pollinizers, and two other cultivars that bloom and seldom have a staminate flower detected by

* Corresponding author.

E-mail address: maki@vos.nagaokaut.ac.jp (S. Maki).

genes involved in persimmon fruit ripening: *1-aminocyclopropane-1-carboxylate synthase 1* (ACCS-1), *ACC oxidase 1* (ACCO-1), and *polygalacturonase* (PG-9) as probes.

We demonstrated that 'M-3' line shares several RFLPs that is specific to 'Saijo' lines, pollinizers, and other cultivars. Also, several morphological features of 'M-3', for example, fruit and leaf shape were similar to 'Saijo' and pollinizer, respectively. These results suggest that 'M-3' line might be the seedlings of 'Saijo' and pollinizer lines.

2. Materials and methods

2.1. Persimmon materials

Eleven 'Saijo' lines ('type-B', 'Izumo', 'Kute', 'Yokozuna', 'Iwami', 'Iwami-2', 'M-1', 'M-2', 'M-2-1', 'M-2-2', and 'M-2-3'), four pollinizers ('Zenjimaruru', 'Akagaki', 'Saefuji', and 'Shogatsu'), and two other cultivars that have a staminate flower (Nishimurawase) and that seldom have a staminate flower ('Fuyu') were used in this study. All persimmon materials were provided by the experimental orchard of the Izumo branch of the Shimane Agricultural Experiment Station, Japan.

2.2. Measurement of fruit weight

Fruit weight of 'M-3' and 'Saijo' (Izumo) lines was measured at December 1, 1999. The 'Saijo' (Izumo) line was used as a control of representative 'Saijo' cultivar. Four replicates were prepared for each line. Statistical analysis of fruits weight was performed by one-way ANOVA followed by the Tukey's post-hoc test ($p < 0.05$).

2.3. DNA extraction and southern blot analysis

Total DNA was extracted from fully expanded fresh leaves of each cultivar of *D. kaki* by polyvinylpyrrolidone extraction methods (Nakamura and Kobayashi, 1994). A 20 μ g sample of total DNA was digested with restriction enzymes (*Hind*III and *Eco*RI), separated by 1.0% agarose gel (Agarose LO3, Takara, Japan) electrophoresis, and transferred to a nylon membrane (Biodyne A membrane, Pall Corporation, CA, USA) by a standard protocol (Southern, 1975). A cDNA clone of *1-aminocyclopropane-1-carboxylate oxidase 1* (ACCO-1, accession number: AB038355), *1-aminocyclopropane-1-carboxylate synthase 1* (ACCS-1, accession number: AB038356), and *polygalacturonase* (PG-9, accession number: AB038358) were used as probes. Probe DNAs were labeled with alkaline phosphatase (AP) using AlkPhos Direct Labelling kit (GE Healthcare, Tokyo, Japan). Hybridization was carried out according to previous procedure (Maki et al., 2001), and signal detection was performed using CDP-Star Detection Reagent (GE Healthcare) according to the manufacturer's protocol.

3. Results

Fruit size is one of the most significant features affecting its economic value. Although the fruit size of 'M-3' line is known to be bigger than other 'Saijo' lines, experimental evidence is lacked. We measured fruit weight of 'M-3' (237.4 ± 19.03 , $n = 4$) and representative 'Saijo' Izumo line (184.4 ± 12.30 , $n = 4$), respectively (values are means [g] \pm SD). Based on these data, fruit weight of 'M-3' line was significantly 1.28 times larger than that of 'Saijo' line (One-way ANOVA, post-hoc Tukey's test, $p < 0.05$). Our previous study reported that combination of genomic DNA digested by *Hind*III and the ACCO-1 probe is appropriate for detection of RFLP patterns in *D. kaki* (Maki et al., 2001). Therefore, to elucidate the origin of 'M-3' line, we conducted the RFLP analysis following previous

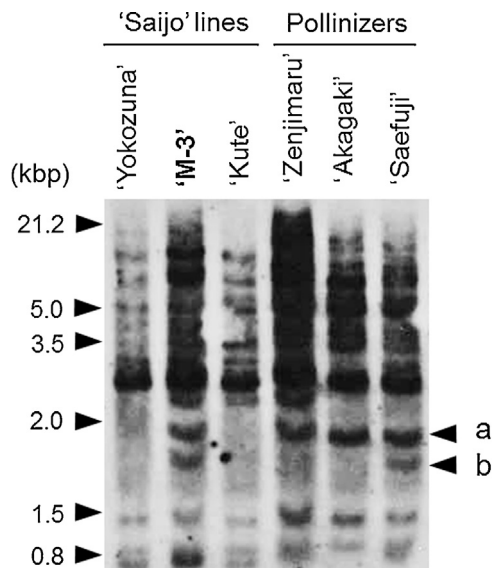


Fig. 1. RFLP patterns of Japanese *Diospyros kaki* cultivars. The ACCO-1 probe was hybridized to *Eco*RI-digested genomic DNA. Arrowheads indicate the molecular markers.

protocol with some modifications (Maki et al., 2001). We found the two pollinizer-specific RFLPs ('a' and 'b' in Fig. 1) using the *Eco*RI digested genome DNA with ACCO-1 probe in 'M-3' line. A specific band 'a' was shared with all pollinizer lines and band 'b' was consistent with 'Saefuji' line (Fig. 1). Moreover, we found the several specific RFLPs using the *Hind*III digested genome DNA with ACCS-1, ACCO-1 and PG-9 probes in 'M-3' line (Fig. 2). In the case of ACCS-1 probe, one band ('c') was detected in 'M-3' and all pollinizer lines (Fig. 2A). Similarly to ACCS-1, we detected several RFLPs using the ACCO-1 probe and revealed these bands ('d' to 'i' in Fig. 2B and 'k' to 'o' in Fig. 3) in 'M-3' and pollinizer lines. Besides, we identified the 'Saijo'-specific RFLP ('j' in Fig. 2C), which is conserved among all 'Saijo' lines, in 'M-3' line using PG-9 probe.

Moreover, fruit and leaf shapes of 'M-3' line seemed to be sharing morphological characteristics between 'Saijo' and pollinizer lines; for examples, fruit shape and color are similar to 'Saijo' line, and black spot pattern of calyx and leaf shape are well consistent of pollinizer characters (Fig. 4).

4. Discussion

One of the most representative Japanese persimmon cultivar 'Saijo' has been reared as the famous local products. To date, its discriminative six lines (Iwami, Izumo, Kute, Hinomisaki, type-A, and type-B) are selected as recommended cultivars (Kono et al., 1980). Although these 'Saijo' lines have a sweet taste and characteristic flavor, their fruit size has relatively small. To make a breakthrough this point, development of attractive novel 'Saijo' cultivars bearing huge fruit has been addressed by screening of bud mutations and successfully established two bud mutants, 'M-1' and 'M-2' (Kurahashi et al., 2000). The fruit weights of 'M-1' and 'M-2' lines were 1.25 and 1.15 times larger than that of 'Saijo', and fruit sizes (transverse diameters) were 1.20 and 1.06 times greater, respectively (Kurahashi et al., 2000). In this study, we demonstrated that fruit weight of 'M-3' was 1.29 times heavier than that of 'Saijo' (Izumo), suggesting that this character becomes noteworthy additional value of 'M-3' as a novel special product.

The elucidation of genealogical origin of novel identified cultivar is an obligatory process for variety registration. Therefore, cultivar identification of Japanese persimmon has traditionally been conducted according to morphological and physiological

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