Efficiency of Sequence Characterized Amplified Region Markers for Selecting Non-Astringent Persimmon (*Diospyros kaki* Thunb.)

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ABSTRACT Persimmon (*Diospyros kaki* Thunb.) is classified into four types based on the fruit traits, astringency and flesh color. Of the four types, the pollination-constant non-astringent (PCNA) fruit is typically most desirable for consumption. In the present study, we used five sequence characterized amplified region (SCAR) markers associated with astringency in persimmon fruit, namely E4/E9r, E4/A2r, 7H9F/AST-R, AST-F/AST-R, and AST-F/PCNA-F/5R3R, to improve the efficiency of PCNA-type persimmon breeding via marker-assisted selection (MAS). A total of 84 cultivars of the four types and their segregating F1 progeny were used to evaluate the association of SCAR markers with the fruit astringency phenotype. Polymerase chain reaction evaluation of each SCAR marker showed that E4/E9r combined with AST-F/PCNA-F/5R3R was appropriate for selecting the *ast* allele responsible for PCNA-type fruit, as the phenotype-genotype match percentages of these two markers were 94% and 99%, respectively. This MAS was verified by the successful use of AST-F/PCNA-F/5R3R to select 107 PCNA-type individuals from 609 F1 hybrid progeny derived from various crosses.

Keywords Marker-assisted selection, Persimmon, Pollination-constant non-astringent, Sequence characterized amplified region

INTRODUCTION

Persimmon (*Diospyros kaki* Thunb.) is a perennial woody plant of the genus *Diospyros* and is a member of the order Ebenales. The origin of this species traces back to East Asia and it has been cultivated since the pre-Christ era (Yonemori *et al.* 2000). Most persimmon cultivars are hexaploid ($2n=6x=90$), although some are akaryotic nonaploid ($2n=9x=135$) (Zhuang *et al.* 1990; Tamura *et al.* 1998). Persimmon is classified as one of the four types based on the presence or absence of astringency in the fruit and the darkening of flesh color around seeds following pollination. These four types are referred to as pollination-constant non-astringent (PCNA), pollination variant non-astringent (PVNA), pollination variant astringent (PVA), and pollination-constant astringent (PCA) (Yamada 1993). Of the four types, PCNA is most preferred by

consumers due to its low astringency and absence of dark flesh in ripe fruit. The first PCNA cultivar in Japan known to the public was ‘Gosho’, which has been broadly cultivated for 200 years (Kikuchi 1948). Various PCNA cultivars have since been created using ‘Gosho’ by natural hybridizations or the discovery and isolation of bud mutations, and cultivated with limited regional distribution. These cultivars share a similar phenotype and genotype, and therefore display high levels of inbreeding depression when further hybridization is performed among the cultivars (Yamada 2005).

In order to prevent genetic erosion and improve genetic diversity, introgression breeding is needed to introduce background traits from non-PCNA into PCNA cultivars. However, PCNA is determined by the homozygous presence of the single recessive allele *ast*, and thus long breeding periods are required due to the low probability of

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selecting for PCNA plants (Ikeda *et al.* 1985; Yonemori *et al.* 2000). Marker-assisted selection (MAS) using *ast*-linked DNA markers may improve the efficiency of breeding programs as PCNA plants could be selected at the seedling stage. Most reported sequence characterized amplified region (SCAR) markers are limited in their use as they detect only the dominant *Ast* allele; therefore, a heterozygous individual (*Ast/ast*) cannot be discriminated (Kanzaki *et al.* 2001; Yonemori *et al.* 2003; Kanzaki *et al.* 2008; Kanzaki *et al.* 2009; Park *et al.* 2013). However, Kanzaki *et al.* (2010) has developed a set of SCARs that are tightly linked to *ast* allele and can thus select for PCNA plants. Compared to other DNA markers, such as random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP), SCARs are advantageous in that they are allele-specific and highly reproducible. The current set of PCNA SCAR markers also aid detection of both *Ast* and *ast* alleles and genotype

heterozygous plants.

It is difficult to breed novel sweet persimmon cultivars because it is time consuming and requires large field space. These limiting factors may be overcome in future breeding programs by increasing selection efficiency through MAS. The objective of this study was to analyze SCAR markers reported by Kanzaki *et al.* (2010) in diverse cultivars and populations from different crosses in Korea, in order to evaluate the suitability of these markers for use in PCNA cultivar MAS.

MATERIALS AND METHODS

Plants materials and DNA extraction

Persimmon accessions included 84 cultivars and breeding lines: 31 of PCNA type, 21 of PVNA type, 11 of PVA type, and 21 of PCA type (Table 1). Also analyzed were 609

Table 1. List of persimmon cultivars and breeding lines (*Diospyros kaki* Thunb.) used in this study and their resulting *ast* locus marker genotyping.

No.	Accession	Type ^{z)}	Origin	SCARs marker ^{y)}	
				E4/E9r (2,200 bp)	AST-F/PCNA-F/5R3R (220 bp)
Cultivars					
1	Maegawajiro	PCNA	Japan	—	—
2	Fujiwaragosho	PCNA	Japan	—	—
3	Jiro	PCNA	Japan	—	—
4	Midai	PCNA	Japan	—	—
5	Mammoth	PCNA	Japan	—	—
6	Sunami	PCNA	Japan	—	—
7	Matsumotowase-Fuyu	PCNA	Japan	—	—
8	Fuyu	PCNA	Japan	—	—
9	Ichikikeijiro	PCNA	Japan	—	—
10	Gosho	PCNA	Japan	—	—
11	Suruga	PCNA	Japan	—	—
12	Okugosho	PCNA	Japan	—	—
13	Yaizuwasejiro	PCNA	Japan	—	—
14	Mikado	PCNA	Japan	—	—
15	Tenjingosho	PCNA	Japan	—	—
16	Ro-19	PCNA	Japan	—	—
17	Hanagosho	PCNA	Japan	—	—
18	WakasugikeiJiro	PCNA	Japan	—	—
19	Daeandangam	PCNA	Korea	—	—
20	IsaHaya	PCNA	Japan	—	—
21	Wakakishiro	PCNA	Japan	—	—
22	kastusa	PCNA	Japan	—	—
23	Youhou	PCNA	Japan	—	—
24	Izu	PCNA	Japan	—	—
25	Uenishiwase	PCNA	Japan	—	—
26	Shinsyu	PCNA	Japan	—	—
27	Kinshu	PCNA	Japan	—	—
28	Taurei	PCNA	Japan	—	—

Table 1. Continued.

No.	Accession	Type ²⁾	Origin	SCARs marker ³⁾	
				E4/E9r (2,200 bp)	AST-F/PCNA-F/5R3R (220 bp)
29	Dongwon1	PCNA	Korea	—	—
30	Dongwon2	PCNA	Korea	—	—
31	Dongwon3	PCNA	Korea	—	—
32	Toyouchi	PVNA	Japan	+	+
33	Johongsi	PVNA	Korea	+	+
34	Nishimurawase	PVNA	Japan	+	+
35	Migatanigosho	PVNA	Japan	+	+
36	Haschiuri	PVNA	Japan	+	+
37	Amahyakume	PVNA	Japan	+	+
38	Koharu	PVNA	Japan	+	+
39	Kyara	PVNA	Japan	+	+
40	Zenjimar	PVNA	Japan	+	+
41	Sanggokuitsy	PVNA	Japan	+	+
42	Akagaki	PVNA	Japan	+	+
43	Tenrubou	PVNA	Japan	+	+
44	Toyoga	PVNA	Japan	+	+
45	Niitsugaki	PVNA	Japan	+	+
46	Mizushima	PVNA	Japan	+	+
47	Shougatsu	PVNA	Japan	+	+
48	Baojiaoz	PVNA	Japan	+	+
49	Sae-Fuji	PVNA	Japan	+	+
50	Kaki Tipo	PVNA	Italy	+	—
51	Wasesaijo	PCA	Japan	—	+
52	Goseung-Tabaegam	PCA	Japan	—	+
53	O-miyawase	PCA	Japan	+	+
54	Sancheong-Danseongsi	PCA	Korea	+	+
55	Sancheong-Kojongsi	PCA	Korea	+	+
56	Sancheong-Kodongsi	PCA	Korea	+	+
57	Yeongju-Kodongsi	PCA	Korea	+	+
58	Saijo	PCA	Japan	+	+
59	Yeongdeong-Weolhasi	PCA	Korea	+	+
60	Yeuseung-Sagoksi	PCA	Korea	+	+
61	Myeongju-Kojongsi	PCA	Korea	+	+
62	Cheongdo-Bansi	PCA	Korea	+	+
63	Gyeongsan-Bansi	PCA	Korea	—	+
64	Haman-Mulgam	PCA	Korea	+	+
65	Haman-Bansi	PCA	Korea	+	+
66	Goesan-Durigam	PCA	Korea	+	+
67	Sangju-Dungsi	PCA	Korea	+	+
68	Eunpungjuns	PCA	Korea	+	+
69	Sureo	PCA	Korea	+	+
70	Mopanshi	PCA	China	+	+
71	Black persimmon	PCA	China	+	+
72	Tonewase	PVA	Japan	+	+
73	Sugitawase	PVA	Japan	+	+
74	Superhiratanenashi	PVA	Japan	+	+
75	O-tanenashi	PVA	Japan	+	+
76	Hiratanenashi	PVA	Japan	—	+
77	Damopan	PVA	Japan	+	+
78	Wasezizya	PVA	Japan	—	+
79	Atago	PVA	Japan	+	+
80	Hachiya	PVA	Japan	+	+
81	Yaoki	PVA	Japan	+	+
82	Rojo Brillante	PVA	Spain	+	+
Breeding lines					
83	Jamisi	PVNA	Korea	+	+
84	Migamjosaeng	PVNA	Korea	+	+

²⁾PCNA: pollination-constant non-astringent, PVNA: pollination variant non-astringent, PCA: pollination-constant astringent, PVA: pollination variant astringent.

³⁾SCAR: sequence characterized amplified region. SCARs were developed by Kanzaki *et al.* (2010). +/— indicate presence/absence of SCAR polymerase chain reaction products, specific for the *AST* locus, respectively.

progeny plants derived from diverse crosses performed 2007 to 2012 between PCNA and non-PCNA cultivars at the breeding field of the Sweet Persimmon Research Institute (SPRI, Gimhae, Korea) (Table 2). Between 2009

and 2012, young leaf tissue was collected for DNA extraction and SCAR marker genotyping when two to three true leaves were fully expanded after foliation stage (between the end of April to May prior to flowering).

Table 2. Results of marker-assisted selection for pollination constant non-astringent (PCNA) persimmon plants (*Diospyros kaki* Thunb.) from various cross combinations using AST-F/PCNA-F/5R3R marker specific for the *ast* allele.

Test year	Cross combination	Total	No. selected PCNA candidates
2010	Youhou × Taishu	3	2
	Hachiya × Taishu	18	6
	Maegawajiro × Taishu	13	12
	Sunami × Taishu	7	6
	Jamisi × Taishu	8	4
	Uenishiwase × Tiashu	3	0
	Jamisi × Sae-Fuji	7	0
	Sae-Fuji × Taishu	10	0
	Youhou × Sae-Fuji	1	0
2011	Izu × 95 (line)	33	4
	Maegawajiro × Taish	39	27
	Youhou × Taish	31	8
	Fuyu × Taishu	3	3
	Sunami × Taishu	27	8
	Migamjosaeng × Taishu	58	4
	Jamisi × Taishu	23	2
	Uenishiwase × Taishu	12	9
	Uenishiwase × Kinshu	5	3
	Migamjosaeng × 3-5 (line)	27	0
	Jamisi × 3-5 (line)	70	0
	Migamjosaeng × Sae-Fuji	84	0
	Migamjosaeng × Kinshu	4	0
	Migamjosaeng × Taishu	6	1
	Uenishiwase × 3-5 (line)	19	0
	Hachiya × Taishu	9	0
	Jamisi × Sae-Fuji	9	0
	Taishu × Sae-Fuji	13	0
	Kinshu × Taishu	1	0
	Youhou × Sae-Fuji	1	1
	3-5 (line) × Taishu	6	0
	Cheongdo-Bansi × Taishu	17	0
	Gillya × 96 (line)	1	0
2012	Uenishiwase × 3-5 (line)	2	1
	Jamisi × Taishu	2	1
	Migamjosaeng × Taishu A	5	1
	Fuyu × Taishu	2	2
	Maegawajiro × Taishu	2	2
	Migamjosaeng × Sae-Fuji	2	0
	Nishimurawase × 3-5 (line)	1	0
	Dongwon3 × 60	17	0
	Uenishiwase × Kinshu	1	0
	Migamjosaeng × Taishu	1	0
	Jamisi × 3-5 (line)	5	0
	Hachiya × Taishu	1	0
Total		609	107

Collected leaf samples were stored at -80°C until further use, and ground under liquid nitrogen before DNA extraction. Genomic DNA were extracted from 1 g ground tissue using a Plant Genomic DNA isolation kit (Core-Bio, Seoul, Korea), and quantified using a spectrophotometer (UV-2450; Shimadzu, Tokyo, Japan or Pico200; Picodrop, Hinxton, England). DNA samples used for polymerase chain reaction (PCR) templates were diluted to 20 ng/ μl .

SCAR marker genotyping

SCAR markers developed by Kanzaki *et al.* (2010) (Table 3) were evaluated for their association with the PCNA type. PCRs of 20 μl total volume were prepared by mixing 10 pmole forward and reverse primer (Table 3) with 4 μl AccuPower PCR PreMix (Bioneer, Daejeon, Korea) containing 0.2 mM dNTPs, 1 U *Taq* DNA polymerase, and 2 μl 10 $\times$ reaction buffer. PCR was conducted using a PCR cyclor (Biometra, Gottingen, Germany) with the following conditions: for markers E4/E9r and E4/A2r, 30 seconds at 94°C , 30 cycles of 1 minute at 94°C , 1 minute at 59°C , and 4 minutes (E4/E9r) or 2 minutes (E4/A2r) at 72°C , and a final 7 minutes (E4/E9r) or 4 minutes (E4/A2r) extension at 72°C ; for markers 7H9F/AST-R and AST-F/AST-R, 30 seconds at 96°C , 30 cycles of 10 seconds at 96°C , 30 seconds at 57°C (7H9F/AST-R) or 55°C (AST-F/AST-R), and 30 seconds at 72°C , and a final 1 minutes extension at 72°C ; for marker PCNA-F/AST-F/5R3R, 30 seconds at 96°C , 35 cycles of 10 seconds at 96°C , 30 seconds at 53°C ,

and 30 seconds at 72°C , and a final 7 minutes extension at 72°C .

PCR products were separated by electrophoresis on a 2% agarose gel (Bioshop Biotechnology, Burlington, ON, Canada) with the addition of RedSafeTM (Intron, Seongnam, Korea) and using 1 $\times$ Tris-borate-EDTA buffer. Gel images visualized using a Gel Documentation System (Bio-Rad, California, CA, USA).

RESULTS

Evaluation of the SCAR markers using F1 cultivars

Five SCAR markers using different primer combinations (Table 3) were evaluated for two F1 cultivars ‘Jamisi’ (PVNA) and ‘Migamjosaeng’ (PVNA) that were developed from a cross performed between ‘Matsumotowase-Fuyu’ (PCNA) and ‘Nishimurawase’ (PVNA) and F1 progeny plants isolated from a cross between ‘Jamisi’ and ‘Taishu’ (PCNA) (Fig. 1). For E4/E9r, a 4.6-kb band specific for the *ast* allele (PCNA) and a 2.2-kb band specific for the *Ast* allele (non-PCNA) were PCR amplified from ‘Matsumotowase-Fuyu’ and ‘Nishimurawase’, respectively, while both PCR products representing a heterozygous *Ast/ast* genotype were amplified from ‘Jamisi’ and ‘Migamjosaeng’. For F1 progeny plants resulting from a cross performed between ‘Jamisi’ and ‘Taishu’, the E4/E9r PCR product specific for the *ast* allele

Table 3. Polymerase chain reaction primer information for sequence characterized amplified region markers used in this study for selecting pollination-constant non-astringent (PCNA) persimmon plants (*Diospyros kaki* Thunb.).

Marker name ²⁾	Sequence (5'→3')	Temperature ($^{\circ}\text{C}$)	Product size (bp)
E4/E9r	E: CCCACTTACCAAAGTAGGCTTCCAACACAAG G	66.7	2,200
	E9r: GCTTAGTCAGCTTAGCCACGCCATTTC	64.8	
E4/A2r	E4: CCCACTTACCAAAGTAGGCTTCCAACACAAG	66.7	2,000
	A2r: CTTGTAGTGAATTAACGGATATGGTG	55	
7H9F/AST-R	7H9F: CAATCACTCATCTCACTTGAC	51.9	410
	AST-R: CCCCTCATGCTTTGCATACTTAATG	59.6	
AST-F/AST-R	AST-F: GTTGCATCGCATAGCGGGTTTGAGG	67.7	190
	AST-R: CCCCTCATGCTTTGCATACTTAATG	59.6	
AST-F/PCNA-F/5R3R	PCNA-F: CCCCTCAGTGGCAGTGCTGC	62.4	350
	AST-F: GTTGCATCGCATAGCGGGTTTGAGG	67.7	220
	5R3R: GAAACACTCATCCGGAGACTTC	54	

²⁾Markers were developed by Kanzaki *et al.* (2010).

was amplified from two plants, but PCR products specific for both *ast* and *Ast* alleles were amplified from another six plants (Fig. 1A). Meanwhile, no E4/A2r PCR product was amplified from these plants (Fig. 1B).

7H9F/AST-R and AST-F/AST-R amplified 410-bp and 190-bp PCR products, respectively, from non-PCNA plants only. No PCR product was amplified from PCNA cultivars and PCR products specific for the *Ast* allele were amplified from both ‘Jamisi’ and ‘Migamjosaeng’ (Fig. 1C, D). AST-F/PCNA-F/5R3R produced a 350-bp PCR product

specific for the *ast* allele from ‘Matsumotowase-Fuyu’ (PCNA) and a 220-bp product specific for the *Ast* allele from ‘Nishimurawase’ (PVNA), while both PCR products representing a heterozygous *Ast/ast* genotype were amplified from eight F1 progeny resulting from the cross between ‘Jamisi’ and ‘Taishu’ (Fig. 1E). These SCAR marker genotyping results indicated that, aside from E4/A2r, all markers were associated with specific phenotypes for ‘Matsumotowase-Fuyu’ and ‘Nishimurawase’, and their F1 cultivars ‘Jamisi’ and ‘Migamjosaeng’. In addition, the

Fig. 1. Agarose gel image showing segregation of E4/E9r (A) and E4/A2r (B) marker in 10 offspring of ‘Matsumotowase-Fuyu’×‘Nishimurawase’, 7H9F/AST-R (C) and AST-F/AST-R (D) in the progeny from ‘Matsumotowase-Fuyu’×‘Nishimurawase’, and multiplex polymerase chain reaction (PCR) (E) by the primer pair AST-F/PCNA-F/5R3R, amplified fragments using primer pair.

M: Kb ladder size marker, N: 100-bp ladder size marker, 1: ‘Matsumotowase-Fuyu’, 2: ‘Nishimurawase’, 3: ‘Jamisi’, 4: ‘Migamjosaeng’, 5-12: progenies from ‘Jamisi’×‘Taishu’. For E4/A2r (B), no PCR band was amplified from several cultivars and their offspring.

Table 4. Pattern of polymerase chain reaction product formation of sequence characterized amplified region (SCAR) markers for selecting PCNA persimmon plants (*Diospyros kaki* Thunb.) observed from F₁ seedlings derived from different cross combinations.

Marker	Cross combination ²⁾							
	J×T	J×S	H×T	Y×T	Y×S	C×T	I×T	Su×T
E4/E9r	+ ^{y)}	+	+	+	+	+	+	+
E4/A2r	—	—	—	—	—	—	—	—
7H9F/AST-R	+	+	+	+	+	+	+	+
AST-F/AST-R	+	+	+	+	+	+	+	+
AST-F/PCNA-F/5R3R	+	+	+	+	+	+	+	+

²⁾J: ‘Jamishi’ (PVNA), T: ‘Taishu’ (PCNA), S: ‘Sae-Fuji’ (PVNA), H: ‘Hachiya’ (PVNA), Y: ‘Youhou’ (PCNA), C: ‘Cheongdo-Bansi’ (PCA), I: ‘Ichikikeijiro’ (PCNA), Su: ‘Sunami’ (PCNA).

^{y)}+ and — indicate presence and absence of the SCAR bands, respectively.

PVNA: pollination variant non-astringent, PCNA: pollination-constant non-astringent, PCA: pollination-constant astringent.

use of the E4/E9r SCAR marker enabled two out of eight progeny plants resulting from a cross between ‘Jamisi’ and ‘Taishu’ to be classified as PCNA, thereby demonstrating the underlying MAS principle.

Evaluation of the SCAR marker using cross populations

F1 progeny populations derived from eight crosses between ‘Jamisi’ (PVNA) and ‘Taishu’ (PCNA) were analyzed using the SCAR markers. E4/E9r PCR products were amplified from plants in all F1 populations, but no E4/A2r PCR products were observed (Table 4). 7H9F/AST-R, AST-F/AST-R, and AST-F/PCNA-F/5R3R PCR products were also amplified from plants in the F1 populations, but there were certain instances where resulting AST-F/AST-R PCR products did not match plant phenotype. Furthermore, SCAR marker phenotype association was confirmed for 7H9F/AST-R and AST-F/PCNA-F/5R3R when these were applied to F1 populations, but inconsistent genotyping results were observed for these two markers (data not shown). Therefore, E4/E9r and AST-F/PCNA-F/5R3R were considered as appropriate markers for use in PCNA MAS.

Evaluation of SCARs using PCNA and non-PCNA progeny

Non-PCNA progeny plants derived from a cross between ‘Jamisi’ (PVNA) and ‘Sae-Fuji’ (PVNA) and PCNA progeny plants from a cross between ‘Maegawajiro’ (PCNA) and ‘Taishu’ (PCNA) were evaluated using three markers, 7H9F/AST-R, AST-F/AST-R, and AST-F/PCNA-F/5R3R, to select the minimum primer combination for selecting PCNA at seedling stage. AST-F/AST-R and 7H9F/AST-R amplified PCR bands (190 bp and 410 bp) specific to non-PCNA (*Ast* allele) only from the non-PCNA progeny. These markers did not amplify any specific band from the PCNA progeny, although there was one plant that showed a 410-bp band specific to non-PCNA by 7H9R/AST-R (Fig. 2A, B). The third marker AST-F/PCNA-F/5R3R amplified two PCR bands in a heterozygous state from all non-PCNA progeny, but amplified a PCNA-specific (*ast* allele) 350-bp band from all PCNA progeny except for one plant (Fig. 2C).

Fig. 2. Agarose gel image showing the segregation of the sequence characterized amplified region (SCAR) markers in breeding population of non-pollination-constant non-astringent (PCNA)-type offspring (I) and PCNA-type offspring (II). For AST-F/AST-R (A) and 7H9R/AST-R (B), the *AST*-specific 190-bp and 410-bp fragment was present in all non-PCNA-type offspring, but absent in PCNA-type offspring, respectively. For a multiplex PCR by the primer pair AST-F/PCNA-F/5R3R (C), all PCNA offspring showed the *ast*-specific 350-bp fragment while non-PCNA-type offspring showed the *AST*-specific 220-bp fragment. N: 100-bp marker.

Evaluation of SCARs using germplasm accessions

E4/E9r and AST-F/PCNA-F/5R3R markers were also evaluated for 82 cultivars and two breeding lines for which the astringency trait has been previously characterized. These 84 accessions included 31 PCNA, 21 PVNA, 11 PVA, 21 PCA types, and depending on the origin, two Chinese, one Italian, one Spanish, 32 Korean, and 59 Japanese accessions (Table 1). From the genotyping results of the two markers, we observed that there was no association of markers with phenotype for six accessions: ‘Wasesaijo’, ‘Goseung-Tabegam’, ‘Gyeongsan-Bansi’, ‘Hiratanenashi’, and ‘Waseziya’ for E4/E9R and ‘Kaki Tipu’ for AST-F/PCNA-F/5R3R. For the Korean PCA heirloom cultivars ‘Goseung-Tabegam’ and ‘Gyeongsan-Bansi’, the expected 2,200-bp PCR product specific for the *Ast* allele was not observed for E4/E9r, which implies that this marker possibly cannot be used for Korean PCA cultivars. Overall, the phenotype match rates of E4/E9r and AST-F/PCNA-F/5R3R markers were 94% and 99%, respectively, indicating that AST-F/PCNA-F/5R3R is a more appropriate marker for selecting PCNA.

Evaluation of marker applicability for MAS

AST-F/PCNA-F/5R3R was integrated into a MAS program performed at the SPRI using segregating breeding populations. These populations comprised of a total of 609 progeny plants that were isolated from 2009 to 2012 from diverse crosses, including that between ‘Youhou’ (PCNA) and ‘Taishu’ (PCNA). As a result of MAS, 107 PCNA candidate plants (17.6%) that displayed the marker result specific to the *ast* allele were isolated (Table 2). In an assessment of marker association with astringency phenotype, fruits from only 154 out of 609 plants could be evaluated, as the remaining plants did not survive, were too young to bear fruits, or displayed deformed or premature small fruits. Our results revealed that of the 154 analyzed plants, 33 PCA, 13 PCNA, 106 PVNA, and two PVA types were present, and marker-trait association was confirmed for all except three plants.

DISCUSSION

In the present study, we used five SCAR markers associated with astringency in persimmon fruit, namely E4/E9r, E4/A2r, 7H9F/AST-R, AST-F/AST-R, and AST-F/PCNA-F/5R3R, to improve the efficiency of PCNA-type persimmon breeding via MAS. To select the minimum primer combination for selecting PCNA at seedling stage, E4/E9r and AST-F/PCNA-F/5R3R were considered as appropriate markers for use in PCNA MAS. But Mitani *et al.* (2014) assessed use of SCAR markers E4/A2r and 7H9F/AST-R for selecting astringency type in segregating populations derived from crossing ‘Taiten’ with PCNA cultivars. However, no E4/A2r PCR products specific for the *ast* allele were observed despite using identical PCR conditions as previously described (Mitani *et al.* 2014). In addition, it is noteworthy that these markers can produce PCR products from plants in a segregating population derived from crossing with a Korean cultivar ‘Cheongdo-Bansi’ (Table 1), even though the markers originated from Japanese cultivars. This indicates that markers can be used in different ways for breeding programs that use Korean germplasm.

In conclusion, we confirmed that AST-F/PCNA-F/5R3R is the optimal SCAR marker for differentiating PCNA from non-PCNA germplasm accessions. Furthermore, our evaluation of progeny plants resulting from diverse crosses indicated that this marker can be used for MAS in sweet persimmon breeding programs.

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