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Development of SCAR Marker for Detection of Sex Expression in Papaya (*Carica papaya L.*) from Several Genetic Backgrounds

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ABSTRACT

Papaya plants are hermaphrodite, pistillate, or staminate. Sex inheritance in papaya is determined by a single gene locus with three alleles of M which is dominant for maleness, MH for hermaphrodites and m which is recessive for femaleness. Only fruits from hermaphrodite plants are marketed since they have the necessary commercial characteristics, i.e., they are pear-shaped and have thicker flesh and a smaller internal cavity. Increased papaya yield has been limited mainly by the ratio of female to hermaphrodite (1: 2) plants normally occurring in orchards. This ratio causes great losses to papaya producers. Identification of seedlings sex during nursery stage is of prime iportance. In order to obtain simple DNA markers to identify sex expression in papaya, five SCAR markers of 20-21 primers were utilized. Examination of these markers into 24 genotypes of papaya from 12 populations of different genetic background revealed that pair of primer PKBT-5 had successfully differentiated male and hermaphrodite plants from female plants. Hence, PKBT-5 pair of primer can be utilized as DNA marker for sex expression character identification in papayas.

Key words: Papaya, sex expression, SCAR marker

INTRODUCTION

Papaya (*Carica papaya L.*) is a polygamous species with three sex types: male, hermaphrodite and female. In most cases, hermaphrodite plants are preferred for commercial use. Since the use of seeds produce seedlings of unknown sex expression, producers have to plant seedlings in groups of three and thin out the female and male plants after 3 to 4 months when it is possible to identify the sex of the seedlings from their floral buds (Lemos *et al*., 2002). Storey (1953) hypothesized that three alleles, M, H, and f, at a single locus Sex1, determine papaya sex. The alleles M and H were assumed to be dominant over the f allele. Thus, the male, hermaphrodite, and female sexes are determined by the Sex1 locus genotypes, Mf, Hf and ff, respectively. Homozygotes of dominant alleles (MM and HH) as well as a heterozygote (MH) were assumed to be lethal. Recently, Sondur *et al.* (1996) suggested a model of sex type based on alternate alleles of a gene encoding a trans-acting factor. They proposed that the male allele of the sex gene, designated as SEX1-M, encodes a trans-acting factor that induces male floral parts while inhibiting carpel development. The hermaphrodite allele, SEX1-H, is intermediate having the ability to induce male structures but only reducing carpel size. The female allele, sex1-f, is incapable of inducing male structures.

Burgeoning development of molecular genetics have revealed several molecular markers linked to the sex of dioecious plants. For example, AFLP (amplified fragment length polymorphism) markers were developed for asparagus (Reamon-Büttner and Jung, 2000), and RAPD markers for *Pistacia vera* (Hormaza *et al*., 1994), and asparagus (Jiang and Sink, 1997). In papaya, RAPD and microsatellite markers linked to sex have been reported (Sondur *et al*., 1996; Parasnis *et al*. 1999; Lemos *et al*., 2002; Urasaki *et al*. 2002), and their conversion to SCAR (sequence characterized amplified region (Urasaki *et al.*, 2002; Deputy *et al.*, 2002).

In this paper, we report an evaluation of several published SCAR markers (Urasaki *et al.*, 2002; Deputy *et al.*, 2002) and new developed SCAR marker from published RAPD sequence (Lemos *et al.*, 2002; Urasaki *et al.*, 2002)) specific to male and hermaphrodite plants into several genetic backgrounds of papaya to elucidate their consistency. Utilization of SCAR allowed rapid and reliable identification of target trait.

MATERIALS AND METHOD

Plant Materials

Plant materials consisted of 12 genetic backgrounds of papaya collections of Center for Tropical Fruit Studies (CETROFS), i.e., California (P-1), Exotica (P-2), Hawaii Solo (P-3), KD-Red (P-4), Ponti (P-5), Red King (P-6), Red King x SW Yellow (P-7), Hawaii Solo (P-8), SW Yellow (P-9), SW-Red (P-10), TW-Red

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(P-11), and Ungu (P-12). Individual plant with different sex expression was selected from each genetic background. List of the plant samples presented in Table 1.

Table 1. List of plant samples and their sex expression subjected to SCAR analysis.

No	Genotype Code	Sex Expression	Genetics background	Origin
1	M1	Male	Exotica (P-2)	Malaysia
2	M2	Male	Exotica (P-2)	Malaysia
3	M3	Male	Ungu (P-12)	Bogor (West Java)
4	M4	Male	SW Yellow (P-9)	Thailand
5	M5	Male	Red King x SW Yellow (P-7)	Crossing
6	M6	Male	Red King (P-6)	Taiwan
7	F1	Female	Exotica (P-2)	Malaysia
8	F2	Female	Red King (P-6)	Taiwan
9	F3	Female	California (P-1)	Bogor (West Java)
10	F4	Female	Hawaii Solo (P-3)	Taiwan
11	F5	Female	Ungu (P-12)	Bogor (West Java)
12	F6	Female	Ponti (P-5)	Pontianak (West Kalimantan)
13	F7	Female	KD-Red (P-4)	Thailand
14	F8	Female	SW-Red (P-10)	Thailand
15	H1	Hermaphrodite	Ungu (P-12)	Bogor (West Java)
16	H2	Hermaphrodite	KD-Red (P-4)	Thailand
17	H3	Hermaphrodite	TW-Red (P-11)	Thailand
18	H4	Hermaphrodite	SW-Red (P-10)	Thailand
19	H5	Hermaphrodite	Ponti (P-5)	Pontianak (West Kalimantan)
20	H6	Hermaphrodite	Red King (P-6)	Taiwan
21	H7	Hermaphrodite	Hawaii Solo (P-3)	Taiwan
22	H8	Hermaphrodite	California (P-1)	Bogor (West Java)
23	H9	Hermaphrodite	Ungu (P-12)	Bogor (West Java)
24	H10	Hermaphrodite	Exotica (P-2)	Malaysia

DNA Isolations

Total DNA from each genotype was isolated using CTAB method (Doyle and Doyle, 1987) with slight modification. Three hundred mg of leaf tissue was frozen in liquid nitrogen and ground into fine powder using mortar and pestle. The pulverized materials were transferred to a microtube and 600 µl of extraction buffer [100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1400 mM NaCl, 2% (w/v) cetyl trimethylammonium bromide (CTAB), and 0.2% (v/v) mercaptoethanol] solution was added. The tubes were vortexed for a few seconds and incubated at 60 ^{0}C for 20 minutes. Following incubation, 570 µl of chloroform: isoamylalcohol (24:1) (v/v) was added and the mixture was shaken vigorously. The extracts were centrifuged for 15 min at 12,000 rpm. The supernatant was transferred to a fresh microtube and then 600 µl of cold isopropanol was added. DNA fibers became visible upon gentle swirling. The DNA was transferred to a fresh tube, rinsed with 70% ethanol and dissolved into 50-100 µl of sterilized deionized water. DNA concentration was measured with UV-spectrophotometer (Shimadzu Corporation, Japan) at wavelengths of 260 and 280 nm.

SCAR Analysis

SCAR analysis was conducted by utilizing published SCAR markers T12, W11 (Deputy *et al.*, 2002), and SPD (Urasaki *et al.*, 2002), and new SCAR markers of PKBT-4 and PKBT-5, which developed from published RAPD fragment sequences of Lemos *et al.* (2002) and Urasaki *et al.* (2002) with criteria of GC ratio $\geq$ 0.5, and annealing temperature equality (Table 2). PCR reaction was conducted in final volume mixture of 25 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 ng of genomic DNA, 10 pM of each primer, 2.5 mM of each dNTPs, and 1 U of Taq polymerase (Perkin-Elmer). In a Perkin Elmer Cetus Thermal Cycler 8700, the reaction mixture was pre-incubated at 94^{0}C for 2 minutes and then PCR was repeated for 30 cycles of 94^{0}C for 30 seconds, 58^{0}C for 30 seconds, 72^{0}C for 1 minutes, followed by final extension at 72^{0}C for 7 minutes.

Table 2. List of SCAR primer pair and their sequences

No	Name	Sequence (5'→ 3')	Source
1	T12-F	GGGTGTGTAGGCACTCTCCTT	Deputy*et al.,* 2002
	T12-R	GGGTGTGTAGCATGCATGATA	
2	W11-F	CTGATGCGTGTGTGGCTCTA	Deputy*et al.,* 2002
	W11-R	CTGATGCGTGATCATCTACT	
3	SDP-1	GCAGGATTTAGATTAGATGT	Urasaki*et al.,* 2002
	SDP-2	GGATAGCTTGCCCAGGTCAC	
4	PKBT4-F	GAGGGCGAGGTTTGAATTTGG	Developed from RAPD sequence of
	PKBT4-R	TTTGGTGTCCTGGTTACCCTC	Lemos*et al.,* 2002
5	PKBT5-F	AGCCAGGGTCGTGGTAAGAG	Developed from RAPD sequence Urasaki
	PKBT5-R	TCCCATGGCGTGTCGCGCTG	*et al.,* 2002

RESULTS AND DISCUSSIONS

Screening five pair of primers were performed by PCR reaction of those primers on three DNA samples of M1 (male), F1 (female), and H10 (hermaphrodite). Pair of primer W-11 and SDP failed to amplify DNA genome of those samples. This results indicated that those two primer pairs did not have complementary sequences in genomic DNA of M1, F1, and H10, therefore they were not selected to amplify remaining samples. Primer T12 amplified clear band in sample M1, and smear band was found in sample F1 and H10, at the same size in Deputy*et al.,* (2002) population of 850 bp. Pair of primer PKBT-4 amplified 350 bp fragment in sample M1, and no band was detected in sample F1 and H10, the obtained fragment size was smaller than the original fragment of 438 bp (Lemos*et al.,* 2002). Pair of primer PKBT-5 has successfully amplified 320 bp fragment in sample M1 and H10, but no band has been detected in sample F1. The obtained fragment size smaller than original fragment of 450 bp (Urasaki*et al*. 2002). These results indicated that PKBT-4 and PKBT-5 successfully differentiated male and hermaphrodite phenotypes to the female one.

Table 3. SCAR marker analysis of three pairs of primer. Presence of amplified band denoted in (1) and absence denoted in (0).

No	Genotype Code	T12-850 bp	PKBT-4 (350 bp)	PKBT-5 (320 bp)
1	M1	1	1	1
2	M2	0	0	1
3	M3	0	0	1
4	M4	0	0	1
5	M5	1	1	1
6	M6	0	0	1
7	F1	1	0	0
8	F2	1	1	0
9	F3	0	0	0
10	F4	1	0	0
11	F5	0	0	0
12	F6	1	0	0
13	F7	1	0	0
14	F8	0	0	0
15	H1	0	0	1
16	H2	1	0	1
17	H3	0	0	1
18	H4	1	0	1
19	H5	1	1	1
20	H6	1	0	1
21	H7	1	0	1
22	H8	1	0	1
23	H9	1	0	1
24	H10	1	0	1

Further analysis was conducted by utilization of three pairs of primer of T-12, PKBT-4, and PKBT-5. Pair of primer T-12 amplified band in two genotypes (M1, M5) out of six male genotypes, seven genotypes (H2, H4, H5, H6, H7, H8, H9, and H10) out nine hermaphrodite genotypes, on the contrary, T-12 primer pair amplified band in female genotype of F1, F2, F4, F6, and F7 (Table 3), these results were not consistent to the results of Deputy et al. (2002). Subsequently pair of primer PKBT-4 amplified band only in two male genotypes (M1, M5), and one hermaphrodite H5, however, also in female genotype F2, and H5 (Table 3), this results were not consistent to the results of Lemos *et al.,* (2002). These results indicated that both pair of primers failed to specify sex expression on papaya consistently.

Primer pairs of PKBT-5 amplified band in all genotypes expressed male flowers and hermaphrodite flowers, but no fragment was amplified in genomic DNA of female phenotype (Figure 1). These results were consistent to the results of Urasaki (2002), indicated that the PKBT-5 pair of primer could be utilized to mark male and hermaphrodite plant of papaya from different genetic background.

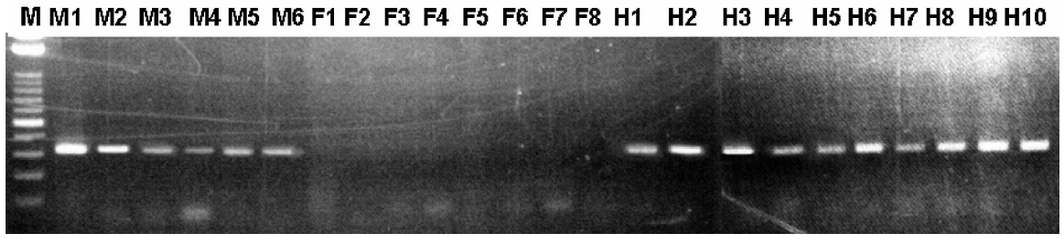

Figure 1. Electrophoresis pattern of PKBT-5 SCAR markers to 24 genotypes of papaya. M is 100 bp DNA ladder (M1-M6, F1-F8 and H1-H10 respectively are male, female and hermaphrodite plants, respectively, regarding to Table 1).

Papaya as polygamous species tends to form heterozygous population which implies genotypes in a population do not have the same genetics properties as revealed by T11 and PKBT-4 in M1 and M2 from Exotica population and by T11 in H1 and H9 from Ungu population. Furthermore, DNA markers linked to sex expression in one population were not consistently successful in determining the sex expression in other populations. It indicated that these markers were located at different site of the chromosome where the MH gene resided. Therefore, these markers did have not have same genetic distance to the target gene, since they were constructed from different population (Lemos *et al.,* 2002; Urasaki *et al.,* 2002; and Deputy *et al.,* 2002). Since pair of primer, PKBT-5 consistently marked sex expression of different populations. It was possible that the marker was located in a conserved region tightly linked to the sex expression gene.

The effectiveness of molecular marker utilization depends on the linkage level of the marker to the target character, and should be reproducible at different populations (Paterson *et al.,* 1991). Primer pairs of T-12, W-11, SDP, and PKBT-4 were able to differentiate sex expression in previous evaluations (Lemos *et al.,* 2002; Urasaki *et al.,* 2002; and Deputy *et al.,* 2002). Nevertheless, in our study, these primers failed to produce the same result in male and hermaphrodite plant indicating that they were not effective in identifying the sex expression of papaya from different genetic background. The consistency of PKBT-5 to in differentiating male and hermaphrodite plant from several different genetic backgrounds indicated that the PKBT-5 were the potential marker for male and hermaphrodite plant of papaya.

CONCLUSSIONS

Examination of five pairs of SCAR markers T-12, W-11, SDP, PKBT-4, and PKBT-5 in 24 genotypes of papaya from 12 populations with different genetic background revealed that pair of primer PKBT-5 had successfully differentiated male and hermaphrodite from female plant. Therefore, PKBT-5 pairs of primer can be utilized as DNA marker for sex expression identification in papaya in seedling stage.

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