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Biochemical properties of yellow and red pulped papaya and its validation by molecular markers

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ABSTRACT

In this study, the carotenoid profiles of yellow and red pulped papayas were analysed by ultra performance liquid chromatography (UPLC) and molecular markers linked to pulp colour were validated. The major carotenoids were the provitamin A carotenoid, β-cryptoxanthin ranging from 0.56 ± 0.12 mg 100 g^{-1} in fresh papaya pulp to 1.13 ± 0.02 mg 100 g^{-1}, β-carotene from 0.12 ± 0.04 mg 100 g^{-1} to 0.82 ± 0.1 mg 100 g^{-1} and α-carotene from 0.07 ± 0.01 mg 100 g^{-1} to 0.38 ± 0.08 mg 100 g^{-1}. The red-pulped papaya contained pro-vitamin A carotenoid along with a significant amount of lycopene (3.05 ± 0.23 mg 100 g^{-1}). Two red pulped genotypes Red Indian and Tainung-1 were found to contain high provitamin A levels and can be useful of breeding for this trait. Two Sequence Characterized Amplified Region (SCAR) markers developed for early identification of pulp colour were found successful in discriminating yellow and red pulp coloured genotypes. These markers can be used in papaya breeding programme where pulp colour is a desired trait.

Key words: UPLC, SCAR markers, pulp colour, papaya, carotene.

INTRODUCTION

Carica papaya L. is a native of Latin America and the most important member of the family Caricaceae. It is widely grown in the tropical and sub-tropical regions of the world. It bears nutritious fruits rich in several minerals and vitamins. It has been estimated that consumption of 200 g of fruit can exceed the adult dietary reference intake (DRI) for vitamin C and provide 10% of dietary requirement of vitamin A (Wall, 14). Papayas have been shown to rank fourth in total carotenoid content and vitamin A content among 38 fruits (Sancho *et al*., 9). Based on pulp colour, papayas are divided into two major types- red and yellow flesh. Red flesh colour is due to accumulation of lycopene, β-cryptoxanthin, β-carotene and ζ-carotene. On the other hand in yellow papayas, β-cryptoxanthin and β-carotene are the major carotenoids, while lycopene gets converted to β-carotene (Yamamoto, 15). The bio-availability of these carotenoids is higher from papayas than that from carrots and tomatoes as reported by Scheweiggert *et al*. (10). However, the nutritional composition of papaya fruit has been shown to vary depending on factors like genotype, time of maturity and ripening. Carotenoids like lycopene have also been shown to range from 0.36 to 3.40 mg 100 g^{-1} DW, β-cryptoxanthin from 0.28 to 1.06 mg 100 g^{-1} DW and β-carotene from 0.23 to 0.50 mg 100 g^{-1} DW (Sancho *et al*., 9).

Genetic studies have shown that flesh colour is determined by a single gene and yellow flesh colour is dominant over red (Storey, 13). Fruit pulp colour has also been linked to shelf-life and red coloured papayas are known to have faster ripening thereby shorter shelf-life (Skelton *et al*., 12). Recently, two alleles linked to dominant yellow pulp and recessive red pulp have been fully sequenced (Blas *et al*., 2). This has lead to identification of chromoplast specific pulp colour gene CpCYC-b (*Carica papaya* chromoplast specific lycopene β-cyclase), which encodes for the enzyme converting lycopene into β-carotene resulting in the observed colour difference between yellow (where gene is functional) and red papayas (non-functional gene). Three SCAR markers CPFC1, CPFC2 and CPFC3 tightly linked to this gene have been developed to facilitate easy identification of yellow and red pulped plants in segregating population at the seedling stage (Blas *et al*., 2).

The present study was designed to generate information about the nutritional, biochemical and molecular profile of important papaya genotypes grown in India. The aim was to evaluate the carotenoid profile in yellow and red pulped papayas and to validate the flesh linked markers for application in marker-assisted selection for pulp colour in papaya breeding work.

MATERIALS AND METHODS

Mature papaya fruits of eight genotypes, 'Line 21', 'Lucknow' with yellow pulp, 'Red Indian', 'Thailand', 'Dwarf Lily', 'Pusa Giant', 'Tainung 1' and 'Tainung 5' with red pulp having colour index 2 (green with trace of yellow) were harvested from papaya fields maintained at the ICAR-IIHR, Bengaluru. The selected fruits were

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free from injuries and insect or pathogen attack. The fruits were left at ambient conditions (25 ± 3°C, 70-80% RH) to ripen for a period of eight days. The experiments were conducted in a completely randomized design with three replications. In each replicate, three fruit were randomly selected for analysis.

The extraction and saponification process were followed as described earlier by Azevedo and Amaya (1). Five gram of papaya pulp from each genotype was added with 25 ml of pre-chilled acetone and left overnight under dark condition. Tissue was homogenized in mortar and pestle and extracted repeatedly till the residue turned colourless. Acetone extract was transferred to a separating funnel for further liquid-liquid extraction with hexane. Hexane fraction containing carotenoids was saponified with 10% potassium hydroxide in alcohol in the dark at room temperature. After completely removing the alkali with distilled water, the pigments were dried in a rotary evaporator at a temperature of 35°C. The residual carotenoids were dissolved in the mobile phase and filtered through 0.2 µm nylon filter prior to injection into UPLC for further analysis.

The Acquity-UPLC system (Milford, MA, USA) consisting of quaternary pump, auto sampler injector, PDA detector equipped with Acquity-UPLC BEH-C18, 1.7 µm, 2.1 × 50 mm column and guarded by Acquity-UPLC BEH-C18, 1.7 µm, 2.1 × 5 mm guard column from Waters (Ireland) was engaged for the profiling of carotenoids. The instrument was controlled and the data was processed by using Mass lynx software. The mobile phase consisted of phase-A acetonitrile:methanol:ethyl acetate (53:7:40) and phase-B methanol, isocratic flow rate of 0.2 ml/ min was used in the ratio of A:B (95:5) for 6 min. with PDA scanning from 200 to 650 nm. The individual carotenoids were identified on the basis of diode array spectral characteristics, retention times (Table 1) and relative elution order compared to standards and literature values. Individual carotenoids were quantified as β-carotene equivalents. The statistical analysis ANOVA was performed by using AGRES software Version 3.01 (Pascal Intl. Software Solutions, USA) following completely randomized design and the mean comparison was done using LSD programmed for descending order and significance at 1 and 5% probability. Statistical analysis was done for comparison of varieties for different compounds.

Marker analysis was done using fresh leaf samples from newly matured leaves of ten yellow and red pulped papaya genotypes namely 'Co1', 'Shillong', 'AC119', 'Nigeria', 'Washington' for yellow flesh and 'CO3', 'Tainung 2', 'Tainung 5', 'Red Indian', 'Thailand' for red pulp were used for isolation of genomic DNA following CTAB method (Doyle and Doyle, 4). Three SCAR (sequence characterized amplified region) markers, *viz*., CPFC1, CPFC2 and CPFC3 developed by Blas *et al.* (1) were used for amplification (Table 2). The integrity and quality of isolated DNA was examined by agarose gel electrophoresis (0.8%). The DNA quantification was carried out using a GeneQuant UV spectrophotometer (GE Health Care Bio-Sciences Ltd., England) and diluted accordingly. Primer sequence and PCR amplification conditions were followed as mentioned in Blas *et al.* (2).

RESULTS AND DISCUSSION

The individual carotenoids were identified on the basis of diode array spectral characteristic, retention times and relative elution order compared to standards and literature values (Table 1). The mean values and standard deviation for major carotenoids found in red and yellow fleshed papayas are shown in Table 3. Analysis of variance detected significant differences ($P \leq 0.05$) in mean carotenoid values among the genotypes. Mean auroxanthin and luteoxanthin values ranged from undetectable level to 0.37 ± 0.09 mg 100 g^{-1} FW in 'Tainung 5' and 'Thailand', and 0.25 ± 0.05 to 1.18 ± 0.31 mg 100 g^{-1} FW in 'Line 21' and 'Tainung 5', respectively. β-cryptoxanthin values ranged from 0.56 ± 0.12 to 1.13 ± 0.02 mg 100 g^{-1} FW of papaya pulp. Lower values were observed in 'Dwarf Lily', 'Line 21', 'Pusa Giant' and 'Tainung 5', while higher values were

Table 1. Retention times of individual carotenoids for identification of compounds by comparing the available values in literature.

RT (min.)	Compound identified	λmax (nm)	λmax reported (nm)	Reference
0.09	Auroxanthin	408,434	381,402,427	Davies (2)
1.26	Luteoxanthin	400,422,450	400,422,450	Pott *et al.* (6)
1.46	β-cryptoxanthin	416,451,471	450,480 446,471	Davies (2)
1.80	Lycopene	446,472,503	446,474,504	Martinez *et al.* (5)
2.57	β-carotene	450	426,452,478	Pott *et al.* (6)
3.19	α-carotene	415,450,469	424,448,476	Rouseff *et al*. (8)

Table 2. Primer sequences of SCAR markers tightly linked to the pulp colour gene.

Marker	Forward (5' – 3')	Reverse (5' – 3')
CPFC1	GACGTGTTAGTGTCCGACAA	GACCAGGAAGCAAATTTTGTAA
CPFC2	GGACCACAGGAGCTGATTAG	TATCTCTGCCACATGCAACC
CPFC3	TGCAAAGAAATGGAGGGTTT	TGAAATCCTTCTGAGCCAAA

Table 3. Mean values of important carotenoids (mg 100 g^{-1}) in red and yellow pulped papaya genotypes.

Genotype	Auroxanthin	Luteoxanthin	β-cryptoxanthin	Lycopene	β-carotene	α-carotene
Line 21	0.16 ± 0.03^{c}	0.25 ± 0.05^{c}	0.68 ± 0.15^{c,d}	0.14 ± 0.03^{e}	0.29 ± 0.09^{d,e}	0.38 ± 0.08^{a}
Red Indian	0.28 ± 0.08^{a,b}	0.48 ± 0.11^{b,c}	0.89 ± 0.22^{a,b,c}	1.15 ± 0.16^{d}	0.82 ± 0.1^{a}	0.07 ± 0.01^{b}
Thailand	0.37 ± 0.09^{a}	0.69 ± 0.32^{b}	0.75 ± 0.18^{b,c,d}	0.95 ± 0.01^{d}	0.48 ± 0.12^{b,c}	0.15 ± 0.03^{b}
Dwarf Lily	0.15 ± 0.04^{c}	1.04 ± 0.22^{a}	0.56 ± 0.12^{d}	1.89 ± 0.46^{c}	0.3 ± 0.06^{d,e}	0.07 ± 0.01^{b}
Lucknow	0.21 ± 0.04^{b,c}	0.35 ± 0.07^{c}	0.98 ± 0.21^{a,b}	0.27 ± 0.06^{e}	0.59 ± 0^{b}	0.33 ± 0.07^{a}
Pusa Giant	0.17 ± 0.03^{c}	0.43 ± 0.02^{b,c}	0.77 ± 0.04^{b,c,d}	1.1 ± 0.02^{d}	0.21 ± 0.04^{e,f}	0.11 ± 0.02^{b}
Tainung 1	0.14 ± 0.03^{c}	0.35 ± 0.09^{c}	1.13 ± 0.02^{a}	2.36 ± 0.28^{b}	0.39 ± 0.08^{c,d}	0.34 ± 0.07^{a}
Tainung 5	ND	1.18 ± 0.31^{a}	0.69 ± 0.21^{b,c,d}	3.05 ± 0.23^{a}	0.12 ± 0.04^{f}	0.33 ± 0.09^{a}

The values of carotenoids are the mean ± SD of three independent measurements. The different small letters in the same column represent significant difference in descending order with 'a' as highest at the 0.05 level and ND indicates not detected.

detected in 'Tainung 1', 'Lucknow', 'Red Indian' and 'Thailand'. This is in agreement with the observations of Rivera-Pastrana *et al*. (7) who have reported that β-cryptoxanthin to increase during storage from 0.0 to 0.80 mg 100 g^{-1} FW corresponding to orange red mature flesh colour. Lycopene mean values ranged from trace in yellow pulped genotypes, *viz*., 'Line 21' and 'Lucknow' to 3.05 ± 0.23 mg 100 g^{-1} FW in 'Tainung 5'. Schweiggert *et al*. (10) also reported trace amount of lycopene (7.0 ± 0.1) µg 100 g^{-1} in yellow pulped papaya fruit pulp, whereas high amount (3046 ± 85 µg 100 g^{-1}) was found in red pulped genotypes. Setiawan *et al.* (11) have reported lycopene value of 5.75 mg 100 g^{-1} fresh pulp in red papaya from Indonesia. Lycopene mean value of 2.23 mg 100 g^{-1} FW has been reported by Rivera-Pastrana *et al*. (7) in papayas belonging to 'Solo' and 'Formosa' groups. They found higher value 3.39 ± 0.32 mg 100 g^{-1} FW lycopene for 'Tainung 1'. Carotenoid levels are known to vary depending on weather conditions, variety, ripening stage, season and geographical area (Setiawan *et al*., 11). Both β-carotene and α-carotene content did not show much difference between red and yellow genotypes. The red pulp genotype 'Red Indian' was found to contain the maximum mean value of β-carotene 0.82 ± 0.1 mg 100 g^{-1} FW followed by yellow pulped 'Lucknow' 0.59 ± 0.0 mg 100 g^{-1} FW. Mean α-carotene values ranged from 0.07 ± 0.01 to 0.38 ± 0.08 mg 100 g^{-1} FW. Maximum amount was detected in yellow genotypes 'Line 21' and 'Lucknow'. Similar observations have been recorded by Schweiggert *et al*. (10) who reported similarity in carotenoid content of red and yellow pulped genotypes for all pigments other than lycopene. Red fleshed genotypes have more consumer acceptance and it is preferred by the juice industry to impart colour in juice blends. Two genotypes, *viz*., 'Red Indian' and 'Tainung1' were found to have high protivamin A compounds along with the desirable red pulp. These genotypes can be useful in breeding for improving the provitamin A contents of papayas. Out of the three SCAR markers, CPFC2 failed to amplify and therefore was not used further for the study. The marker CPFC1 (Fig. 1) showed polymorphism for one genotype 'Shillong' where it was present in co-dominant form. On the other hand, marker CPFC3 (Fig. 2) could detect the co-dominant inheritance in two genotypes 'Tainung

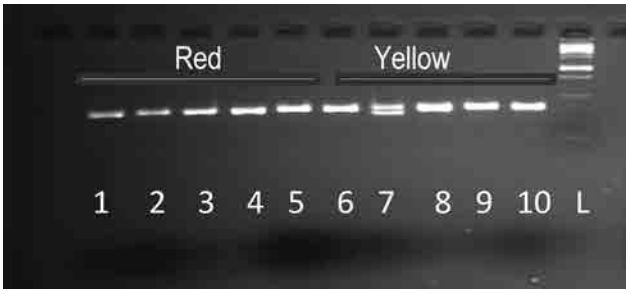

Fig. 1. SCAR marker CPFC1 amplifying the red and yellow pulped papaya genotypes on 1.5% Agarose gel. Red genotypes: 1 = 'CO3', 2 = 'Tainung1', 3 = 'Tainung5', 4 = 'Red Indian', 5 = 'Thailand'; Yellow genotypes: 6 = 'CO1', 7 = 'Shillong', 8 = 'AC119', 9 = 'Nigeria', 10 = 'Washington'; L = 1 kb ladder.

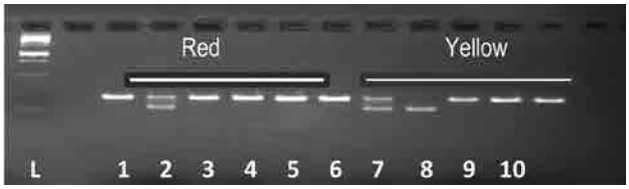

Fig. 2. SCAR marker CPFC3 amplifying the red and yellow pulped papaya genotypes on 1.5% Agarose gel. Red genotypes: 1 = 'CO3', 2 = 'Tainung1', 3 = 'Tainung5', 4 = 'Red Indian', 5 = 'Thailand'; Yellow genotypes: 6 = 'CO1', 7 = 'Shillong', 8 = 'AC119', 9 = 'Nigeria', 10 = 'Washington'; L = 1kb ladder.

1' and 'Shillong', while in 'AC119' it detected the allele for yellow pulp. These markers can be used in simple PCR based screening test to identify individuals in a segregating population with a desired pulp colour. These markers can be adopted in breeding programme to save time and resources for selection for a desired fruit pulp colour and shelf-life, since yellow pulp colour has been linked to longer shelf-life of fruits (Skelton *et al*., 12).

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