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Original Article

Papaya carotenoids increased in Oxisols soils

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A B S T R A C T

The papaya fruit is healthy for humans as it contains high levels of antioxidants and provitamin A due to high lycopene and β-carotenes contents, respectively. The carotenoids were determined from papayas grown in three different locations—Kamphaeng Saen (KS), Sisaket (SK), and Tha Mai (TM)—and also the study investigated whether the Oxisols soil was capable of increasing the carotenoids content in the papaya fruit. The lycopene, β-carotene and β-cryptoxanthin contents were determined from four different ripening stages (mature green to fully ripe). Concomitantly, the transcript levels of five genes involved in carotenoids biosynthesis—phytoene desaturase (*PDS*), carotene desaturase (*ZDS*), lycopene-β-cyclase (*LCY-B1* and *LCY-B2*), and β-carotene hydroxylase (*B-CHX*)—were investigated. Papayas grown at TM had the highest lycopene, which was supported by the high expression levels of *PDS*, *ZDS* and *B-CHX* and the lower expression levels of the two *LCY-B* genes; however the locations did not affect the fruit quality. The 'Plak Mai Lai' papaya was further investigated by being grown in one location but on two different soil types (Oxisols and Ultisols), to compare their carotenoids contents. Higher carotenoid contents were detected in the papaya grown in the Oxisols.

Introduction

Papaya (*Carica papaya* L.) is one of the world most important tropical fruits, serving both the fresh and canning fruit markets due to its firm texture, sweet taste and appealing colors (Food and Agriculture Organization of the United Nations, 2000). Papaya is also a highly recommended healthy food rich in antioxidants and provitamin A (World Health Organization, 2009). 'Khaek Dam' and 'Plak Mai Lai' are the most popular cultivars in Thailand for fresh fruit consumption due to their high yield, firmness, red flesh, preferable flavor and odor (Fuggate et al., 2010).

The main carotenoids in the red-fleshed papaya that interest health-conscious people are lycopene, β-carotene and β-cryptoxanthin; the lycopene content in papaya, found only in the red flesh, varies in the range 1.35–4.31 mg/100 g fresh weight (FW) due to papaya genotypes and geographical locations (Wall, 2006; Schweiggert et al., 2012). Among the red-fleshed genotypes, 'Tailandia', 'Industrial 10G', 'Industrial 10P' and 'Pococi' are distinctively high in carotenoid contents (Kimura et al., 1991; Schweiggert et al., 2011, 2012). Papaya grown in different geographical locations has different carotenoid contents, which has been reported to be due to different climate factors (Shewfelt, 1990; Yadava et al., 1990; Kimura et al., 1991; Almeida et al., 2003; Crane, 2005; Wall, 2006). Brazilian papaya obtained from northeast Brazil (warm climate) had higher carotenoids than that from the southeast (moderate climate) region (Kimura et al., 1991). However, limited data exist on the carotenoids contents of papaya fruits in relation to soil type.

Hawaii and Thailand have tropical climates and have some similarities in geographical and climatic conditions. For example, 'Sunrise' is a popular Hawaiian red-fleshed papaya and Wall (2006) identified carotenoid variations of the 'Sunrise' grown in different plantations. The carotenoid contents of 'Sunrise' papaya harvested from Molloaa were higher than those from Kapoho and the Molloaa and Kapoho plantations had different soil types—Oxisols and Histosols, respectively (Uehara and Ikawa, 2000). Oxisols are also present in Thailand (Bunsompobpan, 1972; Tawornpruek, 2005; Trakoonyingcharoen, 2005; Jaroenchasri et al., 2007). Therefore, it

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was of interest to study whether location and Oxisols could increase the carotenoids content in papaya.

'Khaek Dam Sisaket' (KDS) and 'Plak Mai Lai' (PML), the most popular papaya cultivars of Thailand, were selected for the study. Three locations were specifically chosen for the comparison study of carotenoids content because of their geographical differences and soil types. Kamphaeng Saen (KS) is located in central Thailand, Sisaket (SK) is in the northeast and Tha Mai (TM) is in the east. The soil types of the three locations are Alfisols, Ultisols and Oxisols, respectively. In addition, two soil types, Oxisols and Ultisols, within the same region of TM were investigated for their potential to increase the carotenoids content in PML fruit.

Materials and methods

Papaya cultivars and growing locations

Two red-fleshed papaya cultivars, 'Khaek Dam Sisaket' (KDS) and 'Plak Mai Lai' (PML), were grown at three locations in Thailand: (1) Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom (KS; 14°03′07.58″N; 99°97′46.11″E); (2) Sisaket Horticultural Crop Research Center, Sisaket (SK; 15°10′85.65″N; 10°42′84.83″E); and (3) Tha Mai district, Chanthaburi (TM), where two plantations were chosen 66 km apart, one having Oxisols and the other having Ultisols soil types (12°61′49.58″N; 102°05′34.58″E and 13°07′61.46″N; 101°95′15.27″E, respectively). The papayas grown at different locations were from the same seed stock of each cultivar. Average temperatures, evaporation, annual rainfall and harvesting time are shown in Table 1. Both cultivars grown at the KS and SK sites were harvested in December 2012, while those grown in TM were harvested in March 2013. PML grown in TM was additionally harvested in May and July 2013 for the investigation of effect of Oxisols and Ultisols on carotenoids level and fruit quality. The physical and chemical properties of the Alfisols, Ultisols and Oxisols at KS, SK and TM are described in Table 2.

Fruit sample preparation

Twelve fruits of each cultivar were harvested at four ripening stages—mature green (MG; full green and no color developed), 25%

Table 2
Physical and chemical properties of Alfisols, Ultisols and Oxisols at Kamphaeng Saen (KS), Sisaket (SK) and Tha Mai (TM) study sites.

	Location		
	KS[a]	SK[b]	TM[c]
Latitude	14.030758	15.108565	12.614958
Longitude	99.974611	104.284183	102.053458
Order[1]	Alfisols	Ultisols	Oxisols
Soil series[1]	Kamphaeng Saen	Satuek	Tha Mai
Soil characteristic[2]			
pH (H_20)	7.1	4.8	5.1
pH (KCl)	6.4	4.0	4.6
BS (%)	89	56	9
CEC (cmol/kg) (NH_4OAc)	15	1	11
OM (g/kg)	10	7	45
Available P (mg/kg)	56	18	99
Available K (mg/kg)	185	37	23
Texture	loam	loam	clay
Sand (g/kg)	480	568	237
Silt (g/kg)	340	241	391
Clay (g/kg)	180	190	372

CEC = cation exchange capacity; OM = organic matter.
[1,2] Center for Agricultural Resources Systems Research, n.d.
[a] Ratneetoo, 1995; Jeimjirashat, 1999.
[b] Yodchompoo, 2010.
[c] Jaroenchasri et al., 2007.

Table 1
Climatic data at Kamphaeng Saen (KS), Sisaket (SK) and Tha Mai (TM) locations during plant growth, fruit set to ripe (Meteorological Department, 2014).

Location	Year	Month[1]	Temperature (°C)	Rainfall (mm)	Evaporation (mm)
KS	2012	August	28.0	59.3	4.4
		September	27.6	485.5	4.2
		October	27.9	131.4	3.8
		November	27.6	114.2	3.2
		December	26.8	0.0	3.4
		Average (5 mth[a])	27.5	158.1	3.8
		Average (4 mth[b])	27.6	182.8	3.7
SK	2012	August	27.9	355.8	4.2
		September	27.7	181.2	3.6
		October	27.8	30.3	3.9
		November	27.5	39.5	3.7
		December	26.1	0.0	4.0
		Average (5 mth[a])	27.3	121.4	3.9
		Average (4 mth[b])	27.4	62.8	3.8
TM	2012	November	24.4	204.4	3.6
		December	21.5	0.0	4.2
	2013	January	23.0	66.8	4.1
		February	28.3	43.7	4.0
		March	29.0	61.2	5.0
		April	29.2	223.8	4.3
		May	29.4	140.8	4.4
		June	28.1	562.3	3.9
		July	27.3	1035.4	2.8
		Average (5 mth[c])	26.8	75.2	4.2
		Average (4 mth[d])	25.8	42.9	4.3
		Average (4 mth[e])	29.0	117.4	4.4
		Average (4 mth[f])	28.5	490.6	3.9

[1] Months in bold are harvesting time.
[a] Weather conditions 5 mth before harvest in December 2012 of KDS cultivar.
[b] Weather conditions 4 mth before harvest in December 2012 of PML cultivar.
[c] Weather conditions 5 mth before harvest in March 2013 of KDS cultivar.
[d] Weather conditions 4 mth before harvest in March 2013 of PML cultivar.
[e] Weather conditions 4 mth before harvest in May 2013 of PML cultivar.
[f] Weather conditions 4 mth before harvest in July 2013 of PML cultivar.

breaker (25% B; red color developed on about 25% of cut surface flesh area), 50% breaker (50% B; red color developed on about 50% of cut surface flesh area) and ripe (R; full flesh color developed) (Fig. 1). Six fruits with uniform flesh color, served as six replications, were selected from the total 12 harvested fruits of each cultivar for further investigation. Each fruit was halved transversely in the middle of the fruit. The flesh-colored component, firmness and total soluble solid content were measured from the middle part of each fruit to ensure evenness due to uneven ripening patterns along the length of the fruit. Then, each fruit was stored for further carotenoids analysis. Sample storage was done by cutting the papaya flesh into 0.5–1.0 cm^3 cubes, which were frozen immediately in separate containers with liquid nitrogen, lyophilized and kept at −80°C.

Flesh color and fruit firmness measurement

After harvest, the fruit skin was thinly peeled, the flesh was cut in half along the transverse axis and the seeds were removed. The color components (L^*, a^*, b^*, C, and h) of the flesh in the middle of the fruit were determined at four spots of each fruit using a chroma meter (CR400; Konica Minolta Sensing, Inc.; Osaka, Japan) where L^* signifies the lightness or the general illumination of the color ranging from 0 (black) to 100 (white); a^* signifies the chromaticity colors from green ($-a^*$) to red ($+a^*$); b^* indicates chromaticity from blue ($-b^*$) to yellow ($+b^*$); C represents chroma or ‘saturation’ regardless of lightness or darkness, in which a low chroma is completely dull, gray, or dilute, while high chroma is very luminous or concentrated; and h represents the degree or angle for hue, ranging from 0° (red) through 90° (yellow) and 180° (green) to 270° (blue).

After color determination, the same papaya flesh sample was measured for fruit firmness; four spots on opposite sides of each sample were penetrated using a firmness tester (Chatillon, San Leandro CA, USA) with a 5 mm diameter plunger. The firmness values were reported in Newtons per square meter.

Total soluble solids

The papaya flesh of each ripening stage was homogenized using a homogenizer (Polytron PT2100; Kinematica; Lucerne, Switzerland). A drop of the centrifuged homogenate was placed on a hand refractometer (N-3000E, Atago; Tokyo, Japan). The total soluble solids (TSS) content was expressed in °Brix.

Analysis of carotenoids contents

Carotenoid extraction

The carotenoids extraction was modified from Anthon and Barrett (2007). Frozen papaya flesh (approximately 1 g) was

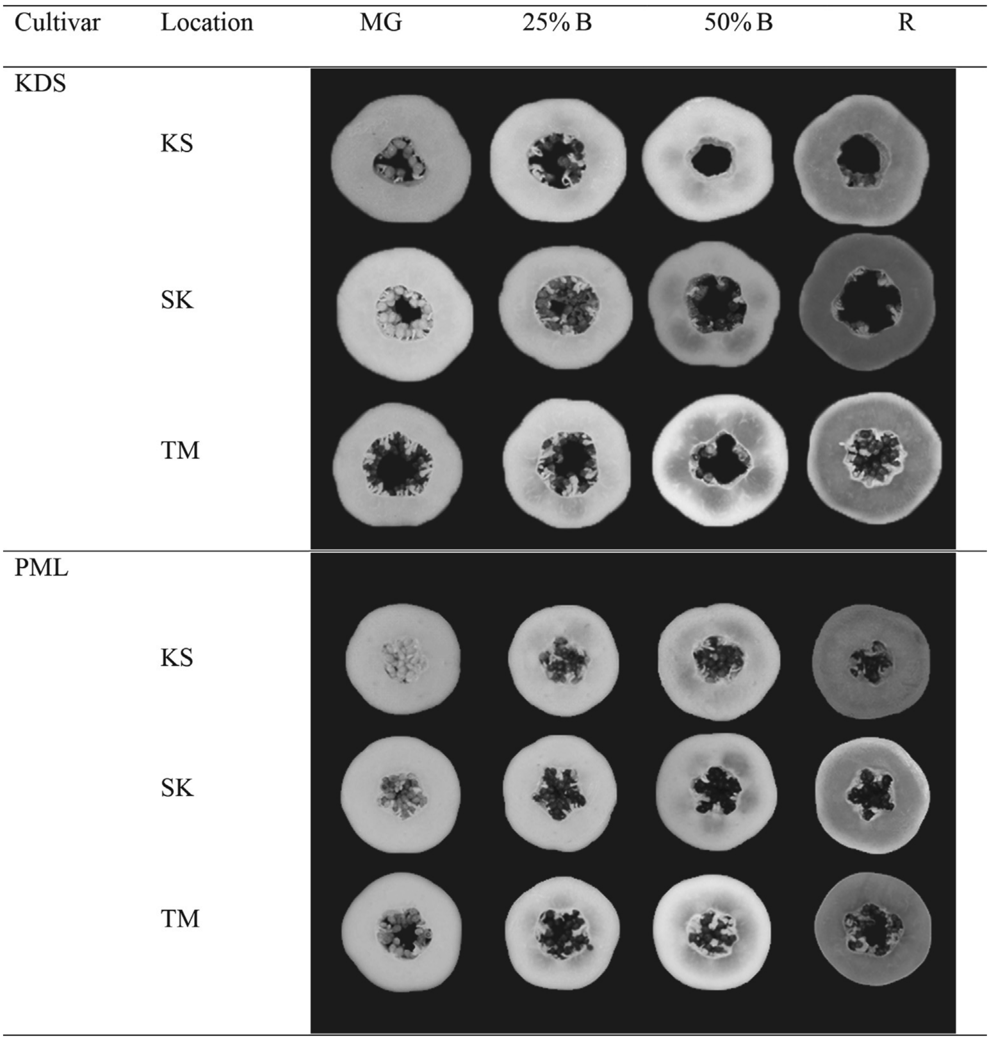

Fig. 1. Flesh color at four ripening stages—mature green (MG), 25% breaker (25% B), 50% breaker (50% B) and ripe (R), of ‘Khaek Dam Sisaket’ (KDS) and ‘Plak Mai Lai’ (PML) grown at three different locations, Kamphaeng Saen (KS), Sisaket (SK), and Tha Mai (TM).

extracted with 25 mL HEA (2:1:1 hexane: ethanol: acetone, volume per volume per volume; v/v/v). The papaya flesh in the HEA extraction buffer was homogenized using a homogenizer at 15,000 rpm for 1 min and then the HEA was added to make 100 mL. The homogenized extract was incubated for 15 min in the dark at room temperature, and 15 mL distilled water was added and incubated for a further 15 min. After the organic phase had separated, the upper layer was transferred into a round-bottomed flask and an aliquot of 4 mL extract was evaporated until dry.

Carotenoids content analysis

The carotenoids contents were analyzed using high performance liquid chromatography (HPLC; Agilent 1100, Hewlett-Packard-Strasse; Waldbronn, Germany). The dried extract was dissolved with 1 mL mobile phase (10: 5: 85 dichloromethane: acetonitrile: ethanol, v/v/v; Krichnavaruk et al., 2008) and filtered through a nylon membrane (0.45 μm pore size). The sample (20 μL) was injected into the HPLC. The HPLC system was equipped with a C30 reversed-phase column (25 cm × 4.6 mm), at a 1 mL/min flow rate at ambient temperature (25°C), and a UV–Vis detector. Absorption spectra for the main peaks were 285 nm for phytoene and 450 nm for other color carotenoids. A chromatographic run lasted 65 min. Each carotenoid was identified by the retention time compared with the external standard. The concentration of each carotenoid was calculated with a standard as described in Hart and Scott (1995). Each carotenoid standard was performed in three different concentrations and linear correlation with a correlation coefficient of 0.99 was considered to ensure the range of concentration accuracy. Lycopene, β-carotene and β-cryptoxanthin standards were purchased from Sigma–Aldrich, (St. Louis, MO, USA). Phytoene, zeaxanthin, violaxanthin and neoxanthin standards were purchased from CaroteNature GmbH, (Lupsingen, Switzerland).

Analysis of carotenoid biosynthesis

RNA extraction and cDNA synthesis

Total RNA was extracted from the frozen papaya flesh (the same fruit used for carotenoids analysis), using a modified CTAB extraction protocol (Chang et al., 1993). Three biological replicates per cultivar were investigated; each cDNA sample was run in triplicate. The RNA was treated with DNase I using a Turbo DNA-free™ kit (Ambion; Austin, TX, USA) following the manufacturer's protocol. The first cDNA strand was synthesized from 1 μg total RNA using an iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA).

Quantitative real-time polymerase chain reaction

Gene expressions of *PDS*, *ZDS*, *LCY-B1*, *LCY-B2* and *B-CHX* were investigated using quantitative real-time polymerase chain reaction (qPCR). The primer pairs (forward and reverse) of *PDS*, *ZDS*, *LCY-B*, *LCY-B2* and *18S*, adopted from previous works, are displayed in Table 3. The *B-CHX* and *ACTIN* primers were designed based on 3′-untranslated regions of each gene from available sequences using the Primer 3 free software (www.ncbi.nlm.nih.gov) as shown in Table 3. The qPCR was performed with a real-time qPCR thermal cycler (version c1000 Touch; Bio-Rad; Hercules, CA, USA). Each reaction consisted of 40 ng of cDNA template, 2 μM of each primer and 10 μL iTaq™ Universal SYBR Green Supermix (Bio-Rad; Hercules, CA, USA), in a final volume of 20 μL. The real-time PCR conditions were performed as initial denaturation for 3 min at 95°C, followed by 24 cycles of 95°C for 10 s, 55°C for 15 s, 95°C for 10 min and completed by melt curve analysis. A negative control (no DNA template) for each primer pair was included in each run. Actin and 18S genes were used as references. Specific gene expression was normalized to the internal control gene 18S and Actin.

Statistical analysis

The experiment was set up in a completely randomized design with six replications (one fruit per replicate) unless otherwise mentioned. Analysis of variance was performed and significant means were compared using Tukey's test at $p < 0.05$. Statistical analysis was performed using the SPSS software (version 19.0; SPSS Inc.; Chicago, IL, USA). A Student's t test at $p < 0.05$ was also performed to compare two groups.

Results

Effect of growing location on carotenoids content and fruit quality of papaya

Carotenoids during fruit ripening

The peaks of three known major carotenoids—lycopene, β-carotene and β-cryptoxanthin—and a few unknown peaks were found in the HPLC chromatogram profiles of samples of both the KDS and PML papaya flesh (Fig. 2). Lycopene was absent in MG papaya. β-Carotene and β-cryptoxanthin were present in all mature stages of the fruit samples. The saponification and unsaponification of carotene and xanthophyll did not give significantly different results (Fig. 3). Lycopene was predominant in both KDS and PML papaya since the 25% B stage. In KDS, a pronounced lycopene level was detected during the transition from 50% B to ripe fruit. In PML, an increase in the lycopene level was found from the 25% B through to the ripe stage. β-Carotene and β-cryptoxanthin were the most abundant carotenoids detected in the MG stage in both KDS and PML flesh. Both β-carotene and β-cryptoxanthin increased continuously from MG to the ripe stage and

Table 3
Quantitative real-time polymerase chain reaction (PCR) primers, PCR product sizes and references used in the analysis of gene expressions of carotenoid biosynthesis in papaya flesh.

Primer	Sequence (5′–3′)	Product size (bp)	Reference
PDS-F	GAAGTTTGCGATTGGGCTTC	99	Yan et al. (2011)
PDS-R	CCCTGCTTTCTCATCCACTCTT		
ZDS-F	CACGGTGCAACTTAGGTATAATGG	126	
ZDS-R	AAGCAGGAGAAATCGGCATC		
Lcy-B1_F	TGGCTATATGGTGGCACGAACTCT	53	Devitt et al. (2010)
Lcy-B1_R	CAAGGAACCGAACAATGGAATCTG		
Lcy-B2_F	CAGATGCGATTGCGGAGTGC	66	
Lcy-B2_R	TGGCCTACCCCTGATCATTCTTGT		
18SrRNA _F	CTCCGGCGTTGTTACTTTGAAGAA	113	
18SrRNA _R	CCCGAAGGCCAACAGAATAGGA		
b-chx_F	CGCTGTGGGTATGGAGTTTT	188	NCBI www.ncbi.nlm.nih.gov/nuccore/HQ998850.1
b-chx_R	CGAGGCCTTTGTTGAAGAAG		
Actin_F	TTGATTTTGAGCAGGAGCTTGA	138	NCBI www.ncbi.nlm.nih.gov/nuccore/FJ696416
Actin_R	TGAGTGATGGCTGGAAGAGAAC		

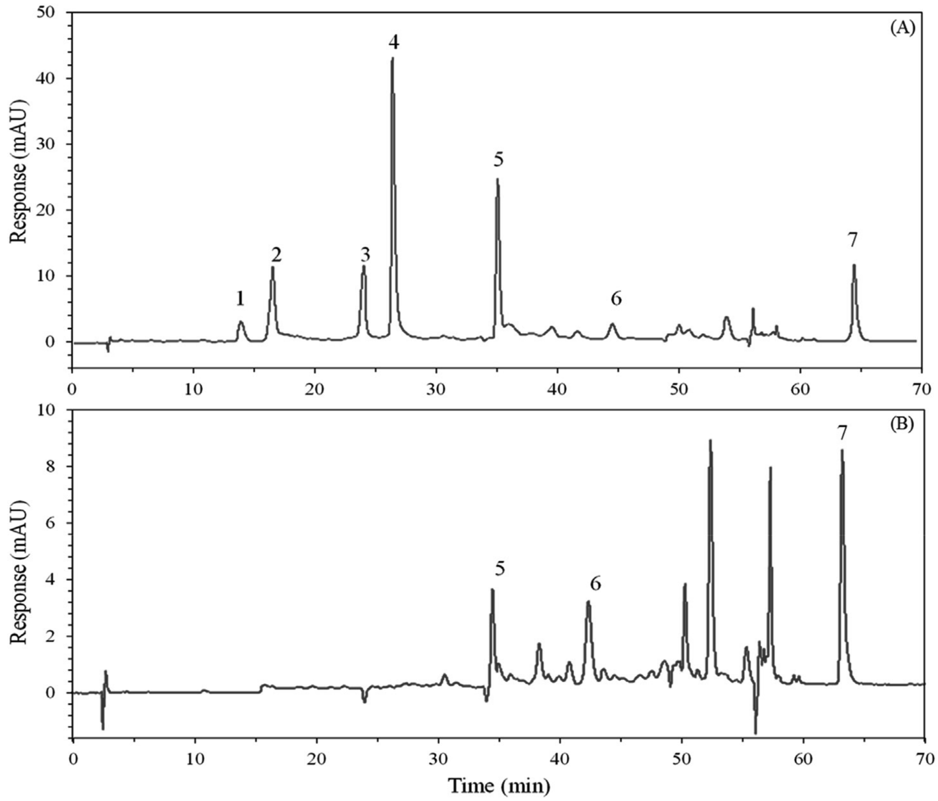

Fig. 2. High performance liquid chromatogram: (A) standard carotenoids (1 = violaxanthin, 2 = neoxanthin, 3 = lutein, 4 = zexanthin, 5 = β-cryptoxanthin, 6 = β-carotene, 7 = lycopene) and (B) carotenoids extracted from 'Plak Mai Lai' papaya fruit.

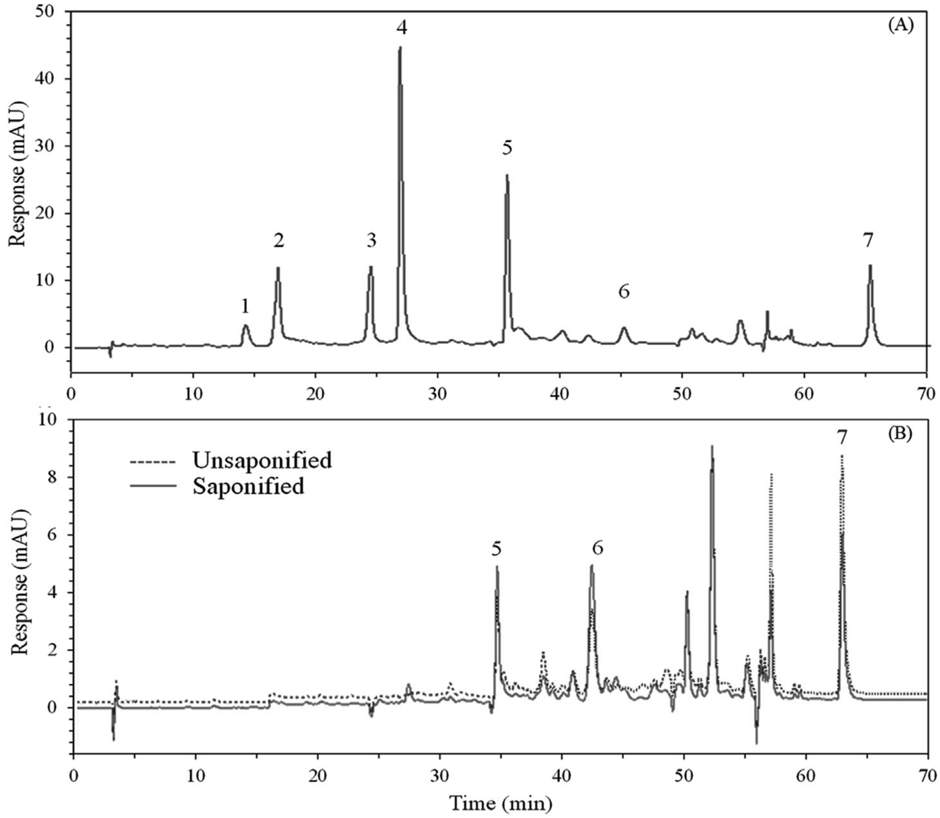

Fig. 3. High performance liquid chromatogram: (A) standard carotenoids and (B) saponified and unsaponified carotenoids extracted from 'Plak Mai Lai' papaya fruit (1 = violaxanthin, 2 = neoxanthin, 3 = lutein, 4 = zexanthin, 5 = β-cryptoxanthin, 6 = β-carotene, 7 = lycopene).

increased significantly during the transition from 50% B to ripe fruit (Table 4). Overall, the increase in carotenoids of both KDS and PML occurred during the transition from 50% B to the ripe stage, resulting in the highest levels in the fully ripe fruit. Therefore, ripe fruit was subsequently the main target of the remainder of the study.

Carotenoids by different locations

Different locations showed significant effects ($p < 0.05$) on lycopene contents in both the KDS and PML cultivars. The lycopene contents of the ripe fruit were 1.5–2 times greater in TM papaya compared with KS (Table 4). However, there were no significant differences in the β-carotene and β-cryptoxanthin levels in both papaya cultivars at the ripe stage among the three locations.

Fruit quality by different locations

Fruit quality during fruit development

The flesh color of both KDS and PML papayas developed from white in MG to orange-red in the ripe stage with decreasing h° (from 100.4–108.3° to 42.8–58.5°, respectively) and increasing a^* value (from −3.2 to −7.5 in MG and from 22.1 to 35.6 in the ripe stage) as shown in Table 5. Fruit firmness decreased throughout the fruit ripening from 31 to 56 N/cm^2 to 16 N/cm^2 and from 30 to 36 N/cm^2 to 8–12 N/cm^2 in KDS and PML papayas, respectively. The TSS of KDS and PML fruit increased continuously from MG (5–6°Brix) to the ripe stage (11–13°Brix) as shown in Table 5. All papaya fruit softened rapidly during the ripe stage, as were the changes in the levels of soluble solids and flesh color development.

Table 4

Carotenoid (lycopene, β-carotene, and β-cryptoxanthin) contents in four ripening stages—mature green (MG), 25% breaker (25% B), 50% breaker (50% B) and ripe (R)—of 'Khaek Dam Sisaket' and 'Plak Mai Lai' papaya cultivars grown in three different locations—Kamphaeng Saen (KS), Sisaket (SK), and Tha Mai (TM).

Carotenoids (μg/100 g dry weight)	Location	'Khaek Dam Sisaket'				'Plak Mai Lai'			
		MG	25% B	50% B	R	MG	25% B	50% B	R
Lycopene	KS	0	0^{a}	65^{a}	652^{a}	0	376^{b}	534	631^{a}
	SK	0	6^{a}	836 ^{b}	918^{ab}	0	0^{a}	493	965^{ab}
	TM	0	88^{b}	733^{b}	1,115^{ab}	0	113^{b}	516	1,692^{b}
	F-test	ns	*	*	*	ns	*	ns	*
β-Carotene	KS	3^{b}	12^{a}	19^{ab}	283	6	72^{b}	145	380
	SK	0^{a}	19^{ab}	86^{ab}	286	12	14^{a}	29	285
	M	9^{c}	20^{b}	134^{b}	531	8	21^{a}	49	236
	F-test	*	*	*	ns	ns	*	ns	ns
β-Cryptoxanthin	KS	10	18^{a}	28^{a}	96	0	47^{b}	71	144
	SK	7	18^{a}	58^{b}	96	18	20^{a}	33	158
	TM	12	35^{b}	65^{b}	191	12	31^{a}	49	159
	F-test	ns	*	*	ns	ns	*	ns	ns

Values are means of three replicates.
ns and * indicate not significantly different and significantly different, respectively, at $p < 0.05$ using one way analysis of variance.
Means in the same column followed by different lowercase letters are significantly different at $p < 0.05$ using Tukey's test.

Table 5

Flesh color (a^* and hue angle), total soluble solids (TSS) and firmness in four ripening stages—mature green (MG), 25% breaker (25% B), 50% breaker (50% B) and ripe (R)—of 'Khaek Dam Sisaket' and 'Plak Mai Lai' cultivars grown in three different locations—Kamphaeng Saen (KS), Sisaket (SK) and Tha Mai (TM).

		Location	'Khaek Dam Sisaket'				'Plak Mai Lai'			
			MG	25% B	50% B	R	MG	25% B	50% B	R
Color	a^*	KS	−3.2	−1.0^{a}	2.6^{a}	28.2^{b}	−3.5^{a}	1.7	15.4	28.0^{a}
		SK	−4.6	8.3^{b}	26.4^{b}	31.1^{b}	−4.7^{b}	−4.1	7.8	35.6^{b}
		TM	−5.7	2.0^{a}	15.3^{b}	22.1^{a}	−7.5^{c}	0.9	7.8	26.7^{a}
		F-test	ns	*	*	*	*	ns	ns	*
	hue angle (°)	KS	100.4	92.2	84.6^{b}	50.9	101.1^{a}	88.3	65.2	53.5^{b}
		SK	104.2	79.3	51.2^{a}	49.1	101.5^{a}	88.4	75.6	42.8^{a}
		TM	105.4	87.5	64.3^{a}	58.5	108.3^{b}	88.4	75.6	57.4^{c}
		F-test	ns	ns	*	ns	*	ns	ns	*
TSS (°Brix)		KS	6.4	6.6	7.8	10.9	6.1	7.9	10.0	13.0
		SK	5.4	8.3	9.1	10.5	6.2	6.5	8.3	11.1
		TM	6.1	7.5	9.2	12.1	6.1	7.9	8.0	13.0
		F-test	ns	ns	ns	ns	ns	ns	ns	ns
Firmness (N/cm^2)		KS	31^{a}	37	30	18	30	30^{a}	28	12
		SK	56^{c}	49	47	29	34	32^{ab}	28	8
		TM	43^{b}	32	31	16	36	40^{b}	24	8
		F-test	*	Ns	ns	ns	ns	*	ns	ns

Values are means of three replicates.
ns and * indicate not significantly different and significantly different, respectively, at $p < 0.05$ using one way analysis of variance.
Means in the same column followed by different lowercase letters are significantly different at $p < 0.05$ using Tukey's test.

Effect of location on color, total soluble solids and firmness

The ripe PML papaya grown at TM had significantly lower red color (lower a* value), resulting in greater h° (57°) than in the papaya grown at SK. Similarly, the a* value of KDS papaya obtained from TM was significantly lower than that obtained from KS and SK, but the h° values were similar. In addition, there were no significant differences in the TSS and firmness among the three locations.

Effect of locations and soil type on carotenoid biosynthesis of 'Plak Mai Lai' papaya

Since the PML papayas grown in KS and TM showed significantly different lycopene content, further studies on the carotenoid biosynthesis and the effect of soil type on carotenoid content were undertaken.

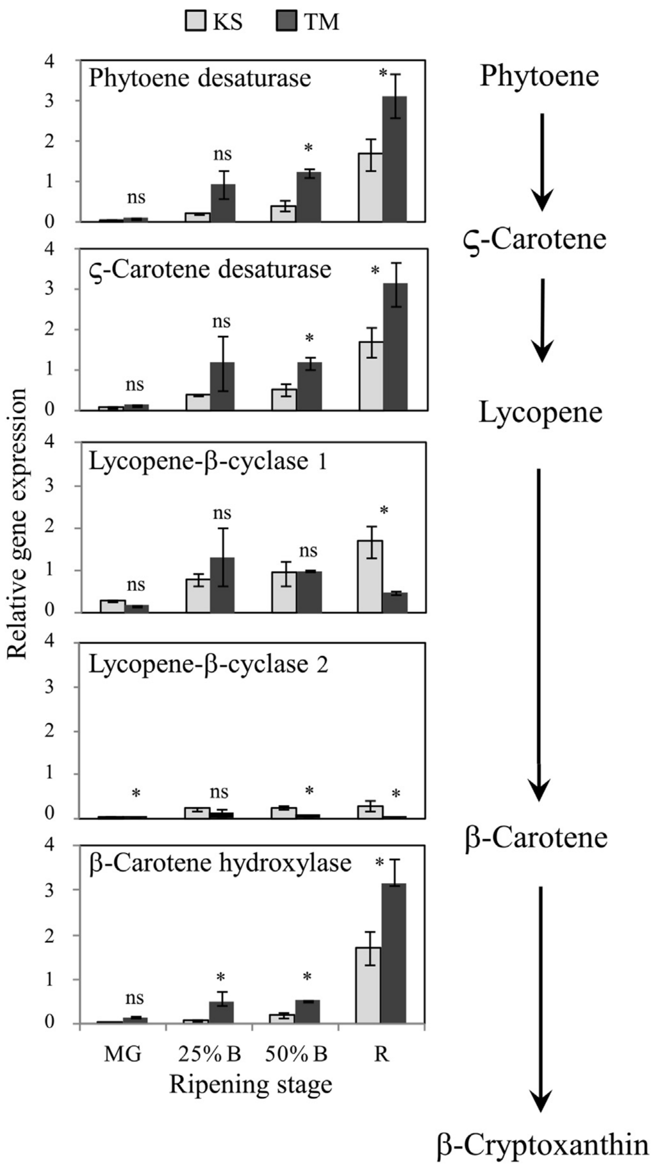

Fig. 4. Gene expressions in carotenoid biosynthesis of 'Plak Mai Lai' papaya grown in Kamphaeng Saen (KS) and Tha Mai (TM) districts for four ripening stages—mature green (MG), 25% breaker (25% B), 50% breaker (50% B) and ripe (R). Means derived from three replicates were compared using a *t*-test; ns indicates not significantly different and * indicates significantly different at $p \leq 0.05$; vertical error bars represent SD.

Carotenoids biosynthesis of the 'Plak Mai Lai' papaya grown in Kamphaeng Saen and Tha Mai

The carotenoid biosynthetic genes of the PML grown at KS and TM were compared. In general, the expression of *PDS*, *ZDS* and *B-CHX* increased throughout fruit development and increased rapidly during the transition from 50% B to the ripe stage. In contrast, expression of both *LCY-B1* and *LCY-B2* was detected at low levels in all ripening stages (Fig. 4). The noticeable changes in all gene expression levels peaked in the ripe stage. The 50% B and ripe PML papayas grown in TM had higher *PDS*, *ZDS*, and *B-CHX* expression than those grown in KS, whereas expression of both *LCY-B1* and *LCY-B2* in the PML ripe fruit from TM were lower (Fig. 4).

Effect of Oxisols and Ultisols on carotenoids level and fruit quality

Carotenoids

The ripe fruit of PML papayas grown in the two TM plantations with the two different soil orders (Oxisols and Ultisols) had significantly different carotenoids profiles (Table 6). Significantly higher β-carotene and β-cryptoxanthin levels in PML papayas grown in Oxisols compared with those in PML papayas grown in Ultisols were observed in the May and July 2013 harvests. Similarly, PML papayas grown in Oxisols also had 35–40% higher lycopene content than those grown in Ultisols, although those harvested in May were not significantly different ($p = 0.07$).

Fruit quality

The a* and h° values of ripe PML papayas grown in Oxisols and Ultisols soils were in the ranges 23.2–27.6 and 56.8–62.6, respectively. The TSS was in the range 11.5–12.5°Brix. There was no significant difference in color and in TSS between papayas grown in Oxisols and Ultisols. In contrast, the fruit firmness showed greater variation. In May 2013 harvesting, the papayas obtained from Oxisols were firmer than those grown in Ultisols while in the July harvesting, the opposite result was observed.

Discussion

Carotenoids and fruit quality by different locations

The color of papaya flesh is determined largely by the presence of carotenoids pigments; red-fleshed papaya fruit contain lycopene, whilst this pigment is absent from yellow-fleshed fruit (Wall, 2006; Schweiggert et al., 2011). The conversion of lycopene (red) to β-carotene (yellow) is catalyzed by lycopene β-cyclase (*LCY-B*) and two forms of *LCY-B*—*LCY-B1* and *LCY-B2*—have been reported in ripe papaya fruit and both are involved in lycopene production with *LCY-B1* being a chloroplast-specific gene, while *LCY-B2* is chromoplast specific and preferentially expressed in fruit (Devitt et al., 2010). The red-fleshed papaya had both *LCY-B* expressions at low levels, thus resulting in the high lycopene accumulation. In contrast, the yellow flesh had low *LCY-B1* but high *LCY-B2* expression levels, resulting in the conversion of all available lycopene to β-carotene and subsequently to β-cryptoxanthin (Devitt et al., 2010). Thus, *LCY-B2* is responsible for the lycopene production in the red-fleshed papaya fruit. Among the three locations, TM appeared to promote only the lycopene in both KDS and PML papayas. The high lycopene content in the TM papayas was supported by the high expression levels of *PDS* and *ZDS*, but low *LCY-B* (Fig. 4). *PDS* and *ZDS* are involved in the upstream lycopene biosynthesis, whereas the *LCY-B*, especially the chromoplast-specific *LCY-B2*, converts lycopene into β-carotene (Devitt et al., 2010; Barreto et al., 2011).

The differences among the three locations were due to weather conditions and soil types, as displayed in Tables 1 and 2. The weather records for the three locations were compared during the

Table 6
Carotenoids (lycopene, β-carotene, and β-cryptoxanthin) contents, flesh color (a* and hue angle), total soluble solids (TSS) and firmness of 'Plak Mai Lai' cultivar grown in two different soil orders (Oxisols and Ultisols) in Tha Mai district.

Harvesting time	Soil order	Carotenoid content (μg/100 g dry weight)			Color		TSS (°Brix)	Firmness (N/cm^2)
		Lycopene	β-Carotene	β-Cryptoxanthin	a*	Hue (°)		
May 2013	Oxisols	1974	417	298	27.6	57.0	11.5	16
	Ultisols	1573	108	148	26.8	56.8	12.0	14
	t-test	ns	*	*	ns	ns	ns	*
July 2013	Oxisols	1689	426	268	23.2	62.6	11.5	13
	Ultisols	1222	115	150	25.8	60.3	12.4	20
	t-test	*	*	*	*	ns	ns	*

Values are means of seven replicates.
ns and * indicate not significantly different and significantly different at $p \leq 0.05$, respectively.

Table 7
Physical and chemical properties of Oxisols and Ultisols soils in Tha Mai district, Thailand.

Soil characteristic	Order	Oxisols				Ultisols
	Series	Tha Mai[a]	Tha Mai[b]	Tha Mai[c]	Tha Mai[d]	Soil group 50[e]
pH (H_2O)		5.9	5.1	4.3	5.1	4.6
pH (KCl)		4.8	4.6	3.7	4.6	4.0
BS (%)		23	9	7	10	15
CEC (cmol/kg) (NH_4OAc)		21	11	11	3	3
OM (g/kg)		54	33	25	45	16
Available P (mg/kg)		90	99	99	99	1
Available K (mg/kg)		130	23	113	23	56
Texture		clay	clay	clay	clay	sandy loam
Sand (g/kg)		244	237	264	237	568
Silt (g/kg)		527	391	301	391	241
Clay (g/kg)		227	372	436	372	190

[a] Bunsompobpan, 1972.
[b] Tawornpruek, 2005.
[c] Trakoonyingcharoen, 2005.
[d] Jaroenchasri et al., 2007.
[e] The Land Development Department, n.d.a.

specific periods that the papaya fruits at each location developed from flowering to ripening. The papayas grown in these locations yielded fruits at different times, and different cultivars took different lengths of time to develop their fruits. The PML fruits took 120 d from flowering to ripen, while the KDS fruits took 150 d. The papayas grown at SK and KS were harvested in December 2012 and those at TM were harvested in March 2013. The TM location had lower rainfall than either KS or SK, while the average temperature and evaporation were similar among the three locations (Table 1). However, the low amount of rainfall in TM was not likely to be responsible for the higher lycopene content because no correlation between amount of rainfall and lycopene content was found (data not shown). In addition, these papaya plantations were irrigated.

Soil type could be the most important factor responsible for the high carotenoids levels. Three different soil types were classified as orders Alfisols, Ultisols and Oxisols at KS, SK and TM, respectively (according to soil taxonomy using search features of the Knowledge of Soil in Thailand webpage by the Center for Agricultural Resources Systems Research—available at http://www.mcc.cmu.ac.th/dinthai/search_taxo.asp). The Alfisols soil at KS had a neutral pH (pH 7.1), while the others were acidic (pH 4.8–5.1). The soil texture of Alfisols and Ultisols is loamy, whereas with Oxisols it is clay (Table 2). Although having a clay texture, Oxisols contain a high content of iron oxides and kaolin resulting in excellent physical properties for water retention and porosity (Trakoonyingcharoen, 2005). A report from Hawaii implied that Oxisols could increase the carotenoids content in papaya (Wall, 2006). Therefore, to prove if the Oxisols could increase the carotenoids, the PML papaya was chosen for further investigation because the PML responded to Oxisols more consistently with increased carotenoids. The location was then limited to only TM in different soil orders (Oxisols and Ultisols). These two plantations were 66 km apart.

Carotenoids in Oxisols

Carotenoids and fruit quality were examined in papaya fruits grown on Oxisols and Ultisols. The carotenoids were significantly higher in the fruit grown on Oxisols; however the fruit quality seemed similar on both soils. The differences in physical and chemical properties of Oxisols and Ultisols at TM were soil texture, organic matter, and available phosphorus (Table 7). The Oxisols composition of sand and clay was 1: 1–1.5; thus they are classified as clay soil, while Ultisols are classified as loamy sand with a higher amount of sand (Table 7). Being clay, the Oxisols have higher organic matter (25–54 g/kg) than the Ultisols (5–21 g/kg) as shown in Table 1. The organic matter was positively correlated with total nitrogen, which was reported to promote carotenoids synthesis (Shewfelt, 1990; Trakoonyingcharoen, 2005).

The chemical properties (soil pH, CEC and available K content) of the Oxisols and Ultisols were similar (Table 7). Although higher levels of P were present in the Oxisols, the P is not available to plants in acidic Oxisols soil (Almeida et al., 2003; Oberson et al., 2001). Therefore, the effect of Oxisols on the increase of the carotenoids in papaya fruit cannot be clearly explained by the soil chemical properties.

PML papayas grown at TM had a higher carotenoids content than those grown at KS. There was no location effect evident in the carotenoids content of KDS papaya. The Oxisols soil promoted lycopene, β-carotene, and β-cryptoxanthin in the PML fruit.

Conflicts of interest

None declared.

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