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SEASONAL VARIATION IN LYCOPENE AND *β*-CAROTENE CONTENT IN *MOMORDICA COCHINCHINENSIS* (LOUR.) SPRENG. (GAC FRUIT) GENOTYPES

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Abstract

Gac fruit (*Momordica cochinchinensis* (Lour.) Spreng.) is a good source of lycopene and *β*-carotene, and it has high potential to be used as functional food. However, phytochemicals could be affected by seasonal variations. The objective of this study was to evaluate seasonal variations in lycopene content, beta-carotene content and agronomic traits in Gac fruit. Three Gac fruit genotypes were arranged in RCBD with three replications and harvested over three seasons. Lycopene and *β*-carotene content were analyzed by HPLC. The season significantly affected concentrations of lycopene, *β*-carotene and total carotenoids in Gac fruits. KKU ac.10-087 and KKU ac.09-030 had the highest lycopene in the summer season whilst KKU ac.10-087 also had the highest lycopene in the dry season. Seasonal and genotypic variations were important sources of variations in lycopene, *β*-carotene and total carotenoids in Gac fruits.

Key words: Carotenoids, *Spiny bitter gourd,* Genotypic variation, G × E interaction.

Introduction

Fruits and vegetables are excellent sources of useful phytochemicals such as flavonols, phenolics, ascorbic acid, anthocyanins and carotenoids. These phytochemicals have antioxidant activity that is beneficial to health (Delgado-Vargas *et al*., 2000; Oomah & Mazza, 2000; Maiani *et al*., 2008). Spiny bitter gourd or Gac fruit has high levels of lycopene and *β*-carotene in red aril of its fruits (Aoki *et al*., 2002; Vuong, 2014). Lycopene plays a role in the prevention of several diseases such as cardiovascular disorders and digestive tract tumors and in inhibiting prostate carcinoma cell proliferation in humans (Levy & Sharoni, 2004). The seed membrane of the Gac fruit also contains a significant amount of oil, which is essential for the absorption and transport of *β*-carotene (Vuong & King 2003). The concentration of lycopene in the aril was about ten-time higher than that in known lycopene-rich fruits, indicating that Gac fruit could be a new and potentially valuable source of lycopene (Vuong *et al*., 2002; Ishida *et al*., 2004; Burke *et al*., 2005; Vuong *et al*., 2006; Ishida & Chapman, 2009). Gac fruit can be easily grown in many countries, such as Thailand, Vietnam, Laos and China (Bootprom *et al*., 2012; Bootprom *et al*., 2015; Kubola *et al*., 2013). In Vietnam, Gac fruit is seasonally harvested from October to February (Nhung *et al*., 2010).

In tomatoes, climatic conditions greatly affect the synthesis of lycopene and *β*-carotene (Panthee, 2012). Temperature, solar radiation, rainfall, relative humidity and genotype are the main factors affecting phytochemical content of tomatoes (Gautier *et al*., 2005). Lycopene and *β*-carotene are influenced by environmental factors, particularly variations in solar radiation and air temperature (Dumas *et al*., 2003; Gautier *et al*., 2005; Lumpkin, 2005). The optimal temperature for lycopene biosynthesis is from 22-25°C (Dumas *et al*., 2003). Temperatures ranging from 27-32°C decreased the lycopene accumulation and lycopene was inhibited above 32°C (Helyes & Lugasi, 2006; Gautier *et al*., 2008). With only *β*-carotene being produced until temperature of 38ºC is reached (Brandt *et al*., 2006). Increased irrigation also led to decreased lycopene content (Helyer *et al*., 2014; Pek *et al*., 2014). As Gac fruit is a new and under-utilized crop, information regarding seasonal effects on the production of *β*-carotene and lycopene is scarce. The effect of temperature on the production of *β*-carotene and lycopene have been studied in many crops e.g. tomato (Dumas *et al*., 2003; Brandt *et al*., 2006; Helyes & Lugasi, 2006; Gautier *et al*., 2008) and sweet potato (Haynes, 2010).

In order to be suitable for production on a commercial scale, Gac fruit varieties should possess high levels of lycopene and *β*-carotene, and have phytochemical stability. Variety and environment are factors determining growth, yield, and quality of Gac fruit (Ishida *et al*., 2009). However, season can also affect the difference in production of *β*-carotene and lycopene in Gac fruit. The aim of this study was to estimate the seasonal variations in lycopene, *β*-carotene content and agronomic traits in Gac fruit. The information obtained in this study will be useful for Gac fruit production and thus providing a good source of raw materials for functional food.

Materials and Methods

Plant materials and sample preparation: Three genotypes (KKU ac.11-148, KKU ac.10-087 and KKU ac.09-030) of Gac fruit normally grown in Thailand were transplanted in RCBD with 3 replications at Nong-Ea-Bood sub-district, Huy-Pung district, Kalasin province (16º38′45″N 103º54′32″E, 138 masl), Thailand. Three plants of each accession, which were propagated by air layering, were planted in each replication with a spacing of 4 m between plants within each row and 4 m between rows. A wood support with 1.8 m in height was constructed for each plot. A fertilizer formula 15-15-15 of N, P and K at a rate of 100 g per plant was applied for three splits at 15, 45 and 90 days after transplanting. Cattle manure was also applied to each plot at the rate of

0.5 kg per plant at 4 month intervals. Furrow irrigation was available as necessary to avoid drought stress. Pests and diseases were not observed in the experiment and therefore the control of pests and diseases was not necessary. Manual weed control was practiced regularly during the growing period. The female flowers were hand pollinated using fresh pollen collected from the male flowers and the pollinated flowers were tagged and labeled to determine maturity dates. As many of the female flowers were pollinated as possible for further random selection of ripe fruits.

At about one year after transplanting, fruits of three genotypes were harvested at ripening stage, indicated as red skin of Gac fruit (Fig. 1). Three fruits in each plot were randomly chosen. The data were recorded for fruit maturity, fruit yield, seed membrane yield at maturity stage in the summer season (March–June), the rainy season (July-October) and the dry season (November-February) in 2012 and 2013. The fruits were cleaned and seed membrane; seeds were removed from the fruit cavity of the ripe fruits. The samples were freeze-dried and stored in a freezer (-20°C) until further phytochemicals analysis.

Extraction of carotenoid: The carotenoid content was extracted using the method described by Kubola & Siriamornpun (2011) with slight modifications. For the aril samples, 0.1 g of each sample was placed in a vessel, protected from light, and mixed with 10 ml of extraction solvent (n-hexane/acetone/ethanol: 2:1:1 v/v/v) until complete color exhaustion. The extract was transferred to a separating funnel and 10 ml of water was added. The upper layer was placed in a volumetric flask. Then the volume of the extracts was adjusted to 10 ml by adding n-hexane. An aliquot of 10 ml of the extract was filtered through 0.45 µm membrane filters, and 20 µl of the sample was injected into high performance liquid chromatography (HPLC) for carotenoids analysis.

Lycopene and *β*-carotene analysis: Lycopene and *β*-carotene content in aril of ripe-fruits were analyzed by HPLC. The composition of solvents and the isocratic conditions used in this study the method described by Kubola & Siriamornpun (2011) was followed. Analysis was performed using Shimadzu LC-20AC pumps, a SPD-20M diode array detector, and inertsil ODS-3 C-18 column reverse phase (4.6 x 250 mm., syringe filter 5 µM). The mobile phase consisted of solvent A (methanol)/solvent B (acetonitrile) and solvent C (dichloromethane). Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 0 to 30% solvent A; from 100 to 30% solvent B; from 0 to 40% solvent C; from 5 to 10 min, 30% solvent A; 30 % solvent B; 40% solvent C; from 10 to 15 min, linear gradient from 30 to 20% solvent A; 30% solvent B; from 40 to 50% solvent C; from 15 to 20 min, linear gradient 20% solvent A; 30% solvent B; 50% solvent C; from 20 to 25 min, from 20 to 0% solvent A; from 30 to 100% solvent B; from 50 to 0% solvent C; from 25 to 30 min, 100% solvent B. Operating conditions were as follows: column temperature, 38°C, injection volume, 20 µl, flow rate of 1 ml/min and UV-diode array detection at 450 nm.

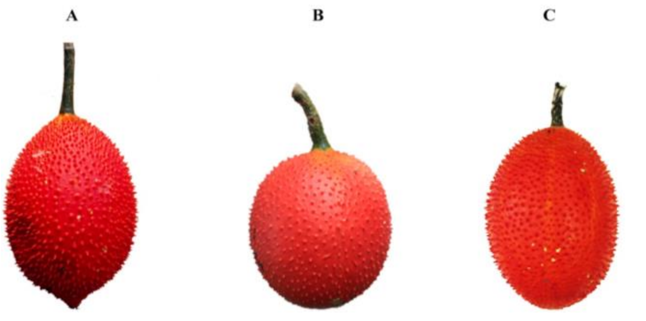

Fig. 1. Fruits at harvested stage of three Gac fruit varieties; KKU ac. 10-087, KKU ac. 11-148 and KKU ac. 09-030.

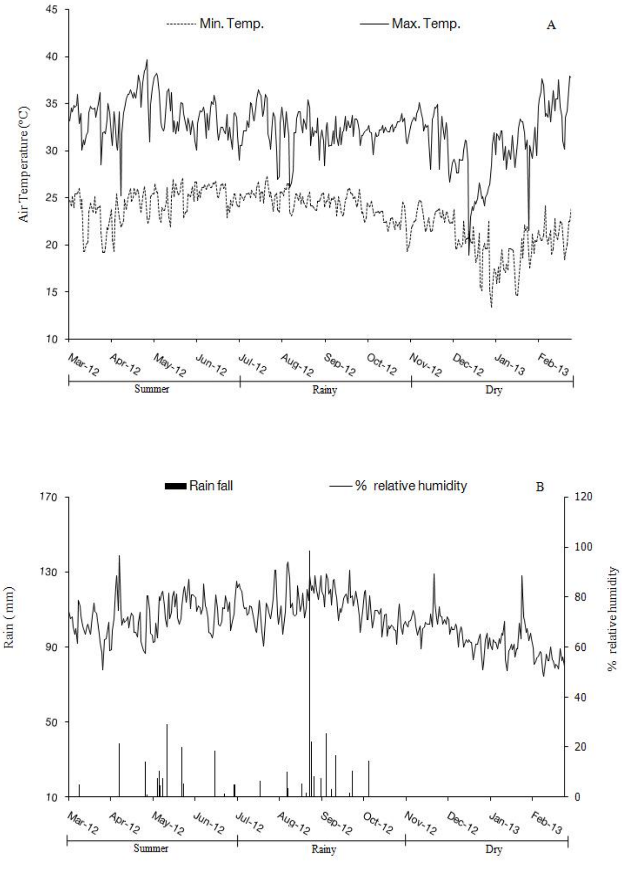

Fig. 2. Maximum temperature, minimum air temperature (°C) (A), rainfall (mm) and relative humidity (B) in March 2012 - February 2013 in Kalasin, Thailand.

Statistical analysis

Individual analysis of variance was performed for each season according to a randomized complete block design (RCBD). Homogeneity of variance was tested for parameters and combined analysis of variance of three season was performed. Calculation was done using STATISTIX 8 software package and LSD test was used to compare means.

Meteorological data: Meteorological data at the experimental site were recorded daily at the nearest weather station in Kalasin province. Maximum and minimum air temperature, rain fall and relative humidity during the month preceding the harvest are presented in Fig. 2.

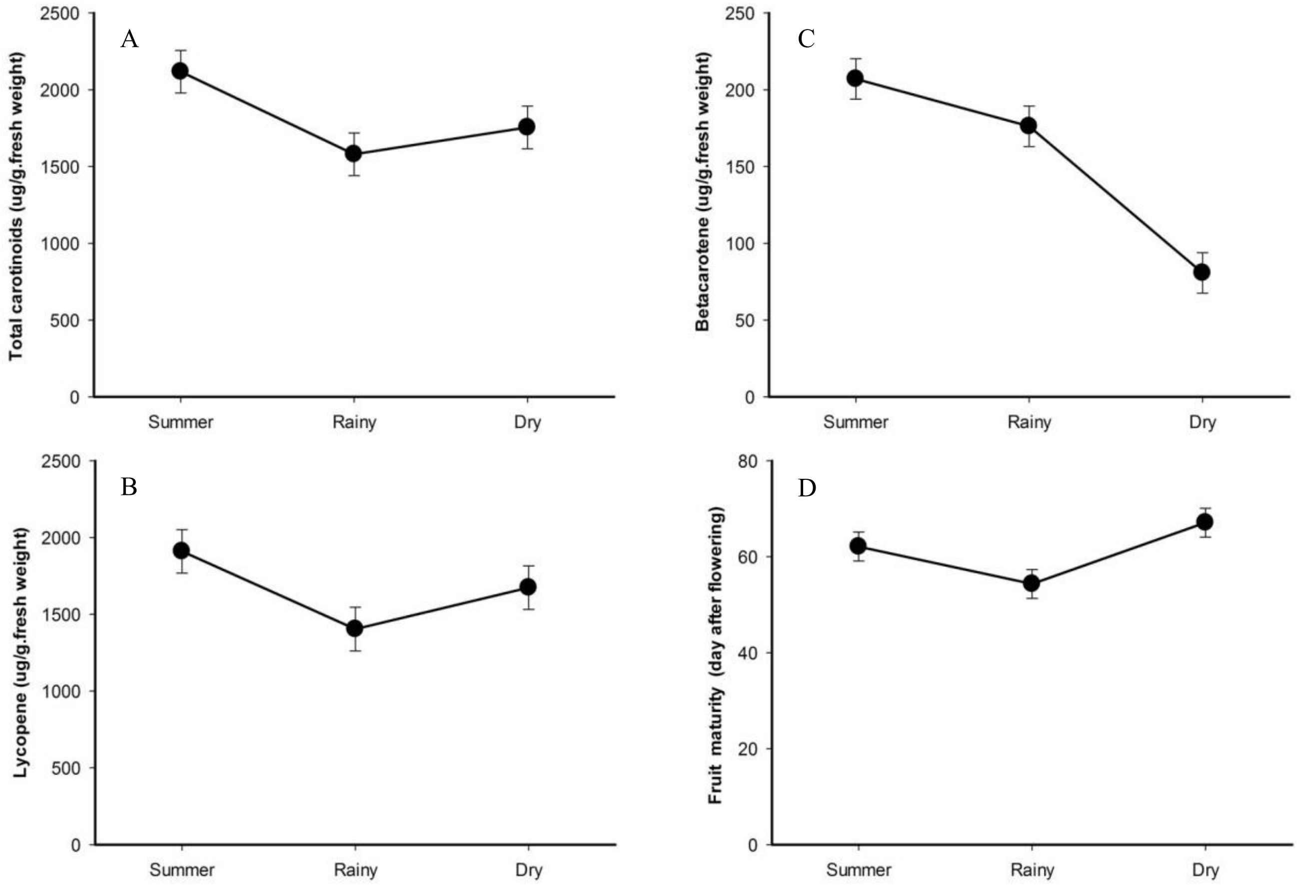

Fig. 3. Total carotenoids (a), lycopene (b), β-carotene content (c) and fruit maturity (d) of Gac fruit genotypes during 2012 to 2013. Bar lines are independent LSD values for mean comparison among seasons.

Results

Weather conditions: Maximum-minimum temperatures, rainfall and relative humidity recorded at the nearest weather station in Kalasin province in the growing seasons 2012 and 2013 are shown in Fig. 2. In summer when the air temperature was highest, average monthly temperature was higher than 28ºC and reached to a maximum of 34ºC in late-April. The maximum and minimum temperatures in the dry season between November and February were 18.9 and 13.4ºC, respectively. The rainfall in the summer was moderate (3.7 mm), whereas the rainfall in the rainy season was highest (5.3 mm). The relative humidity was highest in the rainy season and related to rainfall.

Seasonal variation: Three varieties of Gac fruit were evaluated over three seasons for lycopene, β-carotene, total carotenoid content, fruit maturity, fruit weight, fruit number and aril weight. Season was an important source of variation for lycopene, β-carotene, total carotenoid content and fruit maturity as significant differences ($p \leq 0.05$ or 0.01) between seasons were observed for these parameters, whereas the differences among seasons for fruit weight, fruit number and aril weight were not significant (Table 1 and Fig. 3). Variations among seasons were highest for β-carotene and fruit maturity, whereas variations for Lycopene and total carotenoids content were low although they were significant.

Seasons significantly affected differences ($p \leq 0.05$ or 0.01) for lycopene, β-carotene, total carotenoid content and number of days to maturity, but not significant for fruit weight, fruit number and aril weight. Under growing conditions in Thailand, Gac fruits harvested during summer season (Mach-June) had the highest lycopene content (1910.5 µg/g fresh weight), β-carotene content (207.06 µg/g fresh weight) and total carotenoid content (2117.60 µg/g fresh weight). Gac fruits harvested during rainy season (June-October) had the lowest lycopene content (1403.7 µg/g fresh weight) and total carotenoids content (1580.00 µg/g fresh weight), whereas Gac fruits harvested in the dry season (October-February) had the lowest β-carotene content (80.74 µg/g fresh weight).

Number of days to maturity ranged between 43.33 days in the rainy season to 67.11 days in the dry season, whereas the summer season had an intermediate Fig. 3 (d) the number of day to maturity (62.11 days). Season did not significantly affect fruit weight (551.47 to 647.12 g), fruit number (2.56 to 5.11 fruits per plant) and aril weight (95.82 to 130.98 g per fruit).

Genotypic variability: Although few genotypes recommended for production were evaluated, significant differences ($p \leq 0.05$ or 0.01) among Gac fruit genotypes were found for lycopene, *β*-carotene, total carotenoid content, fruit number and aril weight (except for fruit maturity and fruit weight) in combined analysis of variance (Table 1). When the data of individual seasons were analyzed, Gac fruit genotypes were significantly different ($p \leq 0.05$ or 0.01) at least in one season for lycopene, *β*-carotene, total carotenoid content, fruit maturity, number of fruits, fruit weight and aril weight (Table 2, 3). The data indicated that genotype and genotype by season interaction were important sources of variations in these traits.

G X E interaction: The interactions between season and variety were significant ($p \leq 0.05$ or 0.01) for lycopene, β-carotene, total carotenoid content, fruit maturity, fruit weight, and aril weight, but not for fruit number (Table 1). The highest interactions were found for lycopene and total carotenoids content. KKU ac.10-087and KKU ac.09-030 had the highest lycopene content in the summer season, whilst KKU ac.10-087 was also highest in the dry season. KKU ac.09-030 and KKU ac.11-148 had the highest β-carotene content in the summer season, and KKU ac.09-030 was also highest in the rainy season. KKU ac.09-030 had the highest total carotenoids content in the rainy season, whereas KKU ac.10-087 had the highest total carotenoids in dry season. KKU ac.09-030 was highest for fruit maturity in the dry season and for fruit number in the summer. KKU ac.10-087 was highest for fruit weight and aril weight in the dry season, whereas KKU ac.11-148 was highest for fruit number in the summer season.

Gac fruit genotypes were significantly different in only one season for fruit maturity, fruit weight, fruit number and aril weight (Table 3). KKU ac.09-030 had the highest fruit maturity (72 days) in the dry season and fruit number (6.00 fruits per plant) in the summer season. KKU ac.11-148 also had the highest fruit number (6.67 fruits per plant) in the summer season, which was not significantly different from 6.00 fruits per plant of KKU ac.09-030, whereas KKU ac.10-087 had highest fruit weight (829.33 g) and aril weight (202.47 g per fruit) in dry season.

Table 1. Mean squares for lycopene, *β*-carotene, total carotenoid content, fruit maturity, fruit weight, fruit number and aril weight of Gac fruit genotypes during 2012 to 2013.

Source	df	Lycopene (µg/g fresh weight)	β-carotene (µg/g fresh weight)	Total carotenoids (µg/g fresh weight)	Fruit maturity (days after flowering)	Fruit weight (g/fruit)	Fruit number (fruits/tree)	Aril weight (g/fruit)
Season (S)	2	578772*	39046**	676691*	373**	24357	15	2845
Rep./S	6	90796	781	86740	33	16100	5	739
Genotype (G)	2	1774590**	30624**	1906607**	132**	9583	19**	6945*
S x G	4	1188679**	5476*	1269426**	22	58552*	2	5410*
Pooled error		166016	1361	188696	16	13737	2	1373

* and ** = significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

Table 2. Lycopene, beta-carotene and total carotenoids content of Gac fruit genotypes grown in the summer season, rainy season and dry season during 2012 to 2013.

Genotype	Lycopene (µg/g fresh weight)			*β*-carotene (µg/g fresh weight)			Total carotenoids (µg/g fresh weight)		
	Summer	Rainy	Dry	Summer	Rainy	Dry	Summer	Rainy	Dry
KKU ac.09-030	2363.00a	2175.10	1461.00b	278.05a	272.10	91.47a	2641.10	2447.20a	1552.40b
KKU ac.10-087	2246.80a	829.00	2411.50a	120.94b	111.99	58.81b	2367.70	940.90b	2470.30a
KKU ac.11-148	1121.70b	1207.10	1150.10b	222.19a	144.69	91.94b	1343.90	1351.80ab	1242.00b
Mean	1910.50	1403.70	1674.20	207.06	176.26	80.74	2117.60	1580.00	1754.90
LSD	1000.20	1075.60	634.30	97.84	89.902	57.67	1053.10	1156.20	680.73

Means in the same column followed by the same letter(s) are not significantly different at $p \leq 0.05$ by LSD

Table 3. Fruit maturity (days), fruit weight (g/fruit) and aril weight (g/fruit) of Gac fruit genotypes grown in the summer season, rainy season and dry season during 2012 to 2013.

Genotype	Fruit maturity (days after flowering)			Fruit weight (g/fruit)			Fruit number (fruits/plant)			Aril weight (g/fruit)		
	Summer	Rainy	Dry	Summer	Rainy	Dry	Summer	Rainy	Dry	Summer	Rainy	Dry
KKU ac.09-030	62.00	57.33	72.00a	645.00	644.98	538.69b	6.00a	4.33	4.00	149.58	127.24	112.25ab
KKU ac.10-087	57.33	55.67	63.00b	545.50	433.78	829.33a	2.67b	2.00	1.33	119.11	75.07	202.47a
KKU ac.11-148	67.00	50.00	66.33ab	500.98	575.67	573.33b	6.67a	3.67	2.33	85.29	85.14	78.22b
Mean	62.11	43.33	67.11	563.83	551.47	647.12	5.11	3.33	2.56	117.99	95.82	130.98
LSD	11.75	11.34	5.90	230.01	341.46	205.56	2.72	4.34	3.16	97.65	58.79	90.59

Means in the same column followed by the same letter(s) are not significantly different at $p \leq 0.05$ by LSD

Discussion

Seasonal variations in lycopene, *β*-carotene and total carotenoids: High and uniform raw material quality is very important for production of the functional food products. Gac fruit has lycopene and *β*-carotene level higher than that of any known food crops (Chuyen *et al*., 2015). Seasonal variations in these phytochemicals in Gac fruit can affect the fruit quality of the crop, and information is important for the selection of stable varieties and for production planning.

In this study, seasons were found to significantly affect the levels so lycopene, *β*-carotene and total caroteniods content in Gac fruit. Summer season had the highest lycopene, *β*-carotene and total caroteniods content, rainy season had the lowest lycopene and total caroteniods content, and dry season had the lowest *β*-carotene content. Air temperature (Dumas *et al*., 2003), irrigation (Pek *et al*., 2014), relative humidity (Ehret *et al*., 2013) and light intensity (Ilic *et al*., 2012) can affect the synthesis of *β*-carotene and lycopene. A direct comparison of different studies for Gac fruit is not available as the crop is rather new for researchers. In the tomato, a well-known plant for high lycopene and *β*-carotene, light and temperature were the most important factors affecting *β*-carotene and lycopene synthesis (Dumas *et al*., 2003; Gautier *et al*., 2008). However, differences in seasons did not significantly effect the carotenoids content in cherry tomatoes, and the hot air-temperature of midsummer reduced lycopene (Rosales *et al*., 2006; Raffo *et al*., 2006) and *β*-carotene (Raffo *et al*., 2006). Relative humidity also reduced the accumulation of lycopene and *β*-carotene (Ehret *et al*., 2013). The reduction in *β*-carotene and lycopene in the rainy season in this study was possibly due to high humidity levels in this season.

However, the relationships between *β*-carotene, lycopene and temperature were similar to those reported from other studies. A temperature range between 21 and 26ºC was the optimum for lycopene production (Dumas *et al*., 2003; Gautier *et al*., 2008). The optimal temperature for *β*-carotene accumulation seems to be about 30ºC (Toor *et al*., 2006). The synthesis of lycopene was completely inhibited at 32ºC and as temperature increased from 30 to 35ºC the content of lycopene was reduced, but not that of β-carotene (Baqar & Lee 1978). At temperature higher than 35ºC, lycopene accumulation is inhibited and the conversion of lycopene into *β*-carotene was stimulated (Baqar & Lee 1978; Hamauzu *et al*., 1998; Krumbein *et al*., 2012). In this study, air temperature in the summer season increased from 28 to 32°C and lycopene content was also increased. The reduction in *β*-carotene was possibly due to the negative association between *β*-carotene and lycopene (Nakkanong *et al*., 2012). In previous study, the increase in temperature range from 27-32°C reduced lycopene and *β*-carotene and the authors pointed out that down-regulation of the phytoene syntheses gene played a key role in this reduction (Gautier *et al*., 2008).

In rainy season, the total caroteniods and lycopene contents of Gac fruits were lower than in dry and summer seasons. In rainy season, rain-fall may affect the amount of lycopene (Dumas *et al*., 2003). Similarly, irrigation also decreased lycopene and total caroteniod concentration in the tomato (Helyes *et al*., 2014; Pek *et al*., 2014). In contrast, soil water deficits did not have significant effect on the amount or distribution of β-carotene (Zushi & Matsuzoe, 1998).

In this study, the responses of three Gac fruit genotypes to dry season, rainy season and summer season were reported. As the environment greatly affected lycopene, *β*-carotene and total catotenoids, the three genotypes of Gac fruit performed differently in these seasons. Because the information in literature is limited, direct comparison of the results in different studies is not possible for Gac fruit. However, the results can be compared with those in other crops. For the tomato, lycopene content varied significantly among tomato varieties, and cherry tomato had higher lycopene content than F_1 hybrid tomatoes and round tomatoes (Kuti & Konuru, 2005). In oranges, the genetic variability for carotenoids was observed among sweet orange cultivars (Dhuique-Mayer *et al*., 2009). Results indicate that genetics and environments may have more effect on lycopene content in Gac fruit.

The result obtained in this study is useful for production planning of Gac fruit for use as raw material for functional food products, and for the selection of Gac fruit genotypes with high and stable phytochemicals. Variations in lycopene, *β*-carotene and total carotenoids indicated that improvement of these phytochemicals through breeding is possible. Gac fruit is known to possess these phytochemicals in much higher levels than in the tomato and other crops, and has high potential as a functional food.

Seasonal variations for maturity and fruit number: Days to maturity of Gac fruit in the dry was longer than in summer and rainy season. Differences in temperature affected fruit ripening in Gac fruit, and low temperatures in the dry season delayed maturity. The effect of temperature on fruit ripening was similar to those reported in other crop species. Low temperature increased days to maturity of fruits, and the effect of temperature could be expressed as a thermal time relationship (Adams *et al*., 2001). Difference in temperature is a factor influencing crop development such as fruit maturity, referred to as thermal units or degree days (Hurd *et al*., 1985; Baker & Reddy 2001; Souza *et al*., 2011). The thermal sum accumulated in the plant activates its maturity stage when temperature exceeds a base temperature (Marra *et al*., 2001).

Seasons yielded significantly different results for fruit number, and summer season had the highest fruit number. Gac fruit genotypes were also significantly different for fruit number in the summer seasons, but they were not significantly different in dry season and rainy season. As mentioned earlier the data on Gac fruit for direct comparison is not available, however, in cucumber, which is similar to Gac fruit in terms of fruiting and climbing growth habit, crops planted in April had higher numbers of fruit than those planted in May and June (Eifediyi & Remison, 2009). Low yields of the crop planted in rainy season would possibly be due to high rainfall during flowering and fruiting, leading to an inactivity of bees to pollinate the flowers and the subsequent abortion of flowers (Eifediyi & Remison, 2009).

In the commercial production of Gac fruit, fruit number is an important yield component. However, other yield components such as aril weight and lycopene content are also important and should be used as criteria for the selection of commercial varieties.

Conclusion

In conclusion, season was an important source of variations in lycopene, β-carotene, total carotenoids and fruit maturity in Gac fruit. Differences in varieties were also significant for these parameters. Furthermore, genotype by season interactions was important sources of variations in these traits. As the interactions between season and genotype was significant for most traits, the identification of superior genotypes in this study proved difficult. However, genotypes with good and consistent performance could be identified. KKU ac.09-030 showed high lycopene, β-carotene, fruit number and aril weight in all seasons, whereas in comparison KKU ac.10-087 had low β-carotene content in all seasons. These data are useful for crop management and production planning for high fruit quality and sources of carotenoid contents in the functional food industry. Good agronomic practices associated with production technology may be adopted for farmers, such as pruning scheduling and irrigation levels, in order to control the flowering and ripening of fruits for specific harvest periods, and to design a food supply chain for the functional food industry.

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