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Original Research Article

Determination of main pigments segregation in tangerine and red fruit tomato hybrid

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Abstract: Carotenoids are natural fat-soluble pigments that are common in colorful plants. They act as provitamin A and beneficial for human health, especially for the vision. Lycopene and beta-carotene which belong to carotenoids are quantitative traits that found as main pigments in tomatao. This experiment was conducted to study segregation of lycopene, beta-carotene, chlorophyll content and color values (*L a b*) in F_2 population of tangerine and red fruit tomato. Tangerine tomato (Jinzhu No.1- P1) accumulated the high level of beta-carotene in contrast with red tomato (TTD1003A- P2) whereas red tomato accumulated the high level of lycopene. Tangerine and red tomato crossing produced orange pigmented F_1 fruits with higher lycopene content and beta-carotene similar to those noted in to P_1 parental line. The average lycopene content in F_2 generation was 0.203 mg/100 g Fresh weight (FW) while the beta-carotene content was 0.279 mg/100 g FW. Beta-carotene content was ranged from 0 to 0.57 mg/100 g FW. Mid-parent heterosis of lycopene, beta-carotene, total chlorophyll and color values (*L a b*) were -59.08 44.24 -41.18 -5.28 -16.22 and -7.97 %, respectively. For distribution testing, only beta-carotene and *a*-value fit a normal distribution model (Shapiro-Wilk test: *P*-value; 0.131 and 0.243, respectively). The best estimate for lycopene content was obtained using *a/b* ratio, r = 0.772. Beta-carotene had a linear positive correlation with *L*-value and *b*-value (r = 0.373 ** and r = 0.392**). The results of this study can be utilized for high nutrition tomato breeding programs, as present commercial tomato cultivars focused on red color for the selection criterion, regardless the lycopene content and other properties of consuming.
Keywords: tomato, quantitative trait, mid-parent heterosis, lycopene, color values (*L a b*).

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a major vegetable crop and commonly grown in all over the world. Tomatoes are a good source of nutrients and vitamins. Lycopene is one of the most important natural carotenoids found in tomato, which is the main pigment that giving red color to tomatoes [15, 18]. Lycopene is a very powerful antioxidant and many researches have been reported the healthy benefits of lycopene including heart health, and reducing the risk of some types of cancers [1, 3, 9, 12]. Other carotenoids such as beta-carotene are also presented in tomato. Beta-carotene is a precursor of vitamin A. Several researchers have shown that beta-carotene inhibits the oxidation of other molecules, protects the body from free radicals, and essential for human immune system [2, 6, 8].

The previous studies have been reported that lycopene content in F_2 population derived from a cross between the domestic and wild tomato species (*Solanum lycopersicum* and *S. pimpinellifolium*) was normally distributed [17]. In addition to that, total carotenoids content (phytoene, ζ-carotene, β-carotene, α-carotene, and lycopene) of carrot obtained from crossing with different backgrounds (orange Brasilia × dark orange HCM) in F_2 population also showed normal distribution [4]. It showed that lycopene content and total carotenoids content are agriculturally important quantitative traits which controlled by many genes.

Lycopene and beta-carotene are a good source of potential health beneficial carotenoids in tomato. The objective of this research was to study the main pigments segregation in F_2 population of tomato in order to apply in high nutrition tomato breeding program.

MATERIALS METHODS

Plant materials

Tangerine tomato Jinzhu No.1 (P_1) and red tomato TTD1003A (P_2) were used as parental genotypes. The F_1 generation was derived from a cross between P_1 and P_2. The F_2 generation was obtained by self-pollinating the F_1 plants. All fruits were harvested at full maturity.

The tomatoes were cultivated under greenhouse covered with polymeric film during March-July 2016 at the research green house of the Northwest A&F University, Shaanxi province, China.

Fruit color measurement

Color of each fruit was measured by a Chroma meter CR-400 (Konica Minolta®). The scales for color measurements in the *L a b* color Hunter system, were as follows. *L*-value indicates the level of light or darkness, *a*-value indicates redness or greenness, and *b*-value for yellowness or blueness.

Pigment extraction

The content of lycopene, beta-carotene and chlorophyll were determined based on a spectrophotometric analysis according to the method described by Nagata and Yamashita [13]. Briefly, 1 g of tomato sample was homogenized with 15 ml of Acetone–Hexane (4:6) solvent. After homogenization, two phases were separated and the upper solution was used for the measurements. The absorbance was measured with a UV-3802 spectrophotometer (Unico®) at 663, 645, 505, and 453 nm. Total cholorophyll, lycopene and beta-carotene were calculated using the following equations:

Total chlorophyll = Chlorophyll a + Chlorophyll b
Chlorophyll a = 0.999×A663 – 0.0989×A645
Chlorophyll b = –0.328×A663+ 1.77×A645
Lycopene= $-0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453}$
Beta-carotene = $0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$ (A663, A645, A505 and A453 are the absorbance at 663, 645, 505 and 453 nm, respectively.

Lycopene, beta-carotene and total chlorophyll contents were expressed as mg/100g fresh weight (FW)).

Mid-parent heterosis estimation

The levels of mid-parent heterosis of the F1 hybrid were estimated over the mean value of the two parents using the following formula:

$$\text{Mid-parent heterosis (\%)} = \frac{F_1 - MP}{MP} \times 100$$

(F_1 is mean of F_1 and MP is mean of two parents)

STATISTICAL ANALYSIS

Main pigments content and fruit color data were analyzed using Pearson's correlation coefficients and distribution frequencies among F_2 generations were analyzed by Shapiro–Wilk test. Statistical analysis was performed using SPSS version 23.

RESULTS AND DISCUSSION

The quantity of beta-carotene presented in parental lines of tangerine tomato (P_1) and red tomato (P_2) were 0.253 and 0.077 mg/100g FW respectively. Both F_1 and F_2 progenies produced similar quantities of beta- carotene in tangerine parental line such as, 0.238 and 0.279 mg/100g FW respectively. The result showed the higher beta-carotene content in tangerine fruit than red fruit. These data support a suggestion of dominant allele *B* enhanced the expression of lycopene β-cyclase (Lcy-B) enzyme that converts lycopene to beta-carotene. Due to that beta-carotene, will be increased while decreasing the lycopene accumulation [7, 10]. Beta-carotene content in 152 fruit samples in F_2 generation showed a normal distribution (Shapiro-Wilk test: P-value; 0.131) in the range of 0 to 0.57 mg/100g FW (table1). It showed that beta-carotene content is a quantitative trait which controlled by poly-genes. The measurement of heterosis percentage found that beta-carotene was the only one positive percentage of heterosis (44.24), indicating that the cross combination of tangerine tomato (JinzhuNo.1) × red tomato (TTD1003A) was the best and specific cross combinations for beta-carotene accumulation.

The average lycopene quantity in tangerine tomato and red tomato were 0.011 and 0.424 mg/100g FW respectively. Lycopene content in the F_1 progeny was comparatively higher (0.089 mg/100g FW) than P_1. In F_2 progeny this value was at the intermediate level between the two parents. Tangerine tomato (P_1), red tomato (P_2) and their progeny produced small amounts of total chlorophyll content in all generations and it was not-normally distributed in F_2 generation (table1).

Fruit color measurements were taken using the *L a b* color Hunter system, which *L*-value indicates the level of lightness, *a*-value the redness or greenness and *b*-value yellowness or blueness. The research results showed the fruit color in F_1 generation derived from the cross between tangerine tomato (Jinzhu No.1) and red tomato (TTD1003A) were orange with color value (*L a b*) 37.82, 11.78 and 17.1, respectively and as reported earlier, the *B* allele produced orange fruits instead of red fruit due to the high levels of beta-carotene and low levels of lycopene. F_2 generation showed various colors in orange to red color levels with a wide range of color values (*L*= 29.43-45.37, *a*= 4.14-22.35, *b*= 8.71-23.73). This color variation influenced by genetic control of lycopene and beta-carotene concentration [16]. However, *a*-value which indicates the level of redness showed the distribution resembles to the bell-shaped curve for a normal distribution (Shapiro-Wilk test: *P*-value; 0.243) (table1, figure 2).

Table 1: Color values (*L a b*), beta-carotene, lycopene, total chlorophyll (mg/100g FW) and mid-parent heterosis

	L-value	*a*-value	*b*-value	Beta-carotene	Lycopene	Total chlorophyll
P_1	44.98	10.37	22.78	0.253	0.011	0.046
P_2	34.88	17.75	14.38	0.077	0.424	0.022
F_1	37.82	11.78	17.1	0.238	0.089	0.02
F_2 (mean)	37.66	12.49	16.81	0.279	0.203	0.024
F_2 (ranking)	29.43-45.37	4.14-22.35	8.71-23.73	0 -0.57	0-0.93	0-0.08
F_2 (Shapiro-Wilk value)	0.974	0.988	0.962	0.986	0.715	0.931
F_2 (Shapiro-Wilk: test)	0.006	0.243	0	0.131	0	0
Heterosis (%)	-5.28	-16.22	-7.97	44.242	-59.08	-41.18

Table 2: The correlation analysis of color values (*L a b*) and main pigment content in F_2 population

	L-value	a-value	b-value	a/b ratio	Beta-carotene	Lycopene	Cholophyll
L-value	1						
a-value	-.382**	1					
b-value	.989**	-.363**	1				
a/b ratio	-.792**	.827**	-.795**	1			
Beta-carotene	.373**	-.253**	.392**	-.449**	1		
Lycopene	-.693**	.464**	-.718**	.772**	-.480**	1	
Chlorophyll	-.020	.020	-.001	.004	.050	.041	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

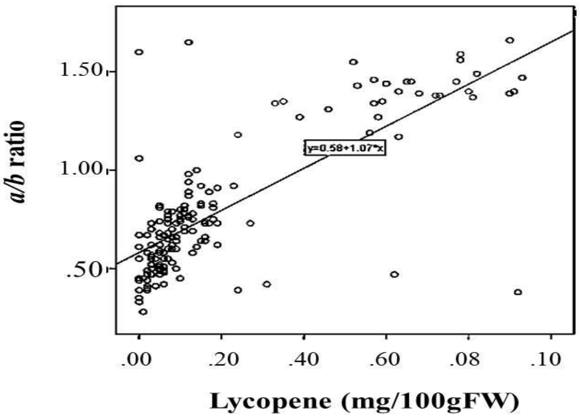

Fig-1: Relationship between the *a/b* ratio of fruit color and lycopene content of tomato flesh

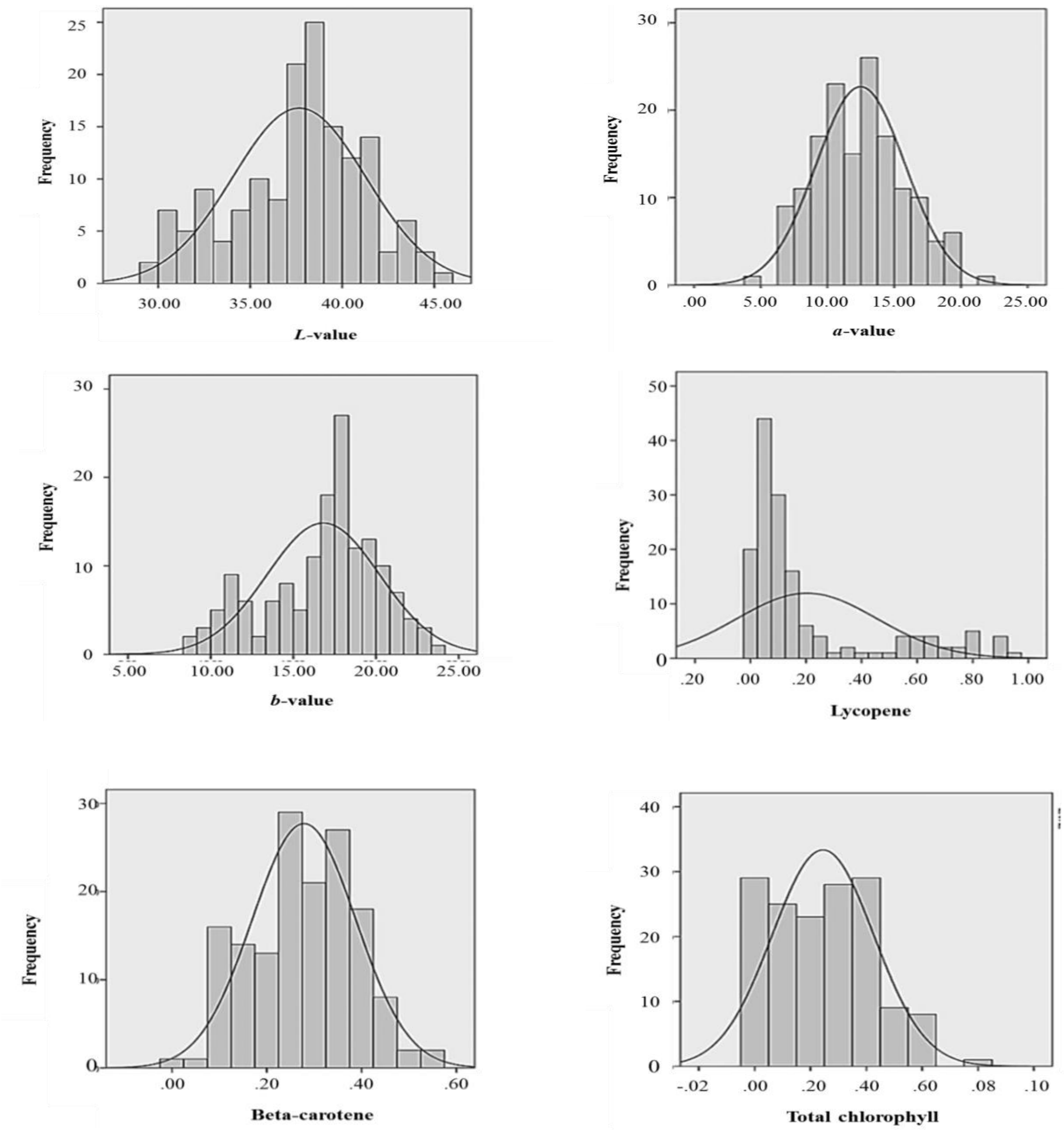

Fig 2: Histogram of the frequency distribution of main pigments and color values (*L a b*) in the F_2 population

To determine the relationships among the analyzed traits, a Pearson correlation coefficient analysis was performed as showed in Table2. Lycopene and *a/b* ratio showed the best positively correlated coefficient of r = 0.772 ** and *a*-value was also correlated with lycopene (r = 0.464**) in contrast with *L*-value and *b*-value (r = -0.693** and r = -0.718**) (Figure 1). As summarized by Liu [11], the correlation between color related traits in the *Lycopersicon pennellii* IL populations, *L** and *a** values were correlated to lycopene content (-0.73 and 0.69). Chen [5] and Saad AG et al.; [14] have been reported, the *a*/b** ratio often used as an indicator for estimate the accumulation of lycopene content. Beta-carotene showed the positive significant correlation with *L*-value and *b*-value (r = 0.373 ** and r = 0.392**). Whereas, beta-carotene was negatively correlated with *a*-value and *a/b* ratio (r = -0.253** and r = -0.449**). Total chlorophyll content was not correlated with any parameter.

CONCLUSION

In this research, we found different quantities of principal carotenoids of beta-carotene and lycopene in two parental tomato varieties. The higher level of beta-carotene was found in tangerine tomato (Jinzhu No.1) while, lycopene was higher in red tomato (TTD1003A). The F1 plants of tangerine and red fruit tomatoes produced fruits with a higher level of both lycopene and beta-carotene. Carotenoid content in F2 plants were identified as a quantitative heritable trait and this cross combination can be utilized for high nutrition tomatoes breeding program. F2 progeny displayed a wide range of color values. These color values (*L a b*) could be useful for estimating beta-carotene and lycopene contents in tomato.

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