

Breeding for Color and Lycopene Content: The Role of Interacting Loci.

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We are interested in improving selection efficiency for color and lycopene content in tomatoes. The correlation between color and lycopene content suggests that selection for improved color may provide an inexpensive alternative to chemical measurement. Objective measurements of color, chromaticity values, have been used to estimate the concentration of lycopene as a tomato matures (D'Souza et al, 1992; Arias et al., 2000). Chemical measurement of lycopene can be accomplished by spectrophotometric methods of extracts and by High Pressure Liquid Chromatography (HPLC) using a polymeric C₃₀ column (Emenhiser et al., 1995). The advantage of HPLC methodology is that individual pigments can be quantified and isomers can be separated. Thus the concentration of all-trans-lycopene, the most significant red pigmented isomer, can be accurately measured. Because the published regression equations are based on maturity stages, it is unclear how well they predict the lycopene content of ripe fruit from different genotypes. The results reported at this meeting were obtained from studies conducted to determine the relationship between color and lycopene content in a breeding population consisting of adapted germplasm, to compare the efficiency of direct selection for lycopene content with selection for color, and to gain insights into the genetic basis of variation for color and lycopene content within adapted germplasm.

Estimating Lycopene Content from Color. We developed regression equations for lycopene content using chromicity values and HPLC measurements of lycopene content obtained for 39 genotypes. Objective color measurements of tomato fruit were based on hue (tint of color), L (darkness or lightness of color), and chroma (vividness of color). These measurements were obtained using a Minolta CR300 colorimeter and the CIELAB color space (CIE, 1978). The a/b relationship common in tomato evaluation shows a strong negative correlation with Hue, but the relationship is truly linear through only a narrow range of Hue values. The content of lycopene for the 39 genotypes ranged from 8 to 14 mg/100 gm dry wt. when data were averaged across trial replicates. Three equations had significant linear correlations between lycopene concentration and objective measures of color:

$$\begin{aligned} [\text{lycopene}] &= -0.425 \times L + 29.36 \quad p = 0.0005 \quad r^2 = 0.66 \\ [\text{lycopene}] &= -0.196 \times \text{Hue} + 21.32 \quad p = 0.0064 \quad r^2 = 0.551 \\ [\text{lycopene}] &= 7.926 \times (a/b) + 4.93 \quad p = 0.0036 \quad r^2 = 0.582 \end{aligned}$$

In order to test the performance of these equations, we applied them to an independent population consisting of 19 selections and 3 high lycopene varieties. The observed range of lycopene was 8 to 25 mg/100 gm dry wt. Previously published equations failed to predict the lycopene content of ripe fruit within 30% of the measured levels (Table 1). In contrast, the three equations we developed had predictive value within 11% to 22% (Table 1). The greater ability to predict lycopene content was noteworthy because the correlation coefficients for our equations are lower than those published by D'Souza (1992) and Arias (2000). It is likely that these equations are biased due to the inclusion of unripe tomatoes, and therefore unsuitable to estimating the lycopene content of populations intended for once over harvest. The effect of including green tomatoes with very little lycopene results in a regression line with influential points that are outside of the portion of the curve relevant for predicting lycopene content in red fruit. At the same time these influential points have the effect of lowering the correlation coefficients by dramatically extending the line over a much larger range of values. The tendency

of both sets of published equations is to over-predict the lycopene content of ripe tomatoes is consistent with low lycopene green tomatoes leveraging the slope of the regression line.

Table 1. Prediction of lycopene content from linear regression equations

Average Difference ²	Regression equation ¹					
	D'Souza (a/b) ²	Arias a/b	Arias (a/b) ²	L	Hue	a/b
Population	5.73	4.66	4.72	1.39	1.69	2.94
Percentage Difference ³						
Population	44.4%	36.1%	36.6%	10.8%	13.1%	22.8%

¹Regression equations are from D'Souza et al, (1992) Lycopene = 7.12 x (a/b)² + 5.60; Arias et al. (2000) lycopene = 11.848 x (a/b) + 1.55 and = 8.707 x (a/b)² + 1.52; and from this manuscript lycopene = -0.425 x L + 29.36, = -0.196 x Hue + 21.32, and = 7.926 x (a/b) + 4.93.

²The absolute value of the average of all differences between the lycopene content estimated using the regression equation and the direct measurement of lycopene content.

³The average of differences as a percentage between the estimated lycopene content based on regression and the direct measurement of lycopene content.

Relative efficiency of selection. Having established that we could estimate the content of lycopene from our color measurements, we wanted to estimate the relative efficiency of selection.

Variance components were estimated for the genotypic component of each trait, and Heritability estimates were obtained according to the relationship $H = \sigma^2_G / [\sigma^2_G + (\sigma^2_E)/rep]$. In these analyses the traits were either objective measurements of color from replicated field trials or direct measurements of lycopene content using HPLC or UV/visual spectrophotometric ratios. Genetic correlations were calculated from the variance components of each pair of traits (x, y) and for the combined trait (x+y). The genetic correlation for traits was calculated as described by Falconer and MacKay (1996) as $Corr = cov(x,y) / \sqrt{[\sigma^2(x) \times \sigma^2(y)]}$ where $cov(x,y) = [\sigma^2(x+y) - \sigma^2(x) - \sigma^2(y)] / 2$ (Equation 19.2). The relative efficiency of selection was then estimated from the ratio of the heritabilities multiplied by the genetic correlation [Relative efficiency of selection = $r_{gen} \times (H_{selected\ trait} / H_{target\ trait})$]. Calculations are based on heritability estimates for two-year single-location data. Estimates are 18% to 21% lower when variance components from multi-year multi-location trials are used to estimate heritability. The relative efficiency of selection is high when L values are used (Table 1).

Table 1. Relative efficiency of selection¹ for lycopene content using chromicity values.

	L	Hue	UV/vis	HPLC
L			0.56	0.72
Hue			0.45	0.40
UV/vis				0.82

Evidence for Interacting Loci within L. esculentum Germplasm. Previous work in our program demonstrated that there is significant genetic variation for color within *L. esculentum* germplasm (Sacks and Francis, 2001). In order to further investigate the genetic basis of this variation, an F₂ population derived from crossing IBL 2349 with Ohio 8245 was evaluated for color in replicated

field trials. Objective color measurements of tomato fruit using the CIELAB color space (CIE, 1978) and the sampling scheme described by Sacks and Francis (2001) provided the phenotypic data for analysis. Realized heritability estimates for hue, L and chroma were moderate to high. By screening the population with molecular markers, two independent *L. esculentum* QTL were found to be associated with the improvement of tomato color. These QTL were linked to RAPD markers UBC-192 and OPBB-09 and linkage was confirmed in F₃ and F₄ families. The QTL marked by UBC-192 maps to chromosome 4 while the QTL marked by OPBB-09 maps to chromosome 11. Total phenotypic variation explained by UBC-192 ranged from 14-80% and phenotypic variation explained by OPBB-09 ranged from 24-44% within and across environments for F₃ and F₄ families.

Epistatic interactions were identified between UBC-192 and OPBB-09 (Table 3). Unexpectedly, the two *L. esculentum* QTL also appeared to interact positively with an introgression from LA407 on chromosome 7 of IBL 2359. This locus was not identified as a QTL in isolation. The QTL identified in this study and their epistatic interactions may provide alternative genes for the improvement of red-fruited tomatoes in breeding programs.

Table 3. Epistatic interactions between pairs of polymorphic RAPD and RFLP markers evaluated in an IBL 2349 x Ohio 8245 derived F₂ population.

Marker ¹	Chrm	Marker ¹	Chrm	p-value		
				Hue	L	Chroma
UBC-192	4	OPBB-09	11	0.0001	0.0002	0.0001
		CT114	7	0.3769	0.7530	0.0001
		TG199	7	0.0150	0.1108	0.0001
OPBB-09	11	UBC-192	4	0.0001	0.0002	0.0001
		CT114	7	0.0004	0.0004	0.4343
		TG199	7	0.0007	0.0002	0.2962

¹ UBC-192 allele of Ohio 8245; OPBB-09 allele of IBL 2349 (Hunt 100); CT114, TG199, TG216 alleles of *L. hirsutum* LA407.

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