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Original Research Paper

Carotenoid Content in Cherry Tomatoes Correlated to the Color Space Values L*, a*, b*: A Non-destructive Method of Estimation

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ABSTRACT

Cherry tomatoes are rich sources of carotenoids. The carotenoids are known to be precursors of vitamin A and also act as an antioxidant. It is important to visually judge the tomato surface color for higher β carotene content since this is the major provitamin A carotenoid. Estimation of carotenoids by HPLC (High Performance Liquid Chromatography) and spectrophotometric methods in tomatoes are very expensive and time consuming. Therefore, colorimeters can be used to describe the color and determine the carotenoid content in a relatively easy and inexpensive manner. The objective of this study was to determine, if the carotenoid content within cherry tomatoes measured by conventional method could correlate with colorimetric CIE (Commission International del'Eclairage) L*, a*, b* color space values. Strong correlations were found between color surface value a* and total carotenoids (0.82) and lycopene content (0.87). We also observed positive correlation for the b* color value with β carotene (0.86). The L* value was negatively correlated (-0.78) with an increase in carotenoids. These close associations between color space values L*, a*, b* and carotenoids will help the breeders to quickly screen large germplasm/ breeding lines in their breeding program for improvement in carotenoid content through this time saving, inexpensive and nondestructive method at fully ripe stage.

Keywords: β carotene, Carotenoid, Lycopene, Tomato.

INTRODUCTION

Color is one of the important quality parameters of fruits and vegetables. The color of tomatoes is the most important quality character to determine the ripeness. The color of tomatoes is the initial external factor that makes them appealing to the consumer's decision for purchasing them. The complexity of tomato color is due to the presence of a diverse carotenoid pigment system with appearance conditioned by pigment types and concentrations, and subject to both genetic and environmental regulation (Radzevicius *et al.,* 2014). Color of tomatoes is an important desired character which can be achieved by genetic improvement of breeding lines with varying concentration of carotenoids. The tomatoes are harvested and consumed at the red ripe stage of ripening, which occurs due to the degradation of chlorophyll at green stage and rapid accumulation of carotenoids particularly lycopene and β carotene. In this study, we have assessed surface color differences among the cherry tomatoes and its relation to their total carotenoid, lycopene and β carotene content. Carotenoid content in fruits can be assessed in laboratory through spectrophotometer measurement of tomato fruit extracts, but this method is time consuming and tedious (Lichtenthaler 1987). Colorimeters can be used to determine the carotenoid content in fruits and vegetables in a quick, easy and in a non-destructive manner. In 1931, the Commission International del'Eclairage (CIE) made possible to express color in exact quantitative and numerical

terms. An improvement of this system was developed in 1976 by CIE, which defines color better related to human perception and where all conceivable colors can be located within the color sphere defined by three perpendicular axes, L* (from white to black),a* (green to red) and b* (blue to yellow). In the present study, an attempt was made to correlate tomato surface color values with actual carotenoids content so as to standardize a fast, inexpensive and non-destructive method.

MATERIALS AND METHODS

Plant material: Nine different cherry tomato lines such as IIHR 2754, IIHR 2857, IIHR 2858, IIHR 2861, IIHR 2862, IIHR 2863, IIHR 2864, IIHR 2865, IIHR 2866 were grown in the open field at ICAR–Indian Institute of Horticultural Research, Bengaluru, India. Fruits were harvested in ripe stages and brought to the laboratory for further examination of color and estimation of carotenoids.

Carotenoid profiling : Total carotenoids and lycopene content were analyzed by spectrophotometry method (Lichtenthaler 1987). Carotenoids were extracted using acetone and partitioned with hexane for the ripe stage.The carotenoids in the extract were estimated by reading absorbance at 470 and 503 nm for estimating total carotenoids and lycopene respectively. Thecarotenoid profiling was done using UPLC, as per themethod reported by Serino *et al*. (2009) with modifications.

Color measurement : The surface color (values of L*,a*, b*, C* and hue angle) was measured on fresh tomatoes using a color Reader, CR-10 (Minolta Co. Ltd, Osaka, Japan; measuring area of 8mm with 8/d viewing geometry using CIE Standard Illuminant D65). Three different measurements were taken at three equidistant points on the equatorial region of individual fruit. The value L*(lightness) indicates the ratio of white and black color, value a* is the ratio of red and green colors, value b* is the ratio of yellow and blue colors. Chroma/Chromaticity (C*) is the saturation or vividness of color. As chromaticity increases, acolor becomes more intense; as it decreases, a color becomes more dull. Hue angle isthe basic unit of color. Both chroma and hue are derived from a* andb* using the following equations: chroma: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$and hue angle: $h^0 = \tan^{-1}(b^*/a^*)^0$ (Itle *et al.*, 2009). It should be noted that all color space values L*, C, a* and b* are measured in NBS units, hue angle h° in degrees from 0 to 360°. NBS unit is a unit of USA National Standard Bureau and corresponds to one threshold of color distinction power, *viz*. the least distinction in color, which the trained human eye can notice (Juskeviciene *et al.*, 2014).

***Statistical analysis*:** Correlation analysis and regression analysis was conducted for total carotenoids, lycopene and β carotene with color space values using statistical package SPSS ver. 19 (SPSS Inc., Chicago, IL, USA) software (Wellman 1998). Microsoft Excel program was used to plot the scatter plot and calculate regression equation. Mean cd and standard error was also calculated.

RESULTS

Colorimetric measurements: Significant differences were observed among the cherry tomato lines for color values L*, a*, b*, C* and hue angle. Beginning with the L* value a range from lightness (48.9) to darkness (37.40) was observed in tomato lines evaluated. Highest L* value of 48.9 NBS units was observed for IIHR 2866 and IIHR 2754 showed the least L* value of 37.40. Mean color space value a* ranged from 35.43 in IIHR 2754 to 18.03 in IIHR 2866. The mean color space value b* ranged from 42.99 in IIHR 2866 to 21.03 in IIHR 2754. C*, Chroma/chromaticity ranged from 46.61 in IIHR 2866 to 34.46 in IIHR 2857. Hue angle ranged from 67.25^0 in IIHR 2866 to 30.69^0 in IIHR 2754 (Table. 1 & Fig. 1). There was a significant difference in the total carotenoid content among the tomato lines. The dark red fruit line IIHR 2754 contained highest carotenoid content with 23.80 mg/100g FW. The lycopene content was also more in IIHR 2754 with 15.10 mg/100g FW and β-carotene content was 3.02 mg/100g FW. IIHR 2865 contained the least amount of carotenoids with 8.20mg/100g FW. IIHR 2866 contained the lowest lycopene content 0.85 mg/100g FW and highest β-carotene content 8.56mg/100g FW (Fig. 2).

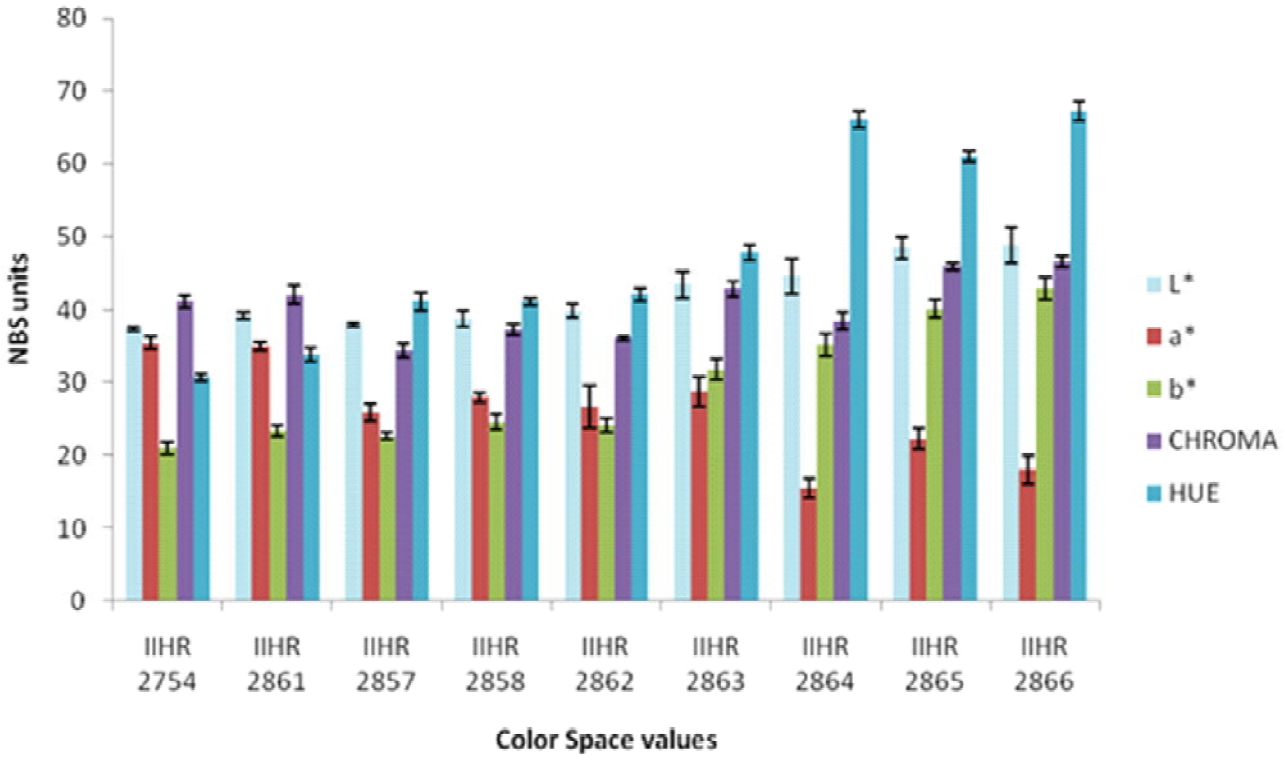


Fig. 1. Color indexes L*, a*, b*, C* and hue angle in cherry tomato lines. Error bars indicate the extent of variation among genotypes.

Table 1. Each observation is a mean ±SD of three replicate experiments of color indexes L*, a*, b*, C*, hue angle (h°) and total carotenoids, lycopene and β-carotene content in cherry tomato lines.

GENOTYPES	L	a*	b*	Chroma angle (h°)	Hue	Tot car	Lycopene	β-carotene
IIHR 2754	37.40 ± 0.49	35.43 ± 0.62	21.03 ± 0.70	41.21 ± 1.25	30.69 ± 0.98	23.80 ± 1.26	15.10 ± 0.69	3.02 ± 1.25
IIHR 2861	39.20 ± 0.29	34.93 ± 1.16	23.37 ± 0.54	42.03 ± 0.96	33.78 ± 1.25	17.90 ± 1.54	11.60 ± 0.87	1.23 ± 0.96
IIHR 2857	37.93 ± 1.08	25.93 ± 0.70	22.70 ± 1.04	34.46 ± 0.69	41.20 ± 0.54	13.70 ± 2.36	8.30 ± 1.06	1.79 ± 0.69
IIHR 2858	38.77 ± 0.94	28.07 ± 2.98	24.53 ± 0.97	37.28 ± 0.25	41.16 ± 0.85	12.10 ± 0.98	5.20 ± 1.89	0.80 ± 0.25
IIHR 2862	39.83 ± 1.89	26.73 ± 1.98	24.20 ± 1.37	36.06 ± 1.02	42.15 ± 1.06	11.00 ± 1.06	6.20 ± 1.65	1.63 ± 1.02
IIHR 2863	43.43 ± 2.36	28.83 ± 1.41	31.80 ± 1.54	42.93 ± 1.26	47.80 ± 1.15	10.70 ± 1.54	6.10 ± 0.84	1.87 ± 1.26
IIHR 2864	44.60 ± 1.47	15.57 ± 1.47	35.13 ± 1.21	38.43 ± 0.48	66.10 ± 0.75	9.74 ± 1.97	3.30 ± 1.78	6.44 ± 0.48
IIHR 2865	48.50 ± 2.50	22.30 ± 1.96	40.13 ± 1.58	45.91 ± 0.78	60.94 ± 1.35	8.20 ± 2.01	2.00 ± 1.30	5.65 ± 0.78
IIHR 2866	48.90 ± 0.69	18.03 ± 1.02	42.99 ± 0.66	46.61 ± 1.36	67.25 ± 1.06	9.50 ± 1.23	0.85 ± 0.56	8.56 ± 1.36

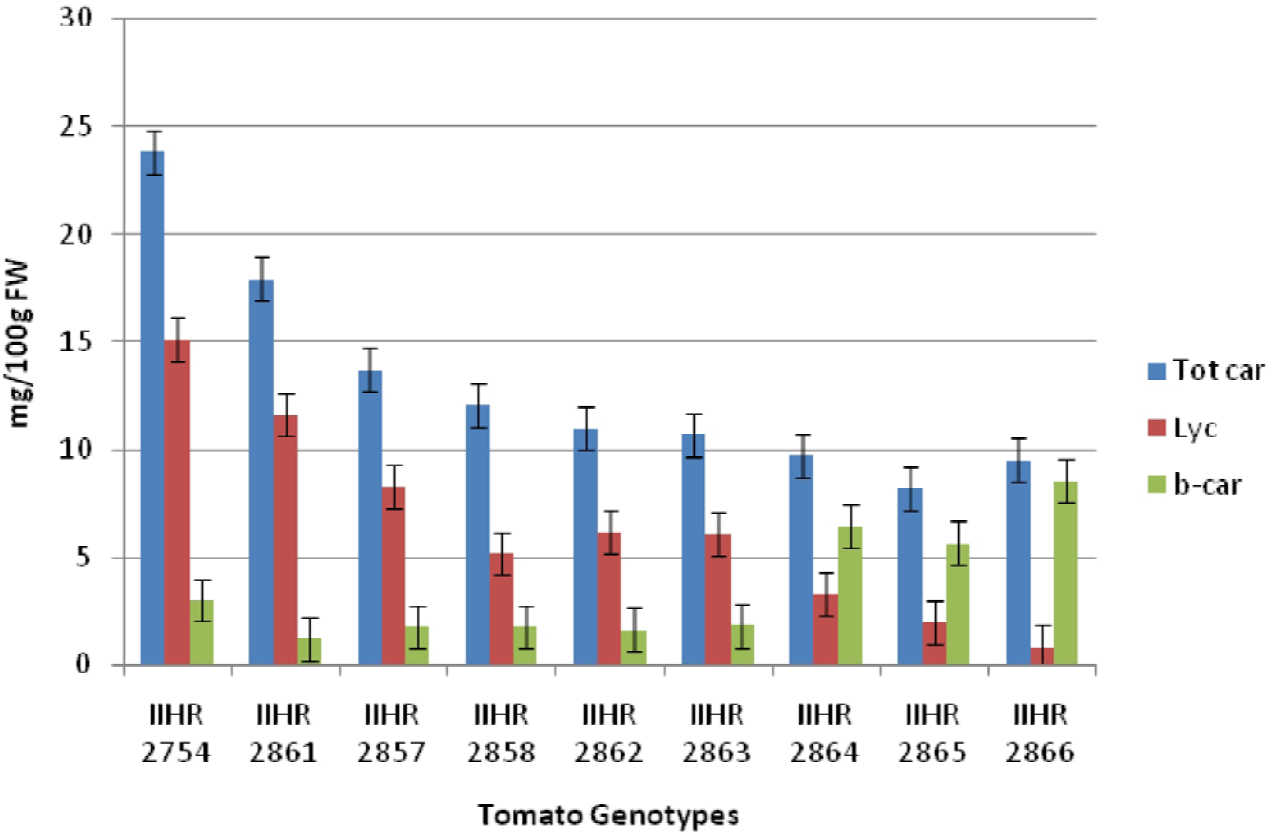


Fig. 2. Total carotenoids, lycopene and β-carotene content in cherry tomato lines. Error bars indicate the extent of variation among genotypes.

DISCUSSION

The color change in tomato is primarily observed from the immature green stage to the red ripe stage. During the process of ripening chlorophyll gets disappeared and carotenoids start accumulating giving the red or the orange color in tomatoes. Color is an important quality attributes in the food and bioprocess industries, and it influences the consumer's choice and preferences (Pathare *et al.*, 2013). Most of the tomato literature defines color in terms of the achromatic descriptors *viz*. L*, a*, b*. The color indexes a* and b* are combined and used by various researchers in different mathematical models to express color changes (Lopez Camelo *et al.*, 2004) in tomato.

In this study, cherry tomato lines were studied for surface color changes associated with carotenoid content in them. The cherry tomato lines used in this study included both red and the orange colored tomatoes. Lightness (L*) values ranged from 48.9 to 37.40. We observed that the L* value which indicates lightness was more in orange fruited tomatoes compared to the red tomatoes, this is because red colored tomatoes synthesize more lycopene and appear darker than the orange colored tomatoes. The L* value of IIHR 2866 was highest (48.9 NBS units) and these tomatoes were lighter than the red colored tomatoes with lower L* values in genotypes such as IIHR 2754 (37.40), IIHR 2861 (39.2).

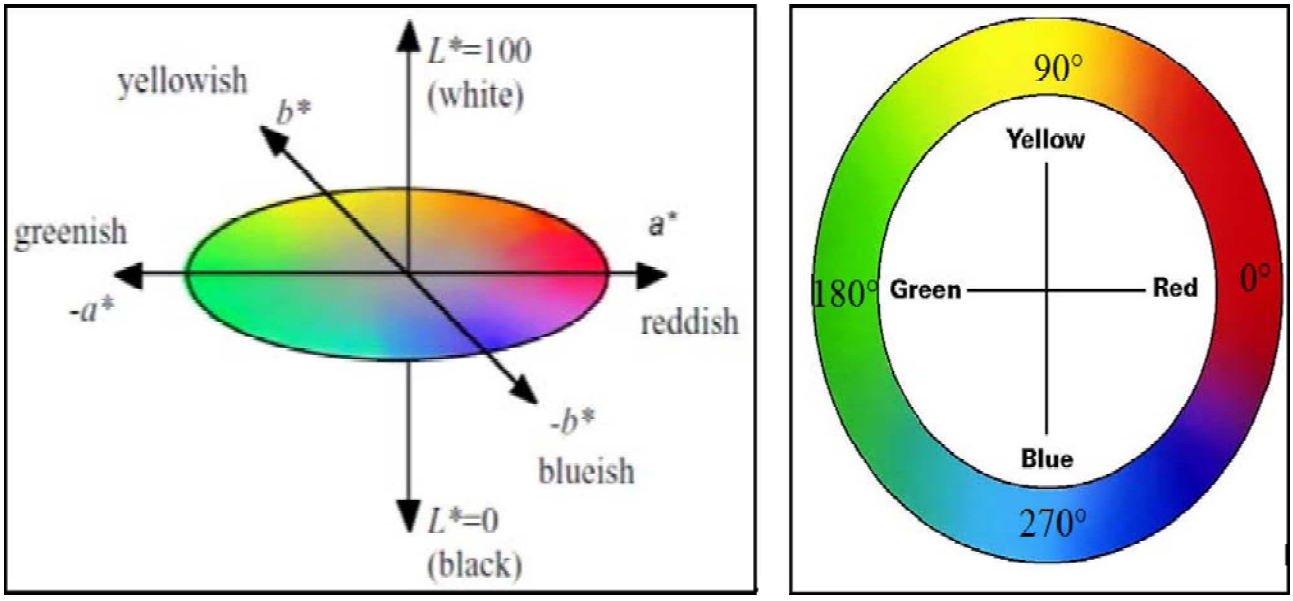


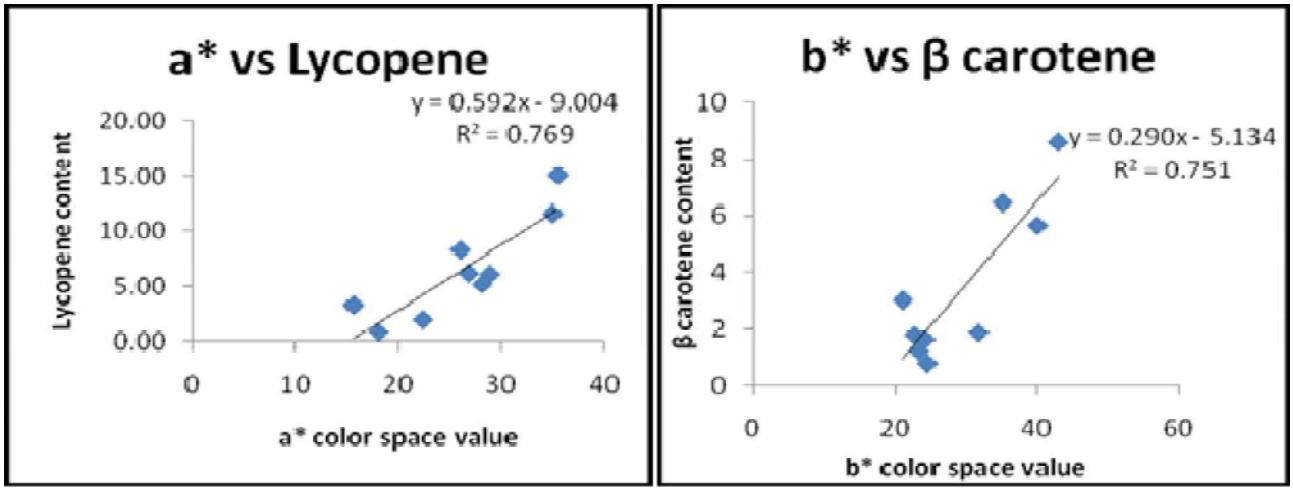
Fig. 3. A three-dimensional representation of CIE (L*, a*, b*) color space. The figure shows horizontal oval disk, with four orthogonal axes radiating out from the center of the disk in the horizontal plane. One set of horizontal axis ranges from -a* (greenish) to +a*(reddish).The other set ranges from -b*(blueish) to +b*(yellowish). Inside the horizontal disk, the range of perceived colors is shown. An orthogonal vertical axis runs through the center of the disk, this vertical axis portrays the lightness dimension, ranging from L*= 100 for white at the top and L*=0 for black at bottom (CIE Publication15.2-1986).

We observed that the correlation between L* and total carotenoids was -0.78($P<0.05$) (Table 2) *viz.* as the total carotenoids in tomato lines increase, the fruit surface L* color space value decreases. A similar study by Itle *et al.*, in 2009 on pumpkins and squashes reported that there was negative correlation between L* and carotenoid content. The color space value a* was found to be higher in IIHR 2754 (35.43) that had high total carotenoids and lycopene content. We observed that, as the a* value decreased in different tomato lines there was concomitant decrease in carotenoids (Table 1). There was a positive correlation between a* value to total carotenoids (0.82) ($P<0.01$) and lycopene content (0.87) ($P<0.01$) where as a negative correlation was observed between a* and β-carotene content (-0.77)($P<0.05$). As indicated in the Table 1, in red colored tomato lines lycopene constitutes major part of total carotenoids which are red color pigments. As higher a* values indicate more redness, the tomato lines with higher surface a* values had more lycopene pigments indicating positive correlation as reported in (Table 2). The orange colored tomatoes showed a* value lower than red tomatoes, as shown in Fig 3 that a* value in horizontal axis is negative for green color and gradually increases with a* value becoming positive as there is change in color from orange to orange red and then to red. The b* value was highest in IIHR 2866 (42.99) which had highest β-carotene of 8.56 mg/100g FW.

Table 2. Pearson correlation coefficients (r) (2 tailed) (n = 10) between color space values (L*, a*, b*, chroma, and hue angle) and total carotenoids, lycopene and β-carotene content. Significant correlations of two-tailed tests are indicated: *, $P < 0.05$; **, $P < 0.01$

	L*	a*	b*	Total carotenoids	Lycopene	β carotene	Chroma	Hue angle(h°)
L*	1	-0.738*	0.993**	-0.788**	-0.817**	0.840**	0.737	0.913**
a*	-0.738**	1	-0.780**	0.822**	0.877**	-0.772*	-0.128	-0.943**
b*	0.993**	-0.780**	1	-0.789**	-0.835**	0.867**	0.709	0.939**
Total carotenoids	-0.788**	0.822**	-0.789**	1	0.976**	-0.507	-0.245	-0.842**
Lycopene	-0.817**	0.877**	-0.835**	0.976**	1	-0.613	-0.286	-0.895**
β carotene	0.840**	-0.772*	0.867**	-0.507	-0.613	1	0.596	0.865**
Chroma	0.737	-0.128	0.709	-0.245	-0.286	0.596	1	0.438
Hue angle	0.913**	-0.943**	0.939**	-0.842**	-0.895**	0.865**	0.438	1

We observed a positive correlation between b* and β-carotene content (0.86) (P<0.01) and there was a negative correlation between b* and total carotenoids (-0.78) (P<0.01) & lycopene content (-0.83) (P<0.01). The surface b* values indicate yellowness and the tomato lines with higher b* values had higher β-carotene content giving positive correlation between b* and β-carotene. Chroma value C showed no significant differences among the genotypes (Table 1). It is reported that although chroma sub model has been proposed (Thai *et al.*, 1990), it is not a good indicator of tomato ripening because it essentially is an expression of the purity or saturation of a single color (differentcolors may have the same chroma values) (Lopez Camelo and Gomez *et al.*, 2004).In the case of tomato ripening, different colors are present simultaneously since chlorophyll is degraded from green to colorless compounds at the same time that carotenoids are synthesized from colorless precursor (phytoene) to β-carotene (pale yellow), lycopene (red), β-carotene (orange) and xanthophylls and hydroxylated carotenoids (yellow) (Giuliano *et al.*, 1993), in a kind of parallel biosynthetic pathway (Horton & Stark,1969). Hue angle, h° was more in IIHR 2866 (67.25) and was less in red colored tomato IIHR 2754 (30.69). Lower hue angle means redness and higher hue angle indicates yellowness. The negative correlation (-0.84) (P<0.01) observed between hue and total carotenoids is perfectly reflected by lower carotenoid readings in tomato lines with higher hue angles. A similar negative correlation was observed by Itle et al., (2009) in pumpkins and squashes; they suggest that as hue angle decrease the carotenoid content increase. But we could observe positive correlation (0.86) (P<0.01) between β-carotene and hue angle. Also, hue angle and b* value strongly correlated (0.93) (P<0.01), as we discussed earlier with increase in β-carotene the b*values also increased and showed positive correlation. So, it can be assumed that b* value and hue angle are clearly associated with β-carotene content in tomatoes.



4(a) 4(b)

Fig. 4(a). Correlation between a* and lycopene content, **4(b).** Correlation between b* and β carotene content (n=10).

CONCLUSION

From this study, it is clear that there was a change in a* value due to accumulation of lycopene. The a* value increased as lycopene content increased and b* value increased with increase in β-carotene content. In the tomato lines selected in our study we observed the total carotenoid content was more in lines where there was more lycopene content, hence there was positive correlation between a* to total carotenoids and lycopene content. Hue angle also showed a strong positive correlation to β-carotene content. Based on these results from this study, we could identify strong correlations between colorimetric values and the carotenoid content. These results confirmed the feasibility of obtaining precise indirect estimation of lycopene and β-carotene content from chromaticity readings. The methodology described here could be useful for large scale selection of tomato lines with improved levels of lycopene without high prices and likewise prevents the residue disposal problems associated with the employment of organic solvents in the standard spectrophotometric methods. The utilization of portable hand held colorimeters for estimation of carotenoids in tomatoes is less clumsy and convenient when compared to other methods.Therefore, the close association between color and carotenoids established through colorimeter readings can be utilized or applied for breeding purposes to improve the nutritional value of tomatoes in very easy, inexpensive, less time consuming and a non-destructive method.

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