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Evaluation of tomatoes fruits flesh colour, beta-carotene and lycopene content

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Abstract The phenotypes of tomatoes in various varieties (lines) were investigated including lycopene and beta-carotenet contents on tomato flesh fruits. Results showed that the fruits of var SL08 (N30B Orange) gave the highest lycopene and tomato var SA (40A Red) gave the lowest lycopene. Moreover, tomato var. SL06 (39B Pink) has the highest beta-carotene content and tomato var. SL02 (33A Yellow-Orange) has the lowest beta-carotene content. It can be concluded that phenotype characters of tomato varieties (lines) in colour of flesh fruits are related to lycopene and beta-carotene content. These preliminary results can be used for further investigation of different genotypes of tomato and relate its fruit flesh colour to the expression of target genes, lycopene beta-cyclase and lycopene epsilon-cyclase genes.

Keywords: Beta-carotene, lycopene, *Solanum lycopersicum*

Introduction

Tomato (*Solanum lycopersicum* L.) is the most popular garden crop grown widely around the world. Tomatoes are commonly consumed in daily diets. It is a major source of antioxidants, like lycopene, and are great sources of vitamin C, potassium, folate, and vitamin K. It is a seasonal crop, and their availability is limited during certain seasons. Tomatoes have numerous uses in both fresh and processed forms. Processed products include ketchup, sauces, pastes and juice. (Gastélum-Barrios *et al*., 2011).

On a global scale, the annual production of fresh tomatoes amounts to approximately 180 million tonnes. In comparison, two times more potatoes and four times more rice production are grown around the world (FAO, 2019). However, about a quarter of those 160 million tonnes are grown for the processing industry, which makes tomatoes the world's leading vegetable for

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processing. Almost 39 million tonnes of tomatoes are processed every year in factories belonging to the greatest labels of the global food industry.

The global tomato market revenue amounted to $190.4B in 2018, rising by 6.5% against the previous year. This figure reflects the total revenues of producers and importers (excluding logistics costs, retail marketing costs, and retailers' margins, which will be included in the final consumer price).

The main production regions are located in temperate zones, close to the 40^{th} parallels North and South, as illustrated on the following map. However, most of this production is based on the Northern hemisphere, where an average of 90% of the world's crop is processed between the months of July and December. The remaining 10% are processed in the Southern hemisphere between January and June. Brazil is an exception, being the only country of the Southern hemisphere to process more than one million tonnes per year at the same time as the Northern hemisphere.

Lycopene is one of the several carotenoid compounds that is rich in tomato fruits. It gives the characteristic red color in plants and has numerous health benefits. Thus, improvement of tomato fruit color has led to indirect improvement of its nutritional value. As known for the genetics involved in carotenoids in tomatoes, loss-of-function mutation in one of the genes encoding one enzyme in the carotenoids biosynthetic pathway is associated with a change in color of a specific plant tissue due to a change in its pigment content (D'Ambrosio *et al.,* 2018).

Carotenoids are a group of lipid-soluble natural pigments which is the precursor of vitamin A and play an essential role as an antioxidant to protect the cells and tissues from peroxides (He *et al.,* 2017). They responsible for the red, yellow and orange pigments in plants are antioxidants known to reduce risk of cancer and cardiovascular diseases and are a factor in attracting pollinators to flowers (Howitt *et al*., 2016). Lycopene is one of the several carotenoid compounds that is rich in tomato fruit. Carotenoid is a plant secondary metabolite synthesized in plastids and responsible for the bright coloration of many reproductive organs in plants like flowers and fruits. Lycopene gives the characteristic red color and numerous other health benefits. Thus, improvement of tomato fruit color has led to indirect improvement of its nutritional value. As for genetics involved in carotenoids in tomatoes, loss-of-function mutation in one of the genes encoding an enzyme of the pathway is almost always associated with a change in the color of a specific organ due to a change in its pigment content (D'Ambrosio *et al*., 2018).

Carotenoids are products of the isoprenoid biosynthetic pathway. Carotenoid biosynthesis begins with the formation of phytoene from geranylgeranyl diphosphate catalyzed by phytoene synthase (PSY). Three enzymes, including phytoene desaturase (PDS), ζ-carotene desaturase (ZDS) and carotenoid isomerase (CRTISO), convert phytoene to lycopene via phytofluene and ζ-carotene. Carotenoid biosynthesis bifurcates after lycopene to produce α- and β-carotenoids through the action of two lycopene cyclase and lycopene β-cyclase. The cyclization of lycopene with lycopene ε- and β-cyclase is a critical branch-point in carotenoid biosynthesis. Lycopene ε-cyclase and lycopene β-cyclase is a critical enzyme that catalyzes the synthesis of α-branch carotenoids through the cyclization of lycopene. (Cazzonelli *et al*., 2010; Shi *et al*., 2014) Overexpression of lycopene β-cyclase in tomato plastids resulted in an unexpected increase in the total carotenoid content, with predominant accumulation of β-carotene in the fruits and xanthophyll carotenoids in the leaves. Downregulation of lycopene ε- cyclase in *Brassica napus* using RNAi resulted in seeds with reduced expression of ε-CYC and increased levels of total carotenoids, including β-carotene, zeaxanthin, violaxanthin and, unexpectedly, lutein. (Apel and Bock, 2009; Yu *et al*., 2008) The lycopene cyclase genes are component of the carotenoid biosynthetic pathway (McQuin, 2018) which is responsible for fruit color in tomato among other quality traits. The cyclization of lycopene with lycopene ε- and β-cyclase is a critical branch-point in carotenoid biosynthesis. Lycopene ε-cyclase and lycopene β-cyclase are the critical enzymes that catalyzes the synthesis of α-branch carotenoids through the cyclization of lycopene.

This research aimed to evaluate lycopene and beta-carotene content of different varieties of tomato with different tomato flesh colors.

Materials and methods

Materials and sample preparation

The eight varieties (lines) of tomato namely: var. 15001, SA, SL06, SL07, SL05, SL08, SL01 and SL 02 were sowed and grown in a plastic greenhouse at Institute of Plant Breeding, University of the Philippines - Los Baños. Tomato fruits were harvested from each plant and the weight, size, and color were compared with Royal Horticultural Society Colour Charts Edition V.Version 2 (http://rhscf.orgfree.com) and forms of tomato fruits (Gastélum-Barrios *et al.*, 2011). After phenotypic evalutaion and frames, they were kept in bio-freezer prior to the measurement of lycopene and beta-carotene content.

Lycopene content measurement

One gram of tomato fresh fruit was extracted twice in a 10ml mixture of acetone: n-hexane at the ration of 4:6, and the mixture was let to stand in an ice bath for 10 min. Then, it was centrifuged at 1370 x g for 10 min. The supernatant was separated using a separatory funnel. The absorbance of the hydrophobic fraction was measured spectrophotometrically at wavelengths of 663, 645, 505 and 453 nm using a UV Vis spectrophotometer, using the acetone/n-hexane mixture as blank (Aguirre and Cabrer, 2012). Three replicates for every fruit were measured. The lycopene concentration was quantified using the equation proposed by Nagata and Yamashita (1992) cited by Aguirre and (Cabrer, 2012) as follows:

[lycopene] (mg/mL) = - 0.0458 A663 + 0.204 A645 + 0.372 A505 - 0.0806 A453

Beta-carotene content measurement

The beta-carotene concentration was determined using a colorimetric assay which developed by Biswas *et al.* (2011). Five hundred (500) mg of dried tomato fresh fruits were extracted twice using 5 mL chilled acetone, and the mixture was let to stand in an ice bath for 15 minutes with occasional shaking. The mixture was mixed vigorously for 10 minutes and centrifuged at 1370 x g for 10 min. The supernatants were pooled and filtered using Whatman filter paper No.42. The absorbance of the extract was read at 449 nm using a UV-Vis spectrophotometer. Three replicates for every fruit were measured.

Experimental design and statistical analysis

The experiment was performed in Completely Randomixed Design (CRD) with three replications. Analysis of variance (ANOVA) was computed, and treatment means were compared by Duncan's New Multiple Range Test (DMRT) at P = 0.01.

Results

Lycopene content

Tomato fruits were measured for lycopene content. Tomato var SL08 expressed N30B Orange color had the highest lycopene content with 574.60 mg/100g DW, followed by tomato var SL05 (N20A Red- Orange), 15001 (42A Red), SL07 (45C Pink), SL06 (39B Pink), SL02 (33A Yellow-Orange), SL01 (N30C Orange), and SL02 (33A Yellow-Orange) with lycopene values 479.56, 378.62, 364.02, 239.25, 182.18, 141.25 and 132.61 mg/100g DW, repectively (Table 1 and Fig.1). Tomato var SL02 with 33A Yellow-Orange expressed color which has the lowest amount of lycopene among all the studied varieties.

Beta-carotene content

Beta-carotene was measured using using a UV-V spectrophotometer. Result showed that tomato var. SL06 with 39B Pink colour registered the highest beta-carotene content with 24.51 mg/100g DW, and followed by 15001(42A red), SL07 (45 C pink), SA (40Ared), SL01 (N30C Orange), SL05(N20A Red-Orange) , and SL02 (33A Yellow-Orange) which beta-carotene values of 19.11, 18.05, 17.20, 9.06, 8.45 and 7.11 mg/100g DW, respectively (Table 1 and Figure 1).

Table 1. Lycopene and Beta-carotene contents of tomato flesh fruits

Accession ID	Fruit colour	Lycopene content (mg/100g DW)	Beta-carotene content (mg/100gDW)
15001	42A Red	378.62 abc[1]	19.11ab
SA	40A Red	132.61 c	17.20 abc
SL06	39B Pink	239.25 bc	24.51a
SL07	45C Pink	364.02 abc	18.05 abcd
SL05	N20A Red- Orange	479.46 ab	8.45 cd
SL08	N30B Orange	574.60 a	NA
SL01	N30C Orange	141.25 c	9.06 bcd
SL02	33A Yellow-Orange	182.18 c	7.44 d
C.V. (%)		**19**	**20**

[1]Means of three replications, means followed by a common letter in each column were not significantly differed by DMRT at P=0.01.

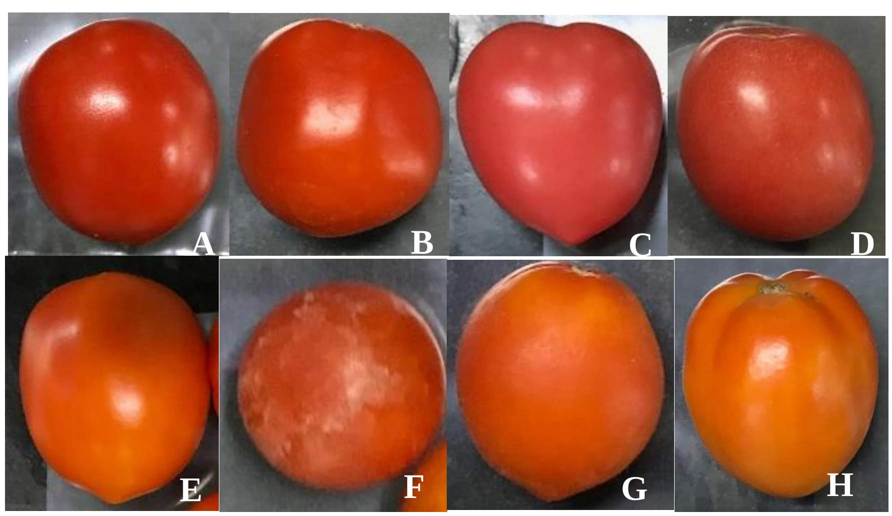

Figure 1. Morphological characteristics of tomato varieties, A= 15001, B= SA, C=Sl06, D=SL07, E=SL05, F= SL08, G=SL01 and H+ SL02

Interestingly, even when the color of the fruits are similar in some varietal lines, it has shown different amounts of lycopene and beta-carotene contents. In addition, the pink fruit fleshed tomato has shown a high amount of both lycopene and beta-carotene. However, red fruit fleshed tomato showed a varying amount of lycopene and beta carotene. Super apollo (SA), which is the red-fruit-fleshed local OPV tomato in the Philippines, has shown the lowest amount of lycopene content but high concentration of beta-carotene. Most orange fruits have shown a high amount of lycopene content, while pink and red-fruit-flesh have shown a high beta-carotene content.

Discussion

The results of this research revealed that tomato flesh fruits with dominant orange color (SL08) expressed the highest lycopene content while the tomato flesh fruits with pink and yellow-orange color registered the lowest in beta-carotene content. This is also seen in the study of Malgorzata *et al.* (2007) which showed that lycopene and beta-carotene occur vary among different tomato fruits and various tomato products. Tomatoes are major sources of lycopene, micronutrient and beta-carotene. The beta-carotene analysis showed that tomato with pink colour expressed the highest beta-carotene content while tomatoes with orange and yellow-orange color had the lowest beta-carotene content. Kondratieva and Golubkina (2016) also reported that the highest lycopene content was found in 2 types of red fruits tomato at 11.5 and 8.7 g/100g. respectively. Tomatoes with orange color had shown highest lycopene content at 6.2 mg/100g and the higher beta-carotene content than in standard. However, the yellow fruits line has shown the highest content of betacarotene at 4.1 mg/100g.

Tomato phenotype, like colour of the flesh, which can be related to the lycopene and beta-carotene accumulation may vary with different environmental settings and will have an effect on the biosynthesis of these compounds. Brandt *et al.* (2006) demonstrated the effect of different environmental conditions in the the biosynthesis of lycopene. If the temperature of the tomato fruits exceeds 30 ℃, the synthesis of lycopene is inhibited. The strong direct radiation on fruits (~2990 μmol m^{-2} s^{-1} for 1.5–4 h) is also harmful to the fruits. Temperature, photosynthetically active radiation (PAR), and harvesting time are the main reasons for the variation of lycopene content in tomato fruits. The high temperature in the Philippines ranging from 40 to 43 ℃ may have caused the low lycopene content among the studied varieties.

Lycopene content of tomato fruit was increased after storage. Tomato fruit color change after storage was estimated with variables of tomato fruit color before storage, storage temperatures and durations by multiple regression (R＝0.76). It showed that the color of tomato fruits stored above 10℃ was increased after storage. With storage condition above 20℃, lycopene content of tomato fruit was increased after storage (Takahashi *et al.,* 2018)

Consumption of tomatoes for the purpose of dietary antioxidant lycopene and pro-Vitamin A could maximally be achieved at fully-ripe stage under field ripening condition. Ibrahim *et al.* (2019) evaluated that the variations of pH, titratable acidity, reducing sugar, total solid, lycopene and beta-carotene contents of these tomatoes were investigated under ambient temperature and field ripening techniques and the mean values of these parameters investigated at different ripening stages and techniques were compared. Lycopene contents were significantly higher ($p < 0.05$) in tomatoes subjected to field ripening compared with those ripened under ambient temperature. The highest lycopene content (17.18 μg/g) was observed in Roma VF cultivar at fully-ripe stage under field ripening technique while the lowest value (0.64 μg/g) was in 4-lobes cultivar at semi-ripe stage under ambient temperature ripening. Similar trend was observed in the variation of betacarotene (a pro-Vitamin A index) among the tomato cultivars. The evaluated reducing sugar contents (ranging from 1.84 to 5.23 μg/g) were significantly higher ($p < 0.05$) in fully-ripe tomatoes compared to semi-ripe ones under field ripening and the trend was reversed for some cultivars under ambient temperature ripening.

The lycopene concentration was quantified using the equation proposed by Nagata and Yamashita (1992) and the absorbance of the extract read at 449 nm using a UV-Vis spectrophotometer was used to quantify beta-carotene. Szuvandzsiev *et al.* (2004) stated that the quality characteristics of processing tomato, which is important for the food industry, are soluble solids and lycopene content. However, lycopene content determination using spectrophotometry is destructive and time consuming and because of this using a portable handheld near-infrared (NIR) spectrometer to predict lycopene content of tomato fruit is a good alternative. Vilbett *et al.* (2019) stated that the extracted tomato pulp by high hydrostatic pressure (HHPE) with different selected factors (high pressure and solvent mixture) had significantly influenced on extraction yield, flavonoid and lycopene content by using response surface methodology (RSM). Tomato pulp which extracted at 450 MPa and 60% hexane concentration in the solvent mixture by HHPE provided a significant the maximum extraction yields (8.71%), flavonoid (21.52 ± 0.09 mg QE/g FW) and lycopene content (2.01 ± 0.09 mg QE/100 g FW).

Alsina *et al*. (2019) demonstated that correlation between lycopene and β-carotene content in tomatoes determined with reflectance spectrometer and extraction of pigments. Linear regression analyses were performed to correlate spectral data with lycopene and β-carotene concentrations measured by pigment extraction and obtained absorption spectra by spectrophotometer UV-Vis -1800 (Ltd. Shimadzu). The best reflectance region for lycopene spectral detection was 570 ± 5 nm, but for β-carotene 487 ± 5 nm. Reflectance indexes for both pigments were worked out. High linear correlation (R2 > 0.9) between spectral parameters and lycopene concentration was detected. Correlation between results obtained with methods used for β-carotene determination was lower and depended on colour of tomatoes fruits.

The genoypes expression of tomato fruit colour are of great interest for valid and stable development of tomato varieties. The sgRNA target sequence of tomato LCY1 and LCYE and design of CRISPR-Cas9 constructs will be further selected and designed. The sgRNA target for lycopene beta-cyclase and lycopene epsilon-cyclase genes will be searched in the entire gene sequence and the annotated gene sequences will be used to screen candidate guide RNA based on parameters (Doench *et al*., 2014). Moreover Li *et al*. (2018) suggested that CRISPR/Cas9 system using Agrobacterium tumefaciens-mediated transformation can be used for significantly improving lycopene content in tomato fruits with advantages such as high efficiency, rare off-target mutations, and stable heredity. It used a bidirectional strategy: promoting the biosynthesis of lycopene, while inhibiting the conversion from lycopene to β- and α-carotene. The accumulation of lycopene was promoted by knocking down some genes associated with the carotenoid metabolic pathway.

The research finding resulted to know the basis investigation of beta-carotene and lycopene contents of the eight tomato varieties (lines) var. 15001, SA, SL06, SL07, SL05, SL08, SL01 and SL 02. These results can be served to find the different genotypes of tomato which related to the target gene expression of lycopene beta-cyclase and lycopene epsilon-cyclase genes through its fruit flesh colour.

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