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ORIGINAL ARTICLE

The Effect of Shrub Pruning and Fruit Thinning on Plant Pigments, Total Soluble Solids (TSS) and Ascorbic Acid in Three Cultivars of Tomatoes (*Lycopersicon Esculentum* Mill)

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Atefeh Tabasi, Hossein Nemati, Ali Tehranifar, Mohammad Akbari: The Effect of Shrub Pruning and Fruit Thinning on Plant Pigments, Total Soluble Solids (TSS) and Ascorbic Acid in Three Cultivars of Tomatoes (*Lycopersicon Esculentum* Mill)

ABSTRACT

Tomato is an excellent source of vitamin C and lycopene. Improving the ways of crop production and their rearing to increase fruit quality and main active ingredients is very important. This study intended to determine the best pruning method for improving fruit quality. The experiment was performed by factorial analysis with 5 replicates in completely randomized design (CRD). First treatment was three cultivars of tomato, second treatment was two types of shrub pruning, in which all of subsidiary branches are removed (type 1) and one cluster and leaf on subsidiary branches are remained and then extras removed (type 2) and third treatment was fruit thinning. Each tomato cultivar was fruit thinned to three different levels (4, 5 and 6 fruit per each truss). In this study, the effects of shrub pruning and the number of fruits on fruit quality (total soluble solids, ascorbic acid content, carotenoid and lycopene levels) were investigated. The results showed that shrub pruning and fruit thinning were significantly difference on the levels of ascorbic acid, soluble solids and carotenoid, while no major difference was observed on fruit Lycopene levels. Generally, Faraon cultivar with four fruits on truss had the highest fruit quality for two types of shrub pruning. Therefore, choosing appropriate cultivars, special pruning techniques and fruit thinning can increase fruit quality of tomato without spending too much money and harmful chemicals.

Key words: Ascorbic acid, Carotenoid, Lycopene, Soluble solids.

Introduction

Lycopene, a participant in the carotenoid family along with a pigment which attributes to the red color of tomatoes, is really a leading factor to its health promoting ability [7,18]. New studies have shown that tomatoes can help decrease the chance of getting lung cancer, because lycopene acts as antioxidant [19]. Qualitative factors of tomato (flavor, color, soluble solids and nutrition value) were affected by cultivar, weather, material storage, fruit

maturity and growth methods [9].

The development of a tomato fruit from a green to a fully ripe red state involves dramatic changes in color. These changes occur within the plastids after the disappearance of chlorophyll from the fruit. The two major groups of pigments found in tomato fruit are chlorophyll and carotenoids.

The most noticeable change during ripening is the remarkable increase in the carotenoid content of the fruit [11].

During ripening the chlorophyll concentration

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decreases while carotenoids, especially lycopene, accumulate in the fruit [11].

Carotenoids also are a major source of vitamin A in the diets of a large proportion of the world population [10]. Luh and Daoud [13] reported that the amount of lycopene in different varieties reveals a considerable difference. The light intensity is influential in the biosynthesis of carotenoids and development of fruit color [16]. Since tomato flavor, is derived from sugar and organic acid, if sugar and acids of tomato is decrease, it will lead to undesirable flavor and platitude. Bradley [3] and Mauz [15] showed that there is a fundamental difference in acidity levels between tomato varieties. They also expressed that there is a negative relation between ascorbic acid and the number of fruit on the plant, while this relation is observed directly between ascorbic acid and leaf fruit ratio. AS suggested by Gould [9] the cultivar of tomato with big fruits has higher soluble solids which due to carpel larger. Mangari [14] and Stevens *et al.* [17] stated that the amount of dry matter has an impact on soluble solids. Therefore, total soluble solid is increased due to dry matter rise.

Materials and methods

Plant Materials and Treatments:

In this study three cultivars of tomato (Faraon, Akdenis, and Dominator) were selected. Shrub pruning and fruit thinning were performed, during the plant growth which was carried out by two styles of shrub pruning, in which all of subsidiary branches are removed (type 1) and one cluster and leaf on branches are remained and then extras removed (type 2). Each tomato cultivar was fruit thinned to three different levels (4,5 and 6 fruit per each truss). Tomato fruit quality was studied in a greenhouse experiment. Traits measured included soluble solids, ascorbic acid, carotenoids and lycopene levels.

Soluble Solids Analysis:

Total soluble solid concentration (TSS, measured as % Brix) of tomato fruits is an important variable which is used to determine fruit quality, because TSS is the most commonly associated with sugar and organic acid concentrations [2,5]. Each % of Brix is equal to 1 gram of soluble solids in 100 g of fresh mass. Total soluble solid contents were determined by extracting and mixing one drop of juice from each fruit into a refractometer (ERMA, TOKYO).

Ascorbic Acid Analysis:

Ascorbic acid was measured according to the method of Jacobs (2005). In order to measure the

ascorbic acid, 10cc from tomato juice was mixed with 20cc distilled water and then 2 cc from (1%) one-percent soluble starch was added. Then vitamin C was determined by titration of 10 ml filtrated juice which contained Iodure de potassium (KI). In fact, it was based on mg ascorbic acid/100gr FW. Ascorbic acid content was calculated using the following formula [1].

$$\text{Ascorbic acid} = \frac{\text{Volume consumed of KI} \times 0.88}{10}$$

Carotenoid Analysis:

To measure carotenoid, method of Lichtenthaler and Welburn was utilized. Therefore 0.05 of fruit tissue was crushed using liquid nitrogen and then 10 ml 80% acetone was added. The solution became smooth with filter paper and the final volume was 20 ml. The solution absorption was measured at 646.8 nm, 663.2 nm and 470 nm with of UV visible spectrophotometer. Equation to determine concentration of chlorophyll a (ca) and b (cb) as well as total carotenoida (Cx+c) were calculated in ugml-1 in %80 acetone with spectrophotometer [12].

$$\text{Ca (mg/ml)} = 12.25 A_{663.2} - 2.79A_{646.8}$$

$$\text{Cb (mg/ml)} = 21.5 A_{646.8} - 5/1 A_{663.2}$$

$$\text{Cx+c (ugml-1)} = (1000 A_{470} - 1.82ca - 85.02cb) / 198$$

Lycopene Analysis:

Lycopene content was an additional biochemical parameter used to measure fruit quality. The lycopene content of the fruits was determined according to the reduced volume method of Davies *et al.* [4].

Ten gram of fruit tissue was added to a test tube containing 10 ml of distilled water (1:1; m/v). The content was homogenized for 30 seconds, using a Polytron electric homogenizer. An aliquot containing 0.5 g of the puree was then added to a test tube containing a mixture of 5 ml 95 % ethanol, 10 ml hexane and 5 ml acetone, containing 0.05 % (w/v) butylated hydroxytoluene (BHT).

The tubes were sealed with Para-film, thoroughly mixed on a bench vortex and extracted on an orbital shaker at 180 rpm for 15 minutes.

After rotations, 3 ml de-ionized water was added to the tubes and replaced on the orbital shaker for 5 minutes.

After extraction, the tubes were left at room temperature (22°C) for 15 minutes in the dark for phase separation to occur before the clear yellowish supernatant (hexane layer) was collected. The tubes were kept in the dark on ice until all samples were extracted. The supernatant (hexane upper layer) was used to determine the absorbance at 503 nm using a CECIL-2502CE Spectrophotometer.

Taking all variables of this specific extraction procedure into account, the lycopene concentration of the tissue was calculated using the following equation [4]:

$$Lycopene = \frac{A503}{17.2 \times 10^4 \text{ M.C}} \times \frac{536.9 \text{ g}}{\text{mole}} \times \frac{11}{10^3 \text{ ml}} \times \frac{10^3 \text{ ml}}{1 \text{ g}} \times \frac{10 \text{ ml}}{\text{kg tissue}}$$

$$\frac{A503 \times 0.0312}{\text{kg tissue}} = \frac{A503 \times 31.2}{\text{kg tissue}} = \text{mg lycopene. gr}^{-1} \text{ tissue}$$

Where A503 (absorbance at 503 nm) is the absorbance at 503 nm and $17.2 \times 10^4 \text{ M.cm}^{-1}$ the molar absorbance coefficient for lycopene. The molecular weight (mw) of lycopene is $536.9 \text{ g.mole}^{-1}$ [4].

Statistical Analysis:

Effect of treatments were verified based on ANOVA using Excel program and the means were compared using LSD test at 5% level.

Results and discussion

Ascorbic Acid:

The experiment results demonstrated that there was a vital difference between three cultivars, shrub pruning and fruit thinning in ascorbic acid content. So the highest content of ascorbic acid was related to Faraon cultivar and shrub pruning of type 1 with 4 fruit.

Our results are similar to Mauz (1966) who reported that increasing light in to the plant leads to raised ascorbic acid. It can be concluded that in pruning of type 1, more light to the crown of the plant was penetrated, which results in increased fruit ascorbic acid.

As shown by Fanasca *et al.* [7] plants with less fruit have boosted source concentration in the fruit which this result is from bigness of fruits. This implies that big fruits have a higher source of ascorbic acid content than small fruits (Table.1).

Table 1: Comparison of the simple effects of traits studied.

Lycopene mg/g-1	Carotenoid ugml-1	Soluble solids %	Ascorbic acid mg/100fw	Cultivar	Shrub pruning
1.02c	10.47c	3.96a	18.38c	Akdenis	
1.65a	16.39a	2.79b	21.66a	Faraon	Cultivar
1.50b	14.65b	3.43b	20.14b	Dominator	
1.41a	15.42a	2.70b	21.19a	Type 1	Shrub pruning
1.37a	12.25b	3.09a	18.94b	Type 2	
1.35a	6.57c	2.96a	21.36a	4	
1.31a	17.02b	2.92a	20.14b	5	Fruit thinning
1.39a	17.92a	2.80b	18.68c	6	

Means with similar letters in each column are not significantly different by LSD multiple range test ($p < 0.05$).

Table 2: Comparison of interaction between variety and shrub pruning for traits studied.

Lycopene mg/g-1	Carotenoid ugml-1	Soluble solids %	Ascorbic acid mg/100fw	Cultivar	Shrub pruning
0.92e	13.14e	2.91a	19.46d	Akdenis	Type 1
1.89a	19.34a	2.73c	22.39a	Faraon	
1.42c	3.77c	2.46d	21.17b	Dominator	
1.13d	7.80f	3.21a	17.30f	Akdenis	Type 2
1.41c	13.43d	2.84b	20.92c	Faraon	
1.58b	13.53b	3.21a	18.58e	Dominator	

Means with similar letters in each column are not significantly different by LSD multiple range test ($p < 0.05$).

Table 3: Comparison of interaction between shrub pruning and fruit thinning for traits studied.

Lycopene mg/g-1	Carotenoid ugml-1	Soluble solids %	Ascorbic acid mg/100fw	Fruit thinning	Shrub pruning
1.65a	4.33f	2.78c	22.59a	4	Type 1
1.40b	24.17a	2.67d	20.83b	5	
1.18c	17.76c	2.66d	20/14c	6	
1.39b	8.81a	3.14a	20/14c	4	Type 2
1.21c	9.87d	2.93b	19.45d	5	
1.52ab	18.07b	3.19a	17.22e	6	

Means with similar letters in each column are not significantly different by LSD multiple range test ($p < 0.05$).

Table 4: Comparison of interaction between cultivar and fruit thinning for traits studied.

Lycopene mg/g-1	Carotenoid ugml-1	Soluble solids %	Ascorbic acid mg/100fw	Cultivar	Fruit thinning
1.3cd	7.64e	3.23b	17.31e	Akdenis	
1.45c	6.06f	2.73d	23.03b	Faraon	4
1.81b	6.01f	2.92c	23.76a	Dominator	
1.15d	13.28b	2.95c	19.95a	Akdenis	
1.41c	29.55a	3.28b	23.90a	Faraon	5
1.37c	8.24d	2.17f	16.87e	Dominator	
0.63e	10.41c	3.0c	18.19d	Akdenis	
2.09a	13.55b	2.35e	18.04d	Faraon	6
13.3cd	29.71a	3.42a	19.81c	Dominator	

Means with similar letters in each column are not significantly different by LSD multiple range test ($p < 0.05$).

Total Soluble Solids (SST):

The study results revealed that there was a significant difference between three cultivars, shrub pruning and fruit thinning in soluble solids. The most soluble solids were obtained from Akdeniz cultivar and shrub pruning of type 2 with 4 fruits. Fernando and Alisdair [8] suggested more number of leaves on the plant increased photosynthesis which results in increased soluble solids. It should be noted that there are a lot of leaves in pruning type 2 in comparison with type 1 thus; pruning type 2 leads to more photosynthesis and soluble solids. Domin and Kempton [6] reported that fruit thinning leads to produce big fruit and raised dry matter of fruit and soluble solids. They also expressed that competition between fruits in the plant is an important factor to increase the soluble solids, large fruits have a better ability to absorb nutrient, and consequently they gain more soluble acids. This could be due to fruit bigness or inhibitory substance production that inhibits the growth of other fruits. These results are in close agreements with our findings.

Carotenoid:

The results of this experiment showed that cultivar, shrub pruning and fruit thinning had central effects on carotenoid level, so that the highest carotenoid content belonged to Faraon cultivar, shrub pruning of type 1 with six fruits (Table 1). Young *et al.* [19] mentioned that there is a prominent difference between cultivar and carotenoid. They also expressed that presence number of carotenoid in some cultivars and their absence in the other varieties is due to differences in their genes controlling biosynthesis. In addition, they reported that light density is a major factor to improve carotenoid. The fruit thinning has a little effect on fruit color. These results are in accordance with the finding of our study. Bradley [3] stated that fruits receiving more light have higher levels of carotenoid. So, it can be concluded that shrub pruning of type 1, revealed a better penetration of light to the crown of the plant that leads to carotenoid content increasing (Table.1).

3.4. Lycopene:

The results demonstrated that cultivar had an essential difference on lycopene level. Thus, the highest level of lycopene was observed in Faraon cultivar. Luh and Daoud [13] reported that the amount of lycopene is influenced by the figure. Shrub pruning and fruit thinning did not reveal major difference on lycopene. Fanasca *et al.* [7] reported that fruit thinning did not considerably affect lycopene level.

The interaction between cultivar and fruit thinning (Table 4), cultivar and shrub pruning (Table 2), fruit thinning and shrub pruning (Table 3) showed

an important difference. This difference may be due to genotype.

Conclusions:

From the current study it was concluded that several factors including: cultivar, shrub pruning and fruit thinning can influence ascorbic acid, soluble solids, carotenoid and lycopene. There was a major difference in the cultivars. The difference between three tomato cultivars was due to genotype. According to this study, light intensity is a very crucial factor on fruit quality. In general, punning bush of type 1 causes more light enter to the plant, so it increases ascorbic acid and carotenoids content. In pruning type 2, more photosynthesis take place because there are more leaves and thus more soluble solids occurs. In addition, Plants with less number of fruits had more soluble solids and ascorbic acid. Shrub pruning and fruit thinning did not demonstrate considerable difference on lycopene.

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