









Preharvest nitrogen application affects quality and antioxidant status of two tomato cultivars

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ABSTRACT: Intensive horticultural crop production usually involves challenges from excessive application of fertilizer, mainly nitrogen (N). The effect of N fertilizer on the fruit quality and antioxidant status of two cherry tomato cultivars (Caballero and Victoria) under greenhouse conditions was performed. Nitrogen treatments were applied ranging from conditions comprising deficiency to toxicity (0, 15, 30, 45 and 60 mmol·L⁻¹). Yield, weight, diameter, fruit quality, phenols, flavonoids, lycopene, ascorbic acid and antioxidant capacity were measured at physiological maturity of the fresh tomato fruits. N treatment with 30 mmol·L⁻¹ produced the highest yield, fruit weight, firmness and diameter in both cultivars. However, increasing N from 30 to 60 mmol·L⁻¹ increased the concentration of phenolics, flavonoids, and antioxidant capacity by 50, 125 and 33% in 'Caballero' tomatoes and by 60, 95 and 24% in 'Victoria' tomatoes, respectively. Lycopene content increased with increasing N doses (45 and 60 mmol·L⁻¹) for 'Caballero' tomatoes, while vitamin C decreased as N concentration increased, suggesting that ascorbic acid acts by protecting against oxidative stress in tomato. The present work shows how N fertilization considerably influences yield and quality of tomatoes, as well as nutritional and healthy values.

Key words: *Lycopersicon esculentum*, greenhouse, ascorbic acid, phenolics, lycopene.

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INTRODUCTION

Tomato (*Lycopersicon esculentum*) is the most important vegetable in the world, ranking first in production (~18 million tons in 2017) and in consumption among vegetables (FAO 2019; Rubatzky and Yamaguchi, 2012; Yildizhan and Taki 2018). Tomato is a valuable food due to its quality and health benefits, the former defined by sensorial attributes such as skin color, firmness, and flavor (Batu 2004), and the latter relating to the fruit as a good source of vitamins, such as ascorbic acid, and phytochemicals, such as lycopene, polyphenols and flavonoids, secondary metabolites with an important antioxidant capacity (Bertin and Génard 2018; Briones-Labarca et al. 2019). Agronomic practices during tomato farming, from growing location to fertilizer amounts, may lead to different levels of quality and different concentrations of bioactive compounds in tomato (Poiroux-Gonord et al. 2010).

Tomato production in greenhouses has attracted attention as a potential adjustment to climate change (Truffault et al. 2019), also because protected conditions increase yield and fruit quality (Peet and Welles 2005) and pest control is easier with a lower need for chemical procedures (Jones Jr. 2007). To achieve good tomato quality in greenhouses, good management of fertilization is important (Gruda et al. 2018).



Tomato production requires optimal fertilization, and nitrogen (N) is one of the major nutrients required for plant growth and development and is often the most limiting nutrient in tomato production (Ren et al. 2010; Elia and Conversa 2012). Nitrogen is a primary constituent of proteins, enzymes and nucleic acids, so it is involved in many physiological and metabolic processes and is fundamental in the structural conformation of tomato plants (Albornoz 2016). This and the fact that fertilizers have relatively low costs often leads producers to over fertilize to minimize any risk of yield reduction and nutrient stress (Scholberg et al. 2000).

An increase of N supply up to a certain level may enhance some nutritional and quality attributes; however, a high N supply may have a negative impact on tomato (Gruda et al. 2018). Bénard et al. (2009) found that low N supply reduced vegetative growth and consequently improved tomato quality (lower acid, higher sugar content); vitamin C tended to be higher in fruit with the lowest N supply, with no significant effect on carotenoid contents. Moreover, some researches indicate that high N application may have significantly negative effects on produce quality as well as secondary plant metabolite and vitamin contents in fruits (Stefanelli et al. 2010; Fernández-Escobar et al. 2014). Moderately high supply seems to improve tomato flavor, but excessive levels can cause fruit deterioration (Wang et al. 2007). Du et al. (2017) found that lycopene increased with N rates up to 250 kg·ha⁻¹ and decreased at 350 kg·ha⁻¹. Further research found that increasing N application increases the concentration of some volatile compounds, titratable acidity, and soluble solids and decreases the firmness of tomato fruits (Wang et al. 2007; Simonne et al. 2007; Bénard et al. 2009).

Applying the correct N rate to maximize tomato yield and quality is not easy because the optimum N rate can vary greatly among cultivars (Jaynes et al. 2011). Kobryn and Hallmann (2005) reported that the effect of N on tomato quality strongly depends on the cultivar. Therefore, it is critical to determine the optimum N application for specific tomato cultivars to optimize their quality and phytochemical compounds. The aim of the present work was to study Caballero and Victoria cherry tomato cultivars grown in a greenhouse to determine the effect of different N preharvest applications on the overall quality and antioxidant status of tomato fruits.

MATERIAL AND METHODS

The study was carried out from July to October 2012 at an experimental greenhouse situated 98 km from Chihuahua, Mexico (28°24'25"N, 106°51'8"W, altitude 2,100 m). The greenhouse consisted of polyethylene, and sheets were 6 m wide, 12 m long and 3 m high. Cultivars Caballero and Victoria were selected for this study since these are cherry tomatoes commonly grown in northern Mexico for the fresh export market. Tomato seeds were sown in peat moss substrate at room temperature (25-30 °C) for 30 days. The plants were transplanted into the greenhouse in hard-plastic 10 L pots that were filled with sand and cultivated under normal conditions, 27/18 °C (day/night) with a photoperiod of 13 h (10 plots per treatment, each plot was considered as a replicate). This experiment included five N fertilization treatments: 0, 15, 30, 45, and 60 mmol·L⁻¹. A solution using CO(NH₂)₂ as the N source was prepared by adding phosphorus (KH₂PO₄), potassium (KCl), and calcium (CaCl₂) to obtain the following mineral concentrations: 10 mmol·L⁻¹ P, 15 mmol·L⁻¹ K, and 7 mmol·L⁻¹ Ca. Urea was used as a source of N, since it is inexpensive and is the major chemical fertilizer used worldwide (Tang et al. 2018). Nutrients were supplied during transplant, vegetative growth, flowering, fruit filling and maturity. Nitrogen fertilization treatments were applied every 15 days according to the method used by Peet and Welles (2005). Irrigation was applied depending on the plant developmental stage three times a week to avoid water stress: 100, 200 and 400 mL of water were applied to each plastic pot at transplant, vegetative growth, and fruit formation, respectively. Fresh tomatoes were harvested when the fruit surface became deep red, and the yield, weight, and equatorial and axial diameters were measured. Mature fruits were harvested on 1, 7 and 13 October. Fruits of each treatment were placed in paper bags and transferred to the laboratory for analysis. Skin color, fruit firmness, titratable acidity, soluble solids, phenols, flavonoids, antioxidant activity, lycopene and vitamin C were evaluated. For each analysis three tomatoes per replicate per treatment were used, which corresponded to the three harvests.

Color, firmness, titratable acidity and soluble solids measurement

Color was assessed using a Minolta CR-300 colorimeter (Konica Minolta, New Jersey, USA). Values were recorded as hue angle (h°) (representing red-purple at an angle of 0° , yellow at 90° , bluish green at 180° , and blue at 270°). The mean values of h° were obtained from three different points along the tomato circumference. The firmness of tomatoes was determined with a Texture Analyser TA-XT2i (Stable Micro Systems, YL, England) equipped with a 4 mm diameter stainless steel probe for the penetration test, with a test speed of $2 \text{ mm}\cdot\text{s}^{-1}$ and a distance of 10 mm into the tomato (Lien et al. 2009). Later, four tomatoes from each treatment were ground in a blender, and juice from the fruit was used to determine titratable acidity (TA) and soluble solids (SS) using a Hanna HI 422 digital pHmeter (Hanna Instruments Inc., Woonsocket, RI, U.S.A.) with 0.1 N NaOH titration and an ATC-1E refractometer (Atago Ltd., Tokyo, Japan), respectively.

Total phenolics and total flavonoid content

A spectrophotometry-based method employing the Folin-Ciocalteu reagent and gallic acid as standard was used to quantify total phenols content (Gao et al. 2011). Briefly, 50 μL of tomato extract and 250 μL of Folin-Ciocalteu reagent were added to 3 mL of deionized water. After 5 min of reaction, 750 μL of 20% Na_2CO_3 solution was added. The mixture was diluted to 5 mL with deionized water and total phenols were measured at 760 nm after 30 min of reaction. Results were reported as mg of gallic acid equivalent (GAE) per 100 g of fresh tomato weight. Flavonoid content was determined employing the method by Zhishen et al. (1999) which consisted in mixing 1 mL of tomato extract with 300 μL of NaNO_2 (5%), and 4 mL of deionized water. 300 μL of AlCl_3 (10%) and 2 mL of NaOH ($1 \text{ mol}\cdot\text{L}^{-1}$) were added after 5 and 6 min of equilibration time respectively.

Total flavonoids content was measured at an absorbance of 415 nm using a UV-Vis spectrophotometer (Varian Cary 50) after the total sample volume was taken to 10 mL with deionized water. Total flavonoids content was expressed as mg of rutin equivalents/100 g of tomato fresh weight.

Lycopene

Lycopene quantification was accomplished following the method of Mejia et al. (1988) with some modifications. Tomato tissue was homogenized in tetrahydrofuran (THF) containing 0.01% butylated hydroxytoluene (3 g tomato/25 mL THF). After 15 min of centrifugation at 10,000 g and filtration (0.45 μm), the sample was analyzed by HPLC (Varian 9012 solvent delivery system, CA, USA) employing a column Microsorb RP-C18 ($4.6 \times 100 \text{ mm}$, 3 μm) and a UV-Vis detector at 460 nm. An acetonitrile:methanol:THF (58:35:7, v/v/v) mixture was employed as mobile phase at a flowrate of $1 \text{ mL}\cdot\text{min}^{-1}$. Lycopene content was expressed as mg lycopene per 100 g of fresh tomato weight.

Ascorbic acid

Ascorbic acid content was determined by HPLC using a microbondapack- NH_2 column ($3.9 \times 300 \text{ mm}$, 10 μm), acetonitrile: 0.05 M KH_2PO_4 (75:25 v/v) at a flow rate of $1.0 \text{ mL}\cdot\text{min}^{-1}$ as mobile phase, and a UV-Vis detector (Varian 9050, CA, USA) set at 268 nm (Dávila-Aviña et al. 2014). Fresh tomato (3 g) was added to 20 mL of a mixture of metaphosphoric acid:glacial acetic acid:water (30:80:890 w/v/v), homogenized, centrifuged (14,000 rpm, 15 min, at 4°C), and filtered (0.22 μm) Ascorbic acid content was expressed as mg of ascorbic acid/100 g fresh tomato tissue.

Radical scavenging activity using the DPPH method

Radical scavenging activity was determined by the method of Kedare and Singh (2011) with some modifications. A solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared by dissolving 2.5 mg of DPPH in 100 mL of methanol and

adjusting its absorbance to 0.7 at 515 nm. 100 μ L of tomato extract (2:8 dilution) were mixed with 3.9 mL of the DPPH solution and stored in the dark with continuous shaking for 30 min.

The absorbance of the incubated solution was determined in a UV-Vis spectrophotometer (Varian Cary 50) at a wavelength of 515 nm, after construction of a standard curve employing 6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic (Trolox) and using 80% methanol as blank. Radical-scavenging activity was expressed as % inhibition.

Statistical analysis

A completely randomized design with five treatments (N applications) and 10 replications per treatment were conducted, which corresponded to 10 pots. Five treatments were used (N fertilization of 0, 15, 30, 45 and 60 $\text{mmol}\cdot\text{L}^{-1}$). The treatment effect was assessed for each variety separately. Statistical analysis of data was performed through analysis of variance (ANOVA) and the Tukey's multiple range test at the 5% probability level. Statistical analyses were performed by using SAS System 9 for Windows (SAS Institute Inc., Cary, NC, USA). Data were presented as mean values for each treatment.

RESULTS AND DISCUSSION

Yield, weight, diameter and color

Tomato yield, weight and diameter were affected by the N fertilization doses. The yield, weight and diameter of fresh tomato fruits increased as N supply increased up to the optimum N level; beyond that, their values were reduced (Table 1). The results indicate that the optimum N dose to achieve maximum tomato yield, weight and diameter is 30 $\text{mmol}\cdot\text{L}^{-1}$. Ozores-Hampton et al. (2012) showed similar trends to this study; tomatoes irrigated at doses of 172 and 298 $\text{kg}\cdot\text{ha}^{-1}$ of N produced maximum marketable yields, and considerably decreasing yields were observed with 22 and 470 $\text{kg}\cdot\text{ha}^{-1}$ of N applied. Another study, in which four N fertilization rates were evaluated (1, 100, 200 and 300 $\text{kg}\cdot\text{ha}^{-1}$), showed results of maximum total yield with 200 $\text{kg}\cdot\text{ha}^{-1}$ (Elia and Conversa 2012). This indicates that there is a threshold for N uptake at certain levels, which if exceeded, drastically decreases yield. According to Wei et al. (2018) at low N level, plants possess lower photosynthetic capacity, and reduced photoassimilates to be translocated from leaves to the fruits, consequently decreasing fruit set and fruit yield. According to Sainju et al. (2003) at high N levels an excessive vegetative growth is promoted which reduces setting of tomato fruits, therefore reducing tomato yield.

Table 1. Effect of N supply on yield, weight, diameter and color of 'Victoria' and 'Caballero' tomato under greenhouse conditions, under greenhouse conditions.

Cultivar	N supply ($\text{mmol}\cdot\text{L}^{-1}$)	Yield (kg/plant)	Weight (g/fruit)	Equatorial diameter (mm)	Axial diameter (mm)	Color ($^{\circ}$ hue)
Caballero	0	0.57 a	54.65 a	43.16 b	48.33 a	33.56 a
	15	0.97 b	48.75 a	41.83 b	51.16 a	33.62 a
	30	1.68 c	106.26 b	58.83 c	63.33 b	33.21 a
	45	0.55 a	44.32 a	40.83 ab	48.58a	33.90 a
	60	0.44 a	40.28 a	38.66 a	46.33a	33.67 a
Victoria	0	0.67 ab	52.72 b	43.16 b	49.83 b	33.48 a
	15	0.95 b	28.79 a	34.83 a	40.83 a	33.52 a
	30	1.70 c	109.68 c	63.33 c	67.66 c	33.49 a
	45	0.49 a	35.26 a	36.66 a	44.41 a	33.62 a
	60	0.42 a	28.50 a	33.5 a	42.16 a	33.26 a

Different letters within a variety and column represent significant differences (Tukey's test, $p < 0.05$).

No significant differences ($p < 0.05$) in the color parameters were observed for the different fertilization treatments. Similarly, for tomato cultivation, Warner et al. (2004), Simonne et al. (2007) and Bénard et al. (2009) found no direct relationship between color intensity and total N dose applied. According to Luh et al. (1973), temperature is more decisive than N for the development of tomato color.

Titrateable acidity, soluble solids and firmness

Soluble solids and titrateable acidity (TA) are highly correlated with the sweetness and sourness of tomatoes (Zushi and Matsuzoe 2011); therefore, these parameters are important indicators of the sensory quality of tomatoes. In this work, significant differences in TA and SS in tomato fruits were observed under different N doses (Fig. 1). The concentrations of TA and SS increased with increasing N rates. The highest concentration was observed with 60 mmol·L⁻¹ of N (Fig. 1). The effects of N application on SS and TA showed similar trends, with a positive slope of ~0.05°Brix/mmol·L⁻¹ of N for SS and ~0.006% citric acid/mmol·L⁻¹ of N for TA ($R^2 > 0.9$). Similar results were obtained by Wang et al. (2007), who reported that increasing the N supply increases the soluble solids and acidity of tomatoes. Bénard et al. (2009) concluded that citric acid content decreased significantly with lower N supply and increased with increasing N concentration. The findings of this work may be related to a stimulation of photosynthetic activity when increasing the N supply, with the consequent increase in photosynthates, some of which are stored as reducing sugars, leading to the accumulation of soluble sugars and soluble solids in tomato fruits (Wang et al. 2007).

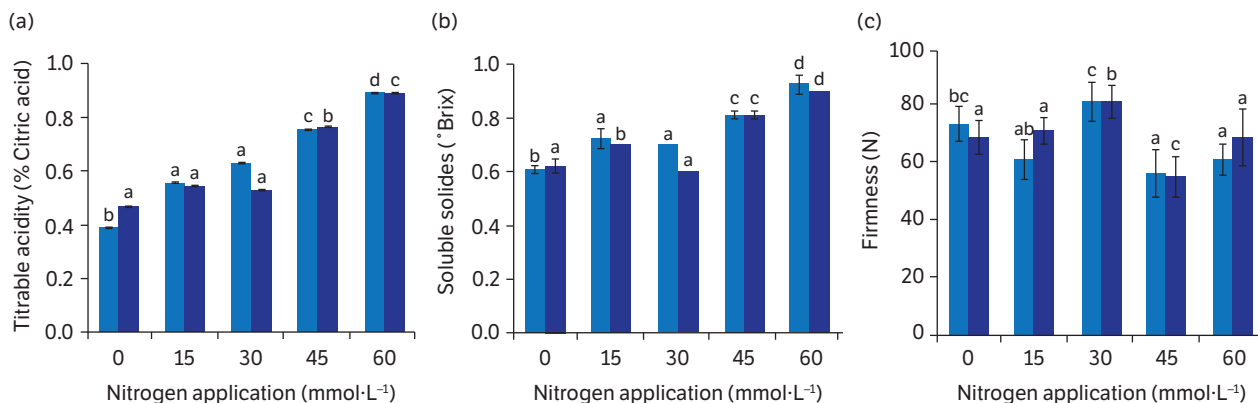


Figure 1. Effect of N supply on titrateable acidity (a), soluble solids (b) and firmness (c) of 'Victoria' and 'Caballero' tomato under greenhouse conditions. Error bars represent the standard deviations calculated from the analysis of three replicates. Different letters within a variety represent significant differences ($p < 0.05$) among treatments.

The firmness of the fruit was affected by N application in both cultivars, increasing with N rates up to 30 mmol·L⁻¹ (Fig. 1), and decreasing at higher N concentration. Similarly, Wang et al. (2007) and Erdal et al. (2007) found that the tomato fruit firmness increased with increasing doses of N until one point, but then firmness decreased. Although a sufficient amount of N is needed to have adequate firmness in tomatoes, higher amounts of N may result to a weaker translocation of Ca to the fruit. A reduction in the accumulation of Ca in the fruit results in the loss of cell wall integrity and loss of firmness (Tavallali et al. 2018, Zhang et al. 2020).

Total phenols, flavonoids and antioxidant activity

Total phenolic content was affected by fertilizer levels in the two cultivars. The highest value was observed at the highest level of N fertilization (60 mmol·L⁻¹). Small differences were observed at 0, 15, 30 and 45mM of N (Fig. 2). Bénard et al. (2009) found that tomato fruits grown with N supply from 4 to 12 mmol·L⁻¹ did not show strong differences in phenolic

compounds. The results of this work may indicate that the application of 60 mmol·L⁻¹ of N can be defined as toxic, as it drastically increased phenolic compounds in tomato. According to Sánchez et al. (2000), the abiotic stress caused in plants by excess of N causes an increase in the activity of phenylalanine ammonia-lyase (PAL), the enzyme responsible of the synthesis of phenolic compounds.

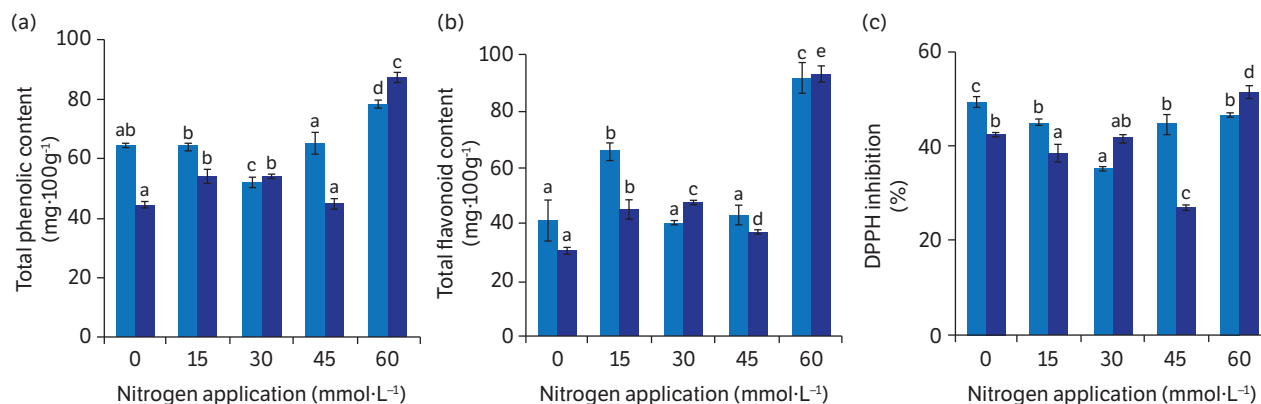


Figure 2. Effect of N supply on phenolic (a), flavonoids (b) and antioxidant capacity (c) of 'Victoria' and 'Caballero' tomato under greenhouse conditions. Error bars represent the standard deviations calculated from the analysis of three replicates. Different letters within a variety represent significant differences ($p < 0.05$) among treatments.

The N treatments affected the accumulation of flavonoids in the Caballero and Victoria cultivars. For both cultivars, plants treated with the highest N application (60 mmol·L⁻¹ of N) showed the greatest accumulation of flavonoids (Fig. 2). These increments could be related to the stimulation of some key enzymes of the phenylpropanoid pathway. In response to N supply, the induction of many enzymes of the phenylpropanoid pathway has been reported, including phenylalanine ammonia-lyase (PAL) in tomato leaves (Løvdaal et al. 2010).

The N treatments affected the antioxidant capacity in the two cultivars. Caballero and Victoria tomatoes showed a higher antioxidant capacity under N stress, connoting N deficiency or toxicity; with a similar behavior to that observed with the total phenolic content (Fig. 2). In 'Caballero', the optimal N concentration of 30 mmol·L⁻¹ was found to result in the lower antioxidant activity. For 'Victoria', 45 mmol·L⁻¹ showed the lowest antioxidant capacity. Bénard et al. (2009) reported that a reduction in the amount of N can be used to improve nutrition and quality by increasing the antioxidant content in fruits.

Increasing N concentration at stress levels (60 mmol·L⁻¹), caused an increase in antioxidant capacity in both cultivars (as well as higher total flavonoid and total phenolic content) (Fig. 2). The accumulation of phenolic compounds, flavonoids and antioxidant phytochemicals occurs first by the accumulation of toxic and harmful N supplements (Kumar and Pandey 2013), because these compounds usually accumulate in response to biotic or abiotic stresses (Reyes and Cisneros-Zevallos 2003).

Lycopene

Lycopene is the most important bioactive compound of tomato due to its beneficial effects on human health (Clinton 1998). The lycopene values (Fig. 3) observed for 'Caballero' were noticeably higher than those for 'Victoria'. The results for 'Caballero' show a tendency for the lycopene values to increase as the N concentration increased. Treatments at 0, 15 and 30 mmol·L⁻¹ of N were statistically similar, with an average of 54 ppm of lycopene, while treatments at 45 and 60 mmol·L⁻¹ of N resulted in accumulation of the highest concentrations of lycopene (63 ppm), respectively. For 'Victoria' the lycopene content of fruits collected from plants grown under different N doses did not show a clear trend (Fig. 3). Elkner et al. (2004) concluded that under a control treatment without N fertilization, lycopene content was the lowest, which agrees with our results obtained for 'Caballero'. However, Benard et al. (2009) found that fruits harvested from plants grown with the

lowest amount of N tended to have higher carotene content. In general, lycopene content is related to the tomato cultivar (Ochoa-Velasco et al. 2016). This was observed in this study since the cultivars evaluated here tended to perform differently.

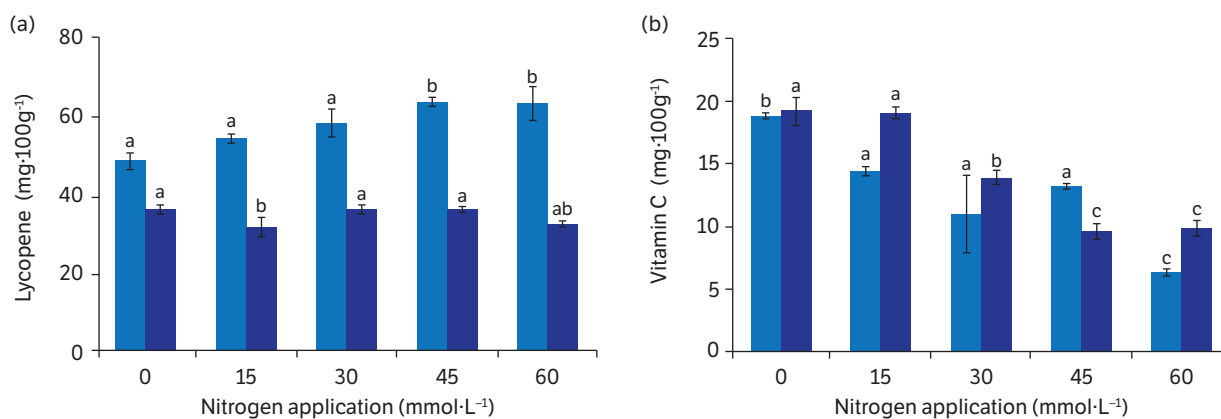


Figure 3. Effect of N supply on lycopene (a) and vitamin C (b) of 'Victoria' and 'Caballero' tomato under greenhouse conditions. Error bars represent the standard deviations calculated from the analysis of replicate plant samples. Error bars represent the standard deviations calculated from the analysis of three replicates. Different letters within a variety represent significant differences ($p < 0.05$) among treatments.

Ascorbic acid

Ascorbic acid is an important vitamin and an essential antioxidant for the human diet (Wheeler et al. 1998). The results of the present work indicate that N fertilization affected the concentration of ascorbic acid. The studied cultivars perform similarly with a tendency to exhibit decreased vitamin C contents as the N rate increases, however, the mechanisms of this reduction are not clear. The greatest accumulation of this vitamin was found in the control treatment and the lowest was found for the high dose N 60 mmol·L⁻¹. The above results are consistent with those found in previous studies: Kołota and Adamczewska (2008) reported that, regardless of the form of N, increasing the dose of 75 kg·ha⁻¹ to 300 kg·ha⁻¹ resulted in a decrease in vitamin C. Similarly, Ochoa-Velasco et al. (2016) observed that the content of vitamin C considerably decreased as the N fertilization increased. The decrease on the vitamin C content as N dose increases may be attributed to a higher oxidative stress potentially caused by N. It seems that as plant tissue are stressed by nitrogen, vitamin C could counteract oxidative damage by scavenging active oxygen species, generating a decrease in vitamin C concentration. Vitamin C apparently can act by regenerating other antioxidants that work against the abiotic stress caused by higher N concentrations used in the study. Furthermore, high N application rates might lower vitamin C levels, probably indirectly, because N might enhance foliage growth and hence increase canopy shading of the fruit on plants unevenly illuminated by direct sunshine. Ibrahim et al. (2011) reported that plants fertilized with high N levels tend to increase their foliage biomass. In general, the higher the intensity of light during the growing season, the greater the vitamin C content in fruit tissues. In order to establish the possible mechanism of reduction of vitamin C caused by toxic N applications; further studies are needed.

CONCLUSION

This paper describes how N application rates affect the fruit quality of two tomato cultivars in terms of weight, diameter, firmness, acidity, and soluble solids and enhances plant defense mechanisms, causing an increase in the production of phenolics, flavonoids and antioxidant capacity. Nitrogen application beyond the optimal concentration led to an increase on the antioxidant status of tomato, supported by an increase in antioxidant capacity, total phenolics, and flavonoids content. The reduction in vitamin C levels observed with increased N application rates apparently can be explained by the possible

action of this vitamin against abiotic stress, and consequently lowering its concentration in the fruit. However, more basic studies in this and other antioxidants must be confirmed in other cultivars to demonstrate the same trend.

AUTHOR'S CONTRIBUTION

Conceptualization, Olivas Orozco G. I.; Methodology, González-Aguilar G. A., Ayala-Zavala J. F. and Parra-Quezada, R. A.; Investigation, Frías-Moreno M. N., Dávila-Aviña J. and Molina-Corral F. J. M.; Writing – Review and Editing, Frías-Moreno M. N., Olivas Orozco G. I., Ayala-Zavala J. F., González-Aguilar G. A. and Parra-Quezada R. A.; Data Analysis, Espino-Díaz M.; Supervision, Olivas Orozco G. I.

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