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Research Article

Nitrogen and potassium supplied by phenological stages affect the carotenoid and nutritive content of the tomato fruit

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Abstract

The effect of nitrogen (N) and potassium (K) supply by phenological stages of horticultural crops such as tomato has been little explored so far. In this study, we evaluated the impact of N supply in the vegetative stage and K in the reproductive stage of tomato, on the carotenoid and nutritive content of fruits of three truss clusters. The concentrations of protein, lycopene, β-carotene, sugars, vitamin C and fruit juice were affected by the N and K application by phenological stages, although the N×K interaction was not significant in the last three variables. Increases in N from 10 to 16 $mol_c\ m^{-3}$ of nutrient solution (NS) in the vegetative stage of the crop increased the concentrations of protein, vitamin C, sugars (temporarily) and fruit juice. Likewise, increases in potassium (5 to 13 $mol_c\ m^{-3}$ NS) in the reproductive stage of the crop raised the concentrations of sugars, vitamin C, protein, lycopene, β-carotene and fruit juice. The concentration of carotenoids and the nutritional value of the tomato fruit were influenced by N and K nutrition by phenological stages, and these effects change slightly depending on the cluster harvested and the temperature during the growing cycle.

Keywords: β-carotene; lycopene; protein; *Solanum lycopersicum* L.; sugars; vitamin C

Introduction

The per capita intake of tomatoes in the world went from 8 kg to 21 kg between 1961 and 2013 (FAO, 2018). Tomato fruit is a source of proteins, vitamins, carotenoids, carbohydrates, and antioxidant substances, among others (Yilmaz, 2001; Bhowmik *et al.*, 2012; Souri and Dehnavard, 2017; Souri and Dehnavard, 2018). Tomato is a significant source of dietary vitamin C and in a daily intake, it can supply 47% of vitamin C (Jones, 2008). Among the carotenoids, lycopene helps reduce the risk of cancer, osteoporosis, and cardiovascular diseases (Burton-Freeman and Reimers, 2011), while β-carotene shows provitamin A activity (Tang, 2010).

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Tomato pulp and juice represent an important source of nutraceutical compounds, albeit the concentration of such compounds may be significantly affected by factors such as the genotype (Prudent *et al.*, 2009), environment (Cebolla-Cornejo *et al.*, 2011), and nutrient supply (Arah *et al.*, 2015). The ratios between sugars and organic acids give the tomatoes special tastes, although the role of genotype and environment cannot be ignored (Beckles, 2012; Souri and Dehnavard, 2017; Souri *et al.*, 2017). Sugars are the major constituents and influence the taste and quality of tomato (Beckles, 2012). Proteins, as the major organic form of nitrogen, are important in foodstuffs (NNDSR, 2018).

Nitrogen (N) and potassium (K) are the most required elements for plant metabolism, with 1 to 6% of the dry matter composition (Hawkesford *et al.*, 2012; Souri and Hatamian, 2019). N is part of proteins, nucleic acids, coenzymes, chlorophyll, and other compounds that affect the physiological and biochemical processes of plants (Leghary *et al.*, 2016). Application of potassium (K) and nitrogen (N) can significantly influence tomato plant growth, yield and quality parameters (Dehnavard *et al.*, 2017; Mardanluo *et al.*, 2018). By increasing the dose of N from 0 to 120 and 180 kg ha^{-1} during cultivation, sugars, lycopene, protein, and vitamin C concentrations in tomato fruit are increased (Kuscu *et al.*, 2014; Hui *et al.*, 2017). However, decreases in the contents of lycopene, β-carotene, and vitamin C in tomato fruits have also been reported when increasing N fertilization (Dorais *et al.*, 2008; Wang *et al.*, 2015).

Potassium (K) has key roles in various physiological and biochemical processes in plants, including photosynthesis, osmoregulation, enzyme activity, secondary metabolism, protein biosynthesis, sugar transport and many quality traits of products (Oosterhuis *et al.*, 2014; Pourranjbari Saghaiesh *et al.*, 2018; Tohidloo *et al.*, 2018). Likewise, in open field tomato cultivation, K doses from 0 to 60 and 120 kg ha^{-1}, or K levels of 200 to 400 mg L^{-1} in the hydroponic solution, increase the content of vitamin C, sugars, lycopene, and protein of the fruit (Almeselmani *et al.*, 2009; Ahmad *et al.*, 2015), although decreases in sugars (42 to 25 g) and vitamin C were also reported due to a K supply of 0 to 200 kg ha^{-1} (Ehsan-Akhtar *et al.*, 2010). Until now, individual fertilizations of N and K, along with combinations of the two nutrients, have been evaluated in tomato. Nonetheless, nutritional requirements of N and K vary according to the phenological stage (Jones, 2008). Furthermore, fruit quality also varies among clusters (Coyago-Cruz *et al.*, 2018).

Tomato mineral nutrition has been extensively studied for many years, though little attention has been paid to N and K nutrition from a phenological point of view. In this study, we hypothesized that N and K nutrition by phenological stages may affect the nutritional value of tomato fruit. Thus, we aimed to study the impact that the supply of N during the vegetative stage and of K during the reproductive stage of tomato have on the concentration of carotenoids and the nutritional value of the fruit in three clusters.

Materials and Methods

Plant material, treatments and experimental design

The research was done in hydroponics using 'tezontle' (red volcanic rock, particles ≤12 mm in diameter) as substrate, under greenhouse conditions, with 37-day-old tomato cv. 'Charleston' (Rogers Seeds®) seedlings. The experiment was carried out in Montecillo, Mexico. The complete experiment was carried out for six months (July to December). In the nutrient solution (NS) of the hydroponic culture, two nutrients were evaluated by phenological stages. In the vegetative stage (limited to the anthesis of the first flower cluster) corresponding to the first 45 days after transplantation (dat), N was supplied at concentrations of 10, 12, 14, and 16 mol_c m^{-3} NS, applying 75% as NO_3^- and 25% as NH_4^+; and in the reproductive stage (46 to 170 dat), K levels of 5, 7, 9, 11, and 13 mol_c m^{-3} NS were evaluated. The experiment was carried out under a factorial arrangement in a completely randomized split-plot design, with N as a large plot and K as a small plot, resulting in 20 treatments with six replicates. The experimental unit (EU) was one plant per pot with 13 L of 'tezontle.'

According to the phenological stage, each experimental unit was irrigated with Steiner's nutrient solution (Steiner, 1961) modified in N and K. The original concentrations of NO_3^- and K^+ in Steiner´s solution

is 12 and 7 $mol_c\ m^{-3}$ NS, respectively. In the first 30 dat, eight irrigations of 5 min each were applied daily at 1 h intervals using 4 L h^{-1} droppers. After this and until the conclusion of the harvest (167 dat), 16 daily irrigations were applied in the abovementioned manner.

The maximum and minimum temperatures during the research were recorded with a Hobo® H8 data logger (Onset Computer Corporation, USA), reporting the values as decennial averages from July to December (Figure 1).

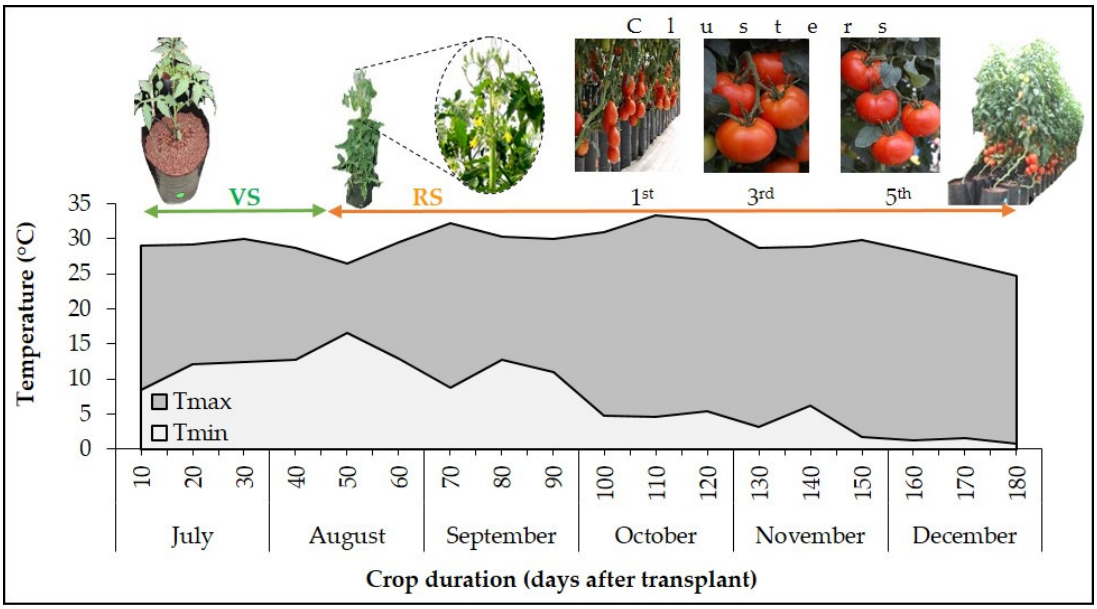

Figure 1. Decennial averages of maximum and minimum temperatures during the vegetative (VS) and reproductive (RS) stages of the tomato crop from July to December

Evaluated variables

The variables were analysed in completely red fruits taken individually from the first (110 dat), third (131 dat), and fifth (167 dat) floral clusters (see details in Figure 1), considering two fruits per experimental unit and per cluster.

Juice. The juice was obtained with an extractor (Tur Mix®, Mexico) considering the initial fruit weight and the final weight of the extracted juice (without seeds or epidermis), expressing the value as a percentage (San Martín-Hernández *et al.*, 2012).

Total sugars in tomato juice. The determination of total sugars was done from 1 g of juice by means of the anthrone colorimetric method (Witham *et al.*, 1971) and by using a D-glucose standard (Sigma-Aldrich®, USA) of known concentration in the calibration curve. The samples were read at 600 nm in a spectrophotometer (Spectronic 20 Baush and Lomb®, USA), obtaining the value in g kg^{-1} of fresh fruit (FF).

Vitamin C. Vitamin C was determined based on the 967.21 official method (AOAC, 2002). One millilitre of tomato juice was mixed with 30 mL 0.5% oxalic acid; then, a 5 mL aliquot was collected and titrated with Tillman's solution (2,6-dichlorophenol indophenol 0.02%, DCIP from Sigma-Aldrich®) until colour change to pale pink (i.e. 15 s after the start of the reaction). The quantification of vitamin C was done by means of L-ascorbic acid standard (Sigma-Aldrich®, USA) in the calibration curve, expressing its concentration in mg kg^{-1} FF.

Protein. The concentration of N in the fruit was analysed using the micro Kjeldahl method and the percentage of N was converted to crude protein by multiplying the percentage of N by 6.63, expressing the value in g kg^{-1} FF (Fujihara *et al.*, 2001; AOAC, 2002).

Lycopene and β-carotene. The carotenoids were extracted following the methods described by Lin and Chen (2003) with some modifications. Two grams of fresh fruit were ground for 1 min in a low volume blender, with 10 mL of extraction solvent (ethanol:hexane, 4:3, v/v), 0.05 g $MgCO_3$, and 1 mL butylated hydroxytoluene (BHT) at 0.025%. The sample was shaken 30 min at 140 rpm on an orbital shaker (Lab-Line®, USA) under reduced light conditions. The upper phase was transferred to a 125 mL flask and the lower phase was extracted again with 16 mL of extraction solvent, shaking as in the previous step. The upper phase was transferred to the same 125 mL flask and the lower part was re-extracted with 5 mL of hexane at 280 rpm for 20 min. In this last step, the sample was filtered with Whatman No. 1 paper, placing the extract in the same 125 mL flask, to which 37.5 mL of distilled water and 25 mL of 10% NaCl were added for a phase partition. The organic phase was collected and evaporated to dryness at 35 °C. The sample was re-suspended with CH_2Cl_2 and filtered on a 0.45 µm membrane, obtaining a final volume of 1 mL of extract, which was placed in an amber vial for storage at -20 °C until analysis by HPLC. The identification of carotenoids was done by comparison of the retention times with the authentic reference standards, which were subjected to a spectral scan. Lycopene showed three absorbance maximums at 448, 508, and 472 nm (Figure 2A), and β-carotene showed two at 456 and 480 nm (Figure 2B). However, in this work the HPLC analysis *per se* was favourable for both compounds at 472 nm (Figure 2C). For these two variables, three replicates were analysed in duplicate in each treatment, quantifying mg kg^{-1} FF according to the calibration curve at concentrations of 10, 30, 100, 200, 300, and 400 µg mL^{-1} in lycopene (Abs = 145.086*[lycopene µg mL^{-1}] + 1466.630, R^2 = 0.996), and 0.5, 1.0, 2.0, 4.0, 30.0, and 60 µg mL^{-1} for β-carotene (Abs = 62.969*[β-carotene µg mL^{-1}] + 30.385, R^2 = 0.987).

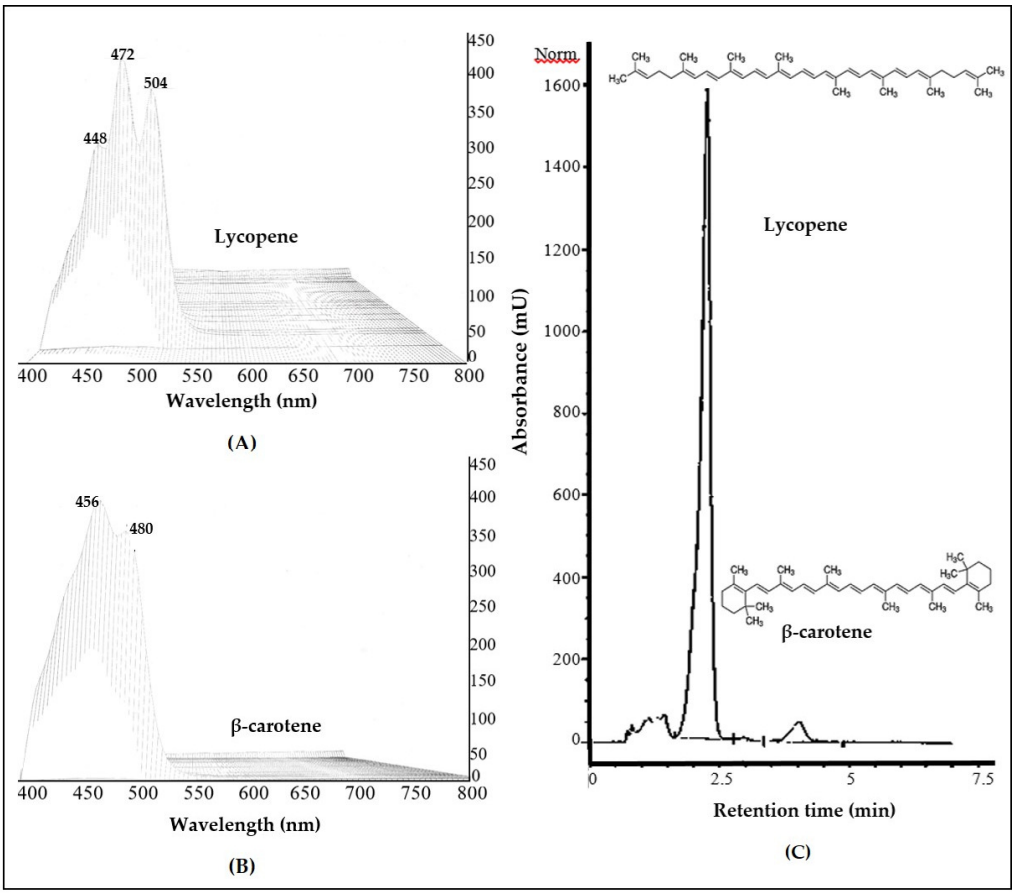

Figure 2. Spectral scanning of carotenoids (A and B); lycopene and β-carotene analyzed by HPLC in completely red tomato fruits (C)

The samples were analysed with the butanol, acetonitrile, and dichloromethane mobile phase in the ratio 29.7:69.3:1 (v:v:v) according to Lin and Chen (2003). The elution was in isocratic mode, injecting 20 µL sample^{-1} at a flow of 2 mL min^{-1} and a duration of 7 min each. The carotenoids were analysed with an Agilent® 1200 HPLC system (Germany) with a diode array detector and a Zorbax Eclipse XDB-C18 4.6x150 mm, 5 µm Ø column (USKH0637359); the solvents ethanol (Fermont®, Mexico), hexane (J.T. Baker®, Mexico), methylene chloride, 1-butanol, and the lycopene and β-carotene standards (Sigma-Aldrich®, USA) were HPLC grade.

Statistical analysis

The statistical analysis was done based on the effects model (1) (Kuehl, 2000; Jones and Nachtsheim, 2009) adapted to a completely randomized split-plot design.

$$Y_{ijk} = \mu + N_i + \varepsilon_a + K_j + N \times K_{ij} + \varepsilon_{ijk} \quad (1)$$

Where: Y_{ijk} = response variable; μ = general mean; N_i = effect of the i-th level of N; ε_a = large plot experimental error; K_j = effect of the j-th level of K; $N \times K_{ij}$ = effect of the N × K interaction; ε_{ijk} = random experimental error. $\varepsilon_a \sim NI(0\ \sigma_a^2)$ and $\varepsilon_{ijk} \sim NI\ (0\ \sigma^2)$ are the assumptions of the model that are assumed to be normal, independent, with zero mean, and common variance σ^2.

The variables determined in the fruits were analysed individually for each of the three clusters evaluated, because their behaviour can change among them (Coyago-Cruz *et al.*, 2018). With the data, the analysis of variance was performed and the means were compared according to the Tukey test ($p \leq 0.05$) considering the standard deviation (SD) with the SAS 9.3 software (SAS, 2011).

Results and Discussion

The main effect of N was significant in the concentrations of juice, sugars, and lycopene in fruits of the first cluster, in the concentrations of vitamin C and protein of fruits of the three clusters, and in the concentration of β-carotene of fruits of the first and third clusters analysed. The main effect of K was significant in all variables, except in the concentration of juice of the first cluster. On the other hand, the N × K interaction was only significant in the protein and lycopene concentrations of the first cluster and for β-carotene in the three clusters evaluated (Table 1).

Table 1. Effects (p-value) of the supply of N, K and N × K interaction in tomato cultivation on the concentrations of total sugars, vitamin C, protein, lycopene, β-carotene, and fruit juice

Variable	Cluster	Source of variation			Coefficient of variation (%)
		N	K	N × K	
Juice	1st	0.0012 *	0.1667 ns	0.2401 ns	4.7
	3rd	0.3198 ns	<.0001 *	0.9973 ns	2.6
	5th	0.0956 ns	0.0031 *	0.9999 ns	5.8
Total sugars	1st	0.0040 *	0.0190 *	0.9288 ns	13.2
	3rd	0.1469 ns	0.0003 *	0.9242 ns	15.3
	5th	0.3619 ns	<.0001 *	0.9986 ns	12.5
Vitamin C	1st	<.0001 *	<.0001 *	0.8113 ns	14.3
	3rd	0.0131 *	<.0001 *	0.0645 ns	20.9
	5th	<.0001 *	<.0001 *	0.4058 ns	15.7
Protein	1st	<.0001 *	<.0001 *	<.0001 *	14.0
	3rd	0.0065 *	0.0166 *	0.0998 ns	12.5
	5th	0.0031 *	0.0179 *	0.3943 ns	17.3
Lycopene	1st	0.0193 *	<.0001 *	0.0005 *	14.8
	3rd	0.5929 ns	<.0001 *	0.7315 ns	14.6
	5th	0.9067 ns	<.0001 *	0.4442 ns	8.2
β-carotene	1st	0.0006 *	0.0002 *	0.0360 *	11.0
	3rd	0.0271 *	<.0001 *	<.0001 *	7.2
	5th	0.1317 ns	<.0001 *	<.0001 *	6.9

*: significant; ns: not significant ($\alpha = 0.05$)

Juice

Between clusters, the effects of N were limited to fruits of the first bunch. Supplies of N from 10 to 16 mol_c m^{-3} NS to the crop increased the juice percentage by 5%, obtaining its lowest percentage (82.2%) in the lowest N concentration in the nutrient solution (Figure 3A). With K, the effects were observed in fruits of the last two clusters. When K went from 5 to 13 mol_c m^{-3} NS, the juice increased by 6 and 7%, although its maximum values of 90 and 88% were obtained with 13 mol_c m^{-3} NS, in the third and fifth clusters, respectively (Figure 3B). These results agree with juice values of 90% in ball tomato produced in hydroponics (San Martín-Hernández *et al.*, 2012). The best doses to increase this attribute were 16 mol_c N m^{-3} NS and from 9 to 13 mol_c K m^{-3} NS. In grapes, the juice content is an attribute that benefits from greater applications of K (Gawek *et al.*, 2000), but its effects occur when enough N is applied at the same time (Ganeshamurthy *et al.*, 2011). Sufficient K concentrations in plant tissues can facilitate an osmotic adjustment that maintains a high turgor pressure (Wang *et al.*, 2013), which is associated with the water content in the cellular tissue and therefore the juice yield can increase as observed in this research.

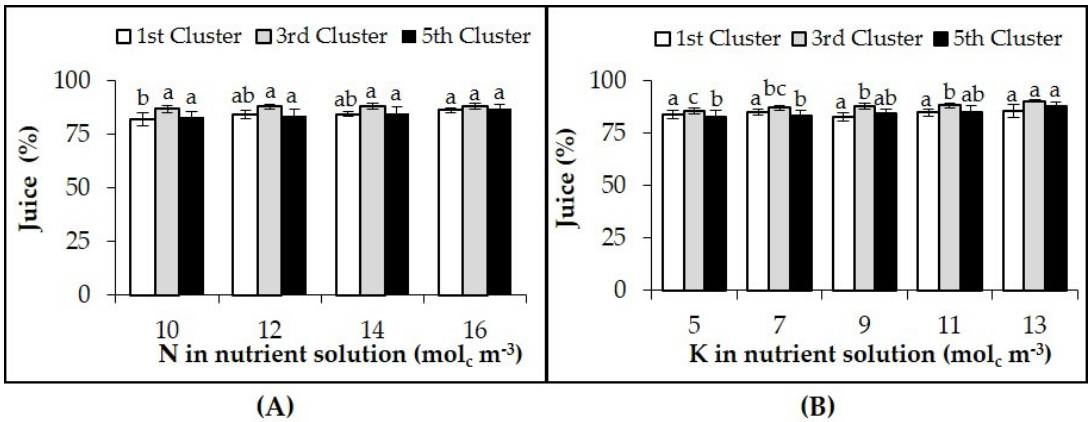

Figure 3. Percentage of fresh fruit juice in three tomato clusters according to the level of supply of N in the vegetative stage (A) and of K in the reproductive stage (B) of the crop

Means ± SD in each cluster and variable with different letters indicate statistical differences (Tukey, $p \leq 0.05$)

Total sugars

An increase in N from 10 to 16 mol_c m^{-3} NS increased the concentration of sugars by 12.5% in the fruits of the first cluster, achieving their highest concentration (32.4 g kg^{-1} FF: fresh fruit) with 16 mol_c N m^{-3} NS (Figure 4A). Contrary to these results, high doses of N in tomato crops limit fruit sugar content (Parisi *et al.*, 2006).

In carbohydrate metabolism, K plays important functions (Jensen et al., 2013), as a companion ion in the release of sugars from mesophyll cells of the leaves to the demand organs (Engels *et al.*, 2012), affecting their distribution (Kanai *et al.*, 2007). High sugar contents depend on the importation of sucrose to the fruit (Balibrea *et al.*, 2006). In this experiment, when this cation changed from 5 to 13 mol_c m^{-3} NS, the concentration of sugars increased by 13, 19, and 20% in fruits of the first, third, and fifth clusters, respectively. Supplies between 9 and 13 mol_c K m^{-3} NS to the crop generated the highest values of sugars in the fruit (Figure 4B). Applications of K equivalent to 3.5 to 11.5 mol_c m^{-3} SN in tomato increase the sugar content of the fruit by 26% (Caretto *et al.*, 2008).

Between clusters, the concentration of sugars was differential and increased during the course of the crop. Roots, leaves, and fruits of clusters in formation and development compete for the supply of photosynthates. When the leaves senesce, the photosynthetic machinery is disorganized and the production of carbohydrates decreases (Falqueto *et al.*, 2009). In tomato, pruning old leaves is part of the intensive

management (Beyers *et al.*, 2014), which could favor the increase of sugars in fruits of the third and fifth clusters (Figures 4A and 4B).

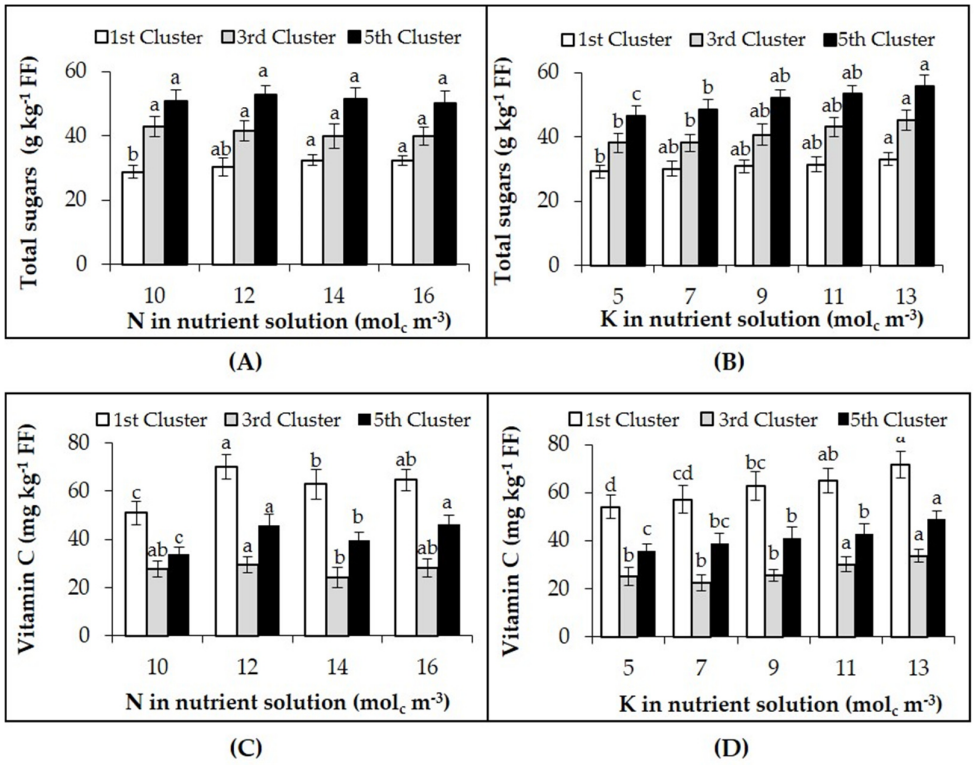

Figure 4. Concentration of total sugars (A and B) and vitamin C (C and D) in fresh fruit (FF) of three tomato clusters according to the level of supply of N in the vegetative stage and of K in the reproductive stage of the crop

Means ± SD in each cluster and variable with different letters indicate statistical differences (Tukey, $p \leq 0.05$)

Vitamin C

Regardless of the N and K treatments evaluated, the vitamin C concentration of the fruit was higher in the first cluster and lower in the third (Figures 4C and 4D).

In tomato, high N applications during cultivation decrease the vitamin C content of the fruit (Dumas et al., 2003), while K exerts an opposite effect (Afzal *et al.,* 2015).

Contrary to the literature, when the supply of N was increased in tomato cultivation (from 10 to 16 $mol_c\ m^{-3}$ NS), the synthesis of vitamin C was favoured, although at 12 mol_c N m^{-3} NS, the highest averages were obtained with 70, 30, and 46 mg kg^{-1} FF, in fruits of the first, third, and fifth clusters, respectively (Figure 4C).

On the other hand, by increasing the application of K from 5 to 13 $mol_c\ m^{-3}$ NS, the concentration of vitamin C increased by 33, 38, and 37% in fruits of the first, third, and fifth clusters, respectively (Figure 4D). L-ascorbic acid is a compound derived from carbohydrates, whose precursors are L-galactose, L-galactone-1,4-lactone, and L-gulose (Lisko *et al.*, 2014). The highest concentration of vitamin C obtained with the highest application of K to the crop can be associated with the transport and accumulation of sugars to the fruit (Bernardi and Verruma-Bernardi, 2013; Vicente *et al.*, 2014), which favours its synthesis (Mengel and Kirkby, 2001).

Protein

Despite the increase in fruit protein due to the supplied nitrogen levels, it decreased between clusters during cultivation. Applications of N from 10 to 16 $mol_c\ m^{-3}$ NS in the vegetative stage of the crop increased the protein content by 21, 16, and 7% in fruits of the first to fifth clusters (Figure 5A). The protein composition of the fruit results directly from the effects that nitrogen nutrition imposes on the crop (Rajasree and Pillai, 2012; Liu *et al.*, 2016) since this nutrient facilitates the requirement for protein synthesis (Wang *et al.*, 2014).

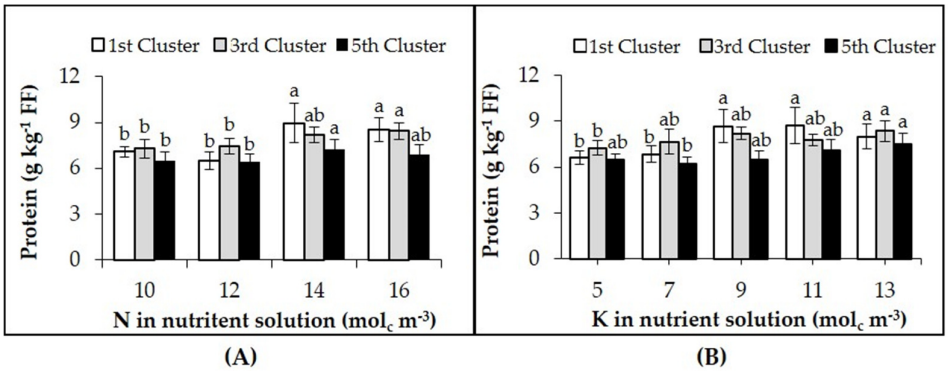

Figure 5. Concentration of protein in fresh fruit (FF) of three tomato clusters according to the level of supply of N in the vegetative stage (A) and of K in the reproductive stage (B) of the crop
Means ± SD in each cluster and variable with different letters indicate statistical differences (Tukey, $p \leq 0.05$)

K supplies of 5 to 13 $mol_c\ m^{-3}$ NS in the reproductive stage of the crop increased the protein concentration of fruits of the first, third, and fifth clusters by 21, 15, and 16%, respectively. However, with K between 9 and 13 $mol_c\ m^{-3}$ NS, the highest protein averages were obtained, being 8.7, 8.4, and 7.5 g kg^{-1} FF in the first, third, and fifth clusters, respectively (Figure 5B). Protein synthesis is the most sensitive process due to the K level in the plant medium (Faust and Schubert, 2016).

In plant metabolism, N and K are closely related and both play a crucial role in protein synthesis (Coskun *et al.*, 2017). The N:K combinations of 14:9 and 14:11 $mol_c\ m^{-3}$ NS were the ones that most increased the protein concentration in the first cluster with 10.5 and 12.2 g kg^{-1} FF, respectively (Table 2).

Lycopene

The increase in N from 10 to 16 $mol_c\ m^{-3}$ NS generated an inverse relationship with the concentration of lycopene in fruits harvested from the first cluster (Figure 6A). On this carotenoid, the literature is contradictory. The lycopene concentration in tomato increases from 38 to 68 mg kg^{-1} FF when the N supplied to the crop decreases from 15.8 to 1 $mol_c\ m^{-3}$ SN (Dumas *et al.*, 2003). Similar responses were found in another research report (Wang *et al.*, 2015). In contrast, small increases in N fertilization during cultivation increase the lycopene content of the fruit (Kuscu *et al.*, 2014; Hui *et al.*, 2017). Indeed, N and K status in the culture media may significantly affect various quality attributes of tomato fruits. Thus, when providing N or K at suboptimal levels, the gradual increase of such nutrients positively affects quality traits such as lycopene content. Conversely, when such nutrients are supplied at sufficient or high levels, quality attributes are negatively affected (Souri *et al.*, 2018; Souri and Hatamian, 2019).

On the other hand, when K increased from 5 to 13 $mol_c\ m^{-3}$ NS in the reproductive stage of the crop, the lycopene concentration increased by 39, 49, and 51% in fruits of the first, third, and fifth clusters, respectively (Figure 6B). The increase in the lycopene concentration has been related to the increase in the supply of K to the tomato crop (Taber *et al.*, 2008). Regarding carbohydrate metabolism, K modulates the enzymes pyruvate kinase and phosphofructokinase, and the formation of acetyl CoA is affected, which is involved in obtaining isopentenyl diphosphate, the first precursor of carotenoids (Fanasca *et al.*, 2006; Vasák and Schabl, 2016).

Table 2. Effect of the combinations of N in the vegetative stage and of K in the reproductive stage (N:K), during tomato cultivation, on the concentration of protein in fruits of the first, third, and fifth clusters

N:K ($mol_c\ m^{-3}$)	Protein ($g\ kg^{-1}$ fresh fruit)		
	1st	3rd	5th
10:5	7.2±0.2cdefg	6.9±0.5a	6.4±0.2a
10:7	7.2±0.2cdefg	6.6±1.0a	5.3±0.6a
10:9	7.3±0.6cdefg	8.3±0.3a	6.0±0.8a
10:11	6.8±0.5defg	7.1±0.3a	7.8±0.5a
10:13	6.8±0.2defg	7.7±0.6a	6.9±0.2a
12:5	6.1±0.4fg	7.0±0.3a	6.1±0.4a
12:7	5.8±0.6g	6.7±0.6a	6.2±0.6a
12:9	6.9±0.8defg	7.5±0.3a	7.1±0.7a
12:11	7.0±0.3defg	8.4±0.4a	6.2±0.3a
12:13	6.8±0.6defg	7.6±0.6a	6.5±0.1a
14:5	6.2±0.1fg	7.3±0.6a	6.9±0.2a
14:7	6.5±0.5efg	8.3±0.5a	6.6±0.2a
14:9	10.5±1.1ab	9.4±0.0a	6.7±0.2a
14:11	12.2±0.1a	7.7±0.2a	7.7±0.7a
14:13	9.4±0.3bcd	8.6±0.5a	8.4±1.0a
16:5	7.0±0.6defg	7.9±0.5a	6.6±0.6a
16:7	7.8±0.1bcdefg	9.0±0.5a	6.7±0.1a
16:9	10.0±0.3abc	7.9±0.3a	6.2±0.2a
16:11	8.8±0.7bcdef	7.9±0.2a	6.8±0.9a
16:13	9.1±1.0 bcde	9.5±0.6a	8.3±0.9a

Means ± SD in each column with different letters indicate statistical differences (Tukey, $p \leq 0.05$)

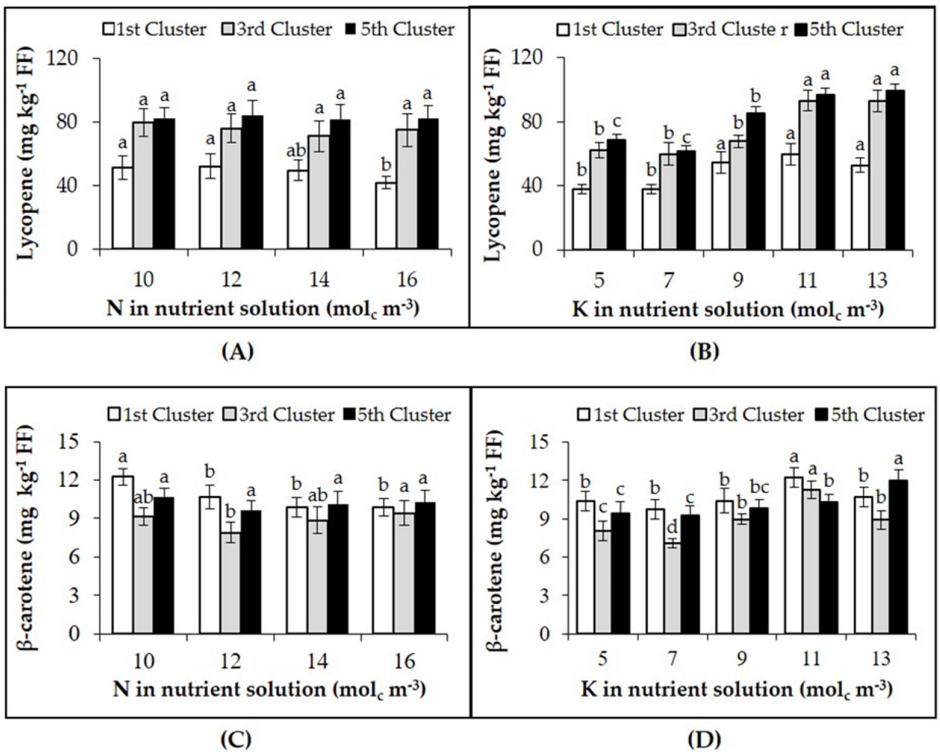

Figure 6. Concentration of lycopene (A and B) and β-carotene (C and D) in fresh fruit (FF) of three tomato clusters according to the level of supply of N in the vegetative stage and of K in the reproductive stage of the crop

Means ± SD in each cluster and variable with different letters indicate statistical differences (Tukey, $p \leq 0.05$)

The highest lycopene concentrations were recorded with K from 9 to 13 $mol_c\ m^{-3}$ NS (Figure 6B) and with N doses of less than or equal to 14 $mol_c\ m^{-3}$ NS (Figure 6A); thus, the best N:K combinations to promote lycopene synthesis were 10:9, 12:11, 14:11, and 10:13 $mol_c\ m^{-3}$ NS (Table 3).

Lycopene synthesis increased in fruits from the first to the fifth cluster. From November to December, temperatures lower than 30 °C were recorded (Figure 5); this coincided with the harvest of the last two clusters. High temperatures inhibit lycopene synthesis, being optimal between 18 and 26 °C; therefore, if the temperature exceeds 30 °C, the lycopene content decreases (Brandt *et al.*, 2006).

Table 3. Effect of the combinations of N in the vegetative stage and of K in the reproductive stage (N:K), during tomato cultivation, on the concentration of lycopene and β-carotene in fruits of the first, third, and fifth clusters

N:K ($mol_c\ m^{-3}$)	Lycopene (mg kg^{-1} fresh fruit)			β-carotene (mg kg^{-1} fresh fruit)		
	1st	3rd	5th	1st	3rd	5th
10:5	38.8±0.8cde	68.0±6.2a	68.6±5.2a	12.1±0.5abcd	8.4±0.1defgh	11.2±0.6bc
10:7	37.5±1.2cde	71.3±0.9a	68.2±2.3a	11.7±0.4abcde	7.7±0.1efgh	10.8±0.3bcd
10:9	73.2±1.1a	66.6±4.2a	80.1±5.0a	13.5±0.1a	9.0±0.2cdefg	8.2±0.6ef
10:11	51.4±6.1abcde	95.4±1.4a	92.5±0.8a	12.7±1.1abc	11.3±0.3abc	9.4±0.1cdef
10:13	55.2±5.3abcde	96.2±12.0a	100.6±0.9a	11.2±0.4abcde	9.3±0.7bcdef	13.8±0.3a
12:5	39.1±4.9cde	62.5±5.8a	71.4±3.0a	9.3±0.7bcde	6.7±0.4gh	8.2±0.4ef
12:7	33.8±2.1de	58.4±9.0a	59.3±2.5a	9.9±0.8abcde	6.1±0.1h	7.6±0.4f
12:9	59.2±1.8abc	75.3±0.5a	92.0±5.2a	9.4±0.4bcde	9.7±0.1bcde	10.4±0.3bcde
12:11	72.2±1.6a	99.3±3.3a	98.5±6.9a	12.9±0.4ab	9.8±0.4bcde	9.4±0.3cdef
12:13	56.3±5.7abcd	84.1±4.8a	100.3±6.2a	12.0±0.9abcde	7.2±0.1fgh	12.4±0.0ab
14:5	41.4±1.7cde	57.7±6.1a	64.0±2.4a	10.8±0.5abcde	6.8±0.1gh	7.1±0.3f
14:7	43.7±4.6cde	53.3±5.8a	57.5±3.3a	8.4±0.6e	7.7±0.2efgh	10.2±0.4bcde
14:9	42.3±3.4cde	64.3±1.2a	86.8±2.9a	9.5±0.3bcde	8.7±0.2defg	11.2±0.5bc
14:11	68.2±4.1ab	87.4±11.2a	100.2±5.6a	11.7±0.7abcde	12.3±0.9a	10.2±0.1bcde
14:13	51.9±6.4 abcde	92.2±4.0a	98.5±4.0a	9.1±0.6cde	8.9±0.3cdefg	11.8±0.7abc
16:5	32.7±1.6 e	61.3±3.3a	71.3±2.1a	9.4±0.4bcde	10.2±0.2abcd	11.1±0.2bc
16:7	36.7±1.7 cde	57.0±8.9a	61.3±3.8a	8.9±0.2de	6.7±0.1gh	8.3±0.4def
16:9	44.1±2.8 cde	65.5±6.1a	83.7±2.6a	9.2±0.2cde	8.4±0.7defgh	9.5±0.1cdef
16:11	47.7±4.1 bcde	90.6±7.3 a	96.3±3.8a	11.7±1.0 abcde	11.6±0.5 ab	12.3±0.2ab
16:13	48.2±1.5 bcde	99.6±3.9 a	98.8±5.3a	10.4±0.3 abcde	10.2±0.5 abcd	10.1±0.6bcde

Means ± SD in each column with different letters indicate statistical differences (Tukey, $p \leq 0.05$)

β-carotene

Although the increase in N from 10 to 16 $mol_c\ m^{-3}$ NS decreased the β-carotene concentration in fruits of the first cluster by 20%, in the third it increased by 19% between 12 and 16 $mol_c\ m^{-3}$ NS (Figure 6C). In contrast, a supply of K from 5 to 13 $mol_c\ m^{-3}$ NS improved the synthesis of β-carotene by 3, 11, and 28%, in fruits of the first, third, and fifth clusters, respectively (Figure 6D). Similar responses have been obtained when K is raised from 8 to 9 $mol_c\ m^{-3}$ NS in tomato (Ramírez *et al.*, 2012). In strawberries and pepper, increasing the K supply from 210 mg L^{-1} to 350 mg L^{-1} in the nutrient solution significantly increased key quality attributes of fruits (Tohidloo *et al.*, 2018).

In general, increases in K at each level of N stimulated the synthesis of β-carotene, achieving its maximum values with K between 9 and 13 $mol_c\ m^{-3}$ NS, while N concentrations less than or equal to 14 $mol_c\ m^{-3}$ NS favoured the best level of this pigment. Therefore, the N:K combinations that most promoted the synthesis of β-carotene were 10:9, 10:13, and 14:11 (Table 3).

The concentration of β-carotene varied between clusters, being higher in the first than in the fifth and third (Figures 6C and 6D). High temperatures can promote the conversion of lycopene to β-carotene (Dorais

et al., 2008). In this study, the harvest of the first cluster coincided with the highest recorded temperatures (Figure 1).

Conclusions

Herewith we demonstrated that the concentration of carotenoids and the nutritional value of tomato fruits were influenced by N and K supply at different phenological stages. Nonetheless, these effects changed slightly according to the origin of the fruit among the three clusters analysed.

Increasing N supply in the vegetative stage of the crop increased the concentrations of protein, vitamin C, sugars (temporarily) and fruit juice. However, at the beginning of production, carotenoid synthesis may have been decreased due to the effects of N. Applications of N from 12 to 16 $mol_c\ m^{-3}$ NS until the anthesis of the first cluster of the crop promoted the highest concentrations of protein and vitamin C of the fruit. Likewise, supplying K from 11 to 13 $mol_c\ m^{-3}$ NS during the reproductive stage of the crop resulted in the highest concentrations of sugars, vitamin C, juice, protein, lycopene and β-carotene. The N x K interaction improved the synthesis of protein, lycopene and β-carotene. In order to get the highest concentrations of such molecules (i.e. protein, lycopene and β-carotene), the combinations N:K 14:11, 10:9 and 10:13 $mol_c\ m^{-3}$ NS, respectively, are suggested. Additionally, among the flower clusters from the first to the fifth, the nutritional constitution of the fruit shows different trends such as increases in sugars and lycopene, reductions in protein and a differential behavior in juice, vitamin C and β-carotene.

Authors' Contributions

Conceptualization: LITT, FCGM; Methodology: LITT, CSMH; Validation: LITT; Formal analysis: CSMH, EAQO, MDMR; Investigation; CSMH, FCGM; Resources; LITT, CSV; Writing-original draft: CSMH, FCGM; Writing-review and editing: LITT, EAQO, MDMR, CSV; Supervision: LITT, FCGM; Project administration: LITT. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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