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IMPACT OF NITROGEN AND WATER STATUS WITHIN PLANT ON DRY MATTER FORMATION AND NUTRITIONAL VALUES OF TOMATOES

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Abstract

Yield and quality of tomato were evaluated under water and nitrogen stress conditions. Tomato plants were cultured under controlled conditions at three different rates of N supply to obtain three N statuses in plants {low (N1), suboptimal (N2) and optimal (N3)}, under three levels of drought stress (severe, moderate and well-watering) until fruit maturity. Tomato plants were subjected to different irrigation regimes (pots were daily watered until moisture reached 60, 80 and 100% of field capacity). Nitrogen levels in plant were controlled by means of daily supplementation of amounts needed to establish three N statuses (1%, 1.5% and 2.5%) based on expectations of plant growth. Dry mass % in leaves were not affected by drought stress (water status) but was reduced with promoted nitrogen status. Soluble sugar concentration in fruits increased significantly under drought stress, N status had no significant effect on soluble sugars concentration in fruits. High water and N status resulted in reduced ascorbic acid and lycopene concentration in tomato fruits. N and water status had significant effects on most macro and micro nutrients in tomato fruits. Low water status resulted in increased K, P Mg, Ca Mn and Zn concentration in tomato fruits. Also, low N status enhanced K concentration in fruits whereas, P and Zn concentration in fruits were increased with increasing N status within plants. However, N status had no significant effect on Mg Ca and Mn concentration in fruits. Adjusting the water and nitrogen status in plant to the needs of each growth stage may produce tomatoes with high nutritional values.

Keywords: Drought stress, nitrogen deficiency, tomato quality, biomass partitioning, lycopene, β-carotene, ascorbic acid concentration.

Introduction

The arid and semi-arid regions of the world are generally characterized by low productivity, which is due to a combination of low availability of both water and soil nitrogen (Hooper and Johnson, 1999; Peek and Forseth, 2003). To improve the efficiency of using limited resources, the negative and positive effect of finite available water and soil N concentrations on the reproductive organs, should assessed. Tomatoes constitute an important agricultural crop worldwide and an integral part of human diet. They are grown for their edible fruits which can be eaten raw in salad or cooked, peeled or made into purees ketchup, soup or powdered or juice in any canning industry (Olajire *et al*., 2007). Tomato is an important source of antioxidants such as polyphenols, ascorbic acid, tocopherols, β-carotene and lycopene (Mostapha *et al*., 2014). In plants, lycopene is an important intermediate in the biosynthesis of many carotenoids, including beta carotene, responsible for yellow, orange or red pigmentation. Due to its strong color and non-toxicity, lycopene is a useful food coloring (Alda *et al*., 2009). Ascorbic acid concentration is one criterion of quality in the tomato fruit. It is an important anti-oxidant (Yang et al., 2003; Rasanu et al., 2005).

It is well established that low water supply results in depressed plant growth and fruit yield (Veit-Kohler *et al*., 1999; Dorais *et al*., 2001). The positive effects of low water supply can be summarized in their effects on fruit quality. (Due to drought stress, the sugar and ascorbic acid concentration in the fruits were significantly increased during fruit ripening (Veit-Kohler *et al*., 1999). Meanwhile, high water supply led to reduced fruit quality due to high fruit, water content of tomato plants (Doris *et al*., 2001). N fertilizer is also important to tomato yield and fruit quality, and has positive effects on yield and quality if the amount is suitable. Secondary plant metabolites which lack N in their structure such as lycopene, β-carotene and phenolics are favored under N-limiting conditions, although photosynthetic activity is not simultaneously reduced; the negative effects of N may also be due to canopy structural changes, as excessive application of N increases the LAI of the crop, resulting in fruit shading (Frossard *et al*., 2000 Montagu and Goh 1990, Simonne *et al*., 2007). Moreover, proper deficit irrigation, improved the total soluble solids, soluble sugars, organic acid and Vitamin C in tomato fruits (Kuscu *et al*., 2014; Wang *et al*., 2015; Chen *et al*., 2013) as well as water use efficiency (Mahajan and Singh, 2006). On the other hand, excessive nitrogen supply may cause reduction in quality of tomato fruits (Yang *et al*., 2006,

Zotarelli *et al*., 2009a- Zotarelli *et al*., 2009b). Several studies have shown that lycopene fruit concentration and ascorbic acid accumulation is increased by sub-optimal nitrogen supply (Dumas *et al*., 2003). Although, moderate N supply increases yield, excessive N supply decreases the concentration of ascorbic acid and carotenoids, (Montagu and Goh 1990; Frossard *et al*., 2000). Therefore, optimum use of water and N is essential in achieving high tomato yield and quality as well as improving water & nitrogen use efficiencies. Our study is set up to evaluate the effect of both water and N stresses on tomato yield and quality through exposing tomato plants to three different levels (almost maintained) of moister and nitrogen to obtain three levels of water and nitrogen within the plant along the growth season.

Materials and Methods

Tomato plants were cultured under controlled conditions at three different rates of N supply to obtain three N statuses in plants {low (N1), suboptimal (N2) and optimal (N3)}, under three different drought stresses (severe, moderate and well-watering) until fruit maturity. Seeds were germinated in peat moss. After one week, seedlings were transferred to plastic pots (one seedling per pot), which contained the soil substrate. Substrate was prepared by mixture of the coarse sand and peat moss (1:1) by volume, the substrate was placed in plastic pots (23 cm diameter and 21 cm height) each pot contained four Kg substrate. Drought and N treatments were started after four weeks from sowing. In this experiment, plants differing in N status were subjected to different intensity of drought stress. Drought treatments were executed by daily adding water to plants up to reach 60, 80 or 100% of field capacity for W1, W2 and W3 respectively. The aim of the experimental treatments was to obtain three groups of plants (Table 1) where, the N status should be different between these groups (for instance, optimal N status within each group should be similar irrespective of water supply (W1, W2 or W3). By monitoring the water use during growth nitrogen was added to different treatments according to prediction of plant growth to achieve three level of nitrogen status (low (N1), medium (N2), and optimal (N3) at each drought stress treatment.

At vegetative growth phase where, exponential growth was assumed the dry mass per plant on each day was calculated according to Eqn 1:

At vegetative growth phase where, exponential growth was assumed the dry mass per plant on each day was calculated according to Eqn 1:

$$DMt = DM0\ P(r + 1)^t \quad ....(1)$$

where:

DMt = DM after t days of treatments

DM0= DM at start of treatments (g plant^{-1})

r = RGR (relative growth rate) g g^{-1} day^{-1}

t = the time (days)

P = P (value was got from table 1)

The daily increment of dry mass per plant was calculated according to Eqn 2:

$$DMd = DMt\ r \quad ...(2)$$

From Eqn 1, it follows that Eqn 2 can be expressed also the following form:

DMt according water consumption was 0.80 and 0.6 for moderate and severe drought stress

$$DMd = DM0\ P(r +1)^t\ r \quad ...(3)$$

The daily nutrient requirement (NRd) of plants on day d was calculated according to Eqn 4 fortiontly

$$NRd = DMd\ C \quad ...(4)$$

where:

NRd= nutrient requirement (mg d^{-1})

DMd = dry mass increment on day d (g d^{-1})

C = the assumed nutrient concentration in the plant dry mass (mg g^{-1} DM)

P = (P values was got from Table 1)

From Eqn 3, It follows that Eqn4 can be expressed also the following form:

$$NRd= DM0\ (r+1)^t\ rC \quad ...(5)$$

The assumed nutrient concentration in the plant dry mass of plants with optimal, medium and low supply were 25, 15 and 10 mg N g^{-1} dry matter respectively.

For the phases of linear fruit growth and fruit maturation, the growth was assumed to be according the question, which was obtained from previous experiment

$$DMt= P(2.59\ t -110) \ldots\ldots R2= 0.99 \quad ...(6)$$

DMt = DM after t days of sowing it is applied when t > 49 days

The daily increment of dry mass per plant was calculated according to Eqn 7:

$$DMd = 2.59\ g\ plant^{-1}\ day^{-1} \quad ...(7)$$

The daily nutrient requirement (NRd) of plants on day d was calculated according to Eqn 8

$$NRd = DMd\ C \quad ...(8)$$
$$NRd = 2.59\ C$$

where:

NRd= nutrient requirement (mg d^{-1})

DMd = dry mass increment on day d (g d^{-1})

C = the assumed nutrient concentration in the plant

dry mass (mg g^{-1} DM)

P = (P values were got from Table 1)

Table 1 : The P value (growth rate %) for all treatments

Treatments	Drought stress		
	Severe (W1)	Moderate (W2)	well watering (W3)
N status	Assumed P values for each treatment		
Low (N1)	0.40	0.65	0.75
Medium (N2)	0.55	0.80	0.90
Optimal (N3)	0.65	0.90	1.00

Each block was paired with pots without seedling that served as a control to correct soil evaporation when determining transpiration. The watering treatments were initiated after the seedlings were established. N was added to plants according to expected growth rate resulting from drought stress and nitrogen supply to maintain the same nitrogen content in plants at different drought stress level. The transpiration water loss was measured gravimetrically by weighting all pots and re-watering with distilled water every other day. The watering amount for each pot was determined according to the difference between the weight of a re-watered pot and the weight of the pot after 24 h (more or less). Mature tomato fruits were harvested at two times and were kept at -18 oC for analysis

Determination of minerals

Total N concentration in different plant organs was analyzed by element analyzer (Elementaranalysator Elementar Vario Max, Hanau, Germany combustion after Dumas Minerals). Total concentrations of K, Mg and other elements in plant material was determined by ICP=OES (IRS/AP) with the pretreatment of dry-ash at 550 °C for 5 h.

Determination of sugars and acidity

The soluble sugar fraction was measured. Soluble sugars in the collected extracts were determined using the anthrone method (Seifter et al. 1950). Acidity of the samples were determined (in triplicate) by acid base titration (Lacey et al., 2009).

Table 2: The mount of N which was added to the plant during growth season.

Treatments	Water status		
	W1	W2	W3
N status	(g N $plant^{-1}$)		
N1	0.99	1.45	1.94
N2	1.96	3.12	3.93
N3	4.02	4.27	7.76

Determination of ascorbic acid, total phenol, RSA (Radical scavenging activity), lycopene and β-carotene

Ascorbic acid measuring by titration of tomato extraction against 0.02% 2.6 Dichloroindophenol dye until the juice turned to permanent pink (Subramanian *et al*., 2006). Total phenols were measured using the Folin-Ciocalteau method (Spanos and Wrolstad 1990), Modified by Lister and Wilson (2001). Radical Scavenging Activity (RSA) of freshly prepared tomato juice was assayed with DPPH (2, 2-diphenyl-1-picrylhydrazyl) according to Ramadan *et al*. (2003). Lycopene and β-carotene contents were calculated according to the Nagata and Yamashita (1992).

Results and Discussion

Effect of water and N status on plant growth

Total dry masses of plants nearly matched our expectations, when plants were exposed to different levels of drought stand nitrogen stresses. Also, low water and nitrogen status reduced dry mass in leaves proportionally with the reduction of total dry mass of whole plants (Fig. 1 a). Due to the successful controlling of plant growth, nitrogen status was fixed at different drought stress. N concentration in leaves was around 2.5, 1.5 or 1.0 % DM for the desired high, medium or low N status respectively (Fig. 1 b). With high water stress (W1), N% decreased in the leaves, where plant growth was higher than expected (Table 3). Dry mass % in leaves were not affected by drought stress (water status) but was affected by nitrogen status, where, leaves DM% was reduced to promote the nitrogen status (Fig. 2 a). In contrast, dry mass in fruits was not influenced by nitrogen status; while, drought stress increases dry mass in fruits. Concerning the interaction between nitrogen and water stress, dry mass in leaves was constant regardless of drought stress for different nitrogen status where, leaves DM% was 15, 17.5 and 20% of high, medium and low nitrogen status respectively. Contrary, dry mass in fruits was constant irrespective of nitrogen status for different levels of drought stress where, fruits DM% was 7.5, 8.5, and 10 % of high, medium and low drought stress respectively.

Disappearance of drought effect on the DM % in leaves was attributed to the immediate transference of photosynthesis from the leaves to fruits keeping the water status constant due to continued transpiration. In case of high nitrogen stress photosynthesis products accumulate in the form of starch and hence led to increased DM % in leaves. Induced accumulation of starch in leaves at low N status was recorded in other studies (Geiger *et al*., 1999). Where Low N status was attributed to higher transcript levels and activity of ADP-glucose pyrophosphorylase, a key enzyme in the regulation of starch biosynthesis. Nitrate suppresses transcription of gene encodes of this enzyme and therefore, low N status is associated with higher transcript levels when nitrate is missing (Scheible *et al*., 1997). Probably, under conditions of low N status,

nitrate concentrations in leaf cells fall below critical values that are needed to suppress extensive starch biosynthesis in leaves

On the other side, DM % in fruits reduced at high water status due to dilution effects of water on accumulated dry material in fruits, because in tomato high amount of water can accumulate in their fruits. N status had no effect on the DM% in fruits, because in low N status, N resorption was range (1- 0.7 %) in leaves that allowed to remain photosynthesis process. The resorption percentage is in this range of most plant species (Killingbeck, 1996). This maintains photosynthate which is exported to fruits. Thus, fruits receive the appropriate amount of photosynthate and thus maintain the same proportion of dry matter. Water and N status within tomato plants and their interaction significantly influenced the fresh biomass of fruits (Table 4). Fruits number was not affected by water and N status. The fresh weight of fruits was reduced dramatically by decreased water and N statuses; however, there was no significant differences among N status treatments at low water status. High N status with low water status had the lowest fruit yield.

Table 3 : Expected and observed P values which are represented the percentage of deficit of plant growth resulting from drought stress and nitrogen status.

N status	(W1)		(W2)		(W3)	
	expected	observed	expected	observed	expected	observed
Low (N1)	0.40	0.43	0.65	0.63	0.75	0.77
Medium (N2)	0.55	0.55	0.80	0.87	0.90	0.95
Optimal (N3)	0.65	0.71	0.90	0.93	1.00	1.00

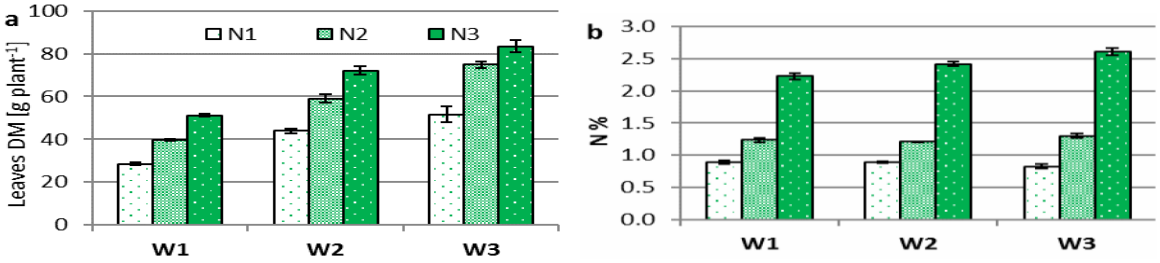

Fig. 1: Effect of water and N status on dry mass and N % in tomato leaves based on leaves dry mass. Vertical lines indicate standard errors of means (n=4)

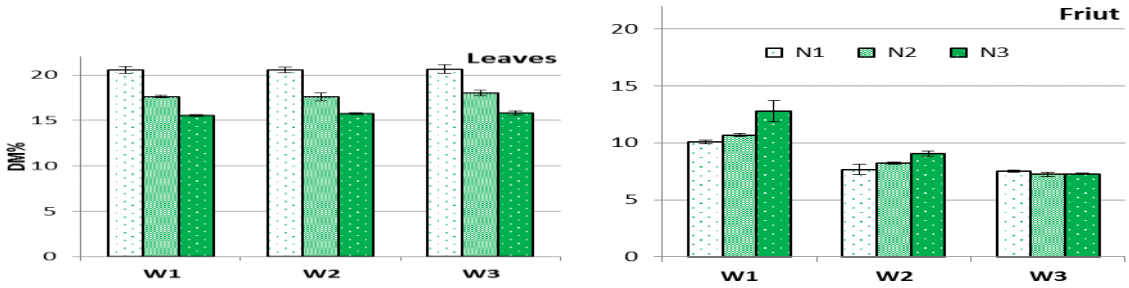

Fig. 2 : Effect of Water and N status on dry mass % in leaves and fruits of tomato plants. Vertical lines indicate standard errors of means (n=4)

Table 4 : fruits number, fresh weight, dry matter content and mean fruit weight of tomato fruits as affected by drought stress and nitrogen status in 8plants. Different letters within a column indicate significant (Tukey-Kramer's test, P<0.05) differences of organ mass among nutrient treatments

Treatments		Fruit No	FM (g plant^{-1})	MFM (g fruit^{-1})	Fruit Index
W1	**N1**	25.8 a	271 e	10.7 cd	0.54 a
	N2	25.5 a	264 e	10.4 cd	0.44 bc
	N3	26.5 a	244 e	09.2 d	0.35 d
W2	**N1**	25.8 a	363 de	14.1 bc	0.50 abc
	N2	29.0 a	508 bc	17.5 b	0.50 abc
	N3	27.8 a	486 cd	17.5 b	0.43 bc
W3	**N1**	26.3 a	448 cd	17.2 b	0.52 ab
	N2	28.0 a	677 a	24.1 a	0.52 ab
	N3	28.0 a	631 ab	22.6 a	0.45 bc

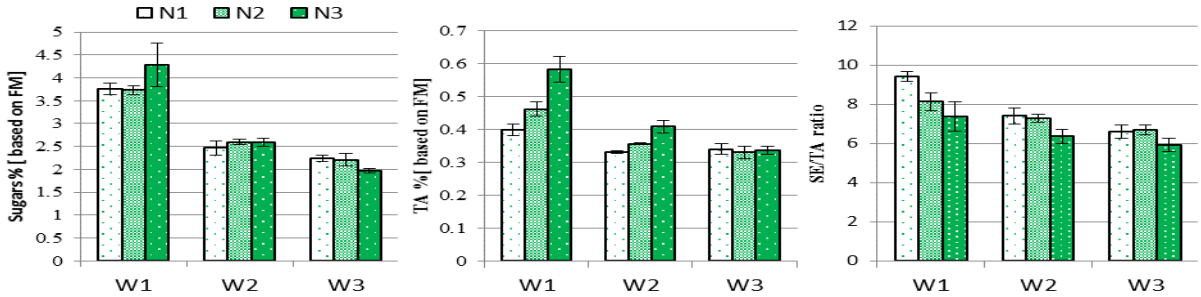

Fig. 3 : Effect of water and N status on soluble sugar, titratable acidity TA and sugar to TA ratio in tomato fruits. Vertical lines indicate standard errors of means (n=4)

Medium and high N with high water status within plants had the highest fruit yield. The mean fruit weight was reduced in different N status in plants under low water status; however, medium and high N status treatments under high water status had the highest fruit weight more than twice compared with low water status (Table 4). Enhanced DM % in fruit associated with reduced (fruit size or mean fruit weight) due to drought which may affect tissue expansion through its effects on the biophysical, metabolic, and hormonal factors involved in the regulation of cell turgor and osmotic pressures and cell-wall extension (Bertin *et al*., 2003; Prudent *et al*., 2010). Fruit index is the proportion between fruit fresh mass (FM) to shoot fresh mass. Fruit index had positive relationship with water supply, because drought reduced fruit index. In contrast, negative relationship was found between N concentration and fruit index; where, increased N concentration within plant (high N status) resulted in reduced fruit index. Concerning the interaction between water and N status within plants, high N status was associated with lower fruit index particularly under low water status. The effect of N status on fruit index at low water status was clear; whereas, under moderate or high-water status, fruit index was not influenced by N status within plant (Table 4).

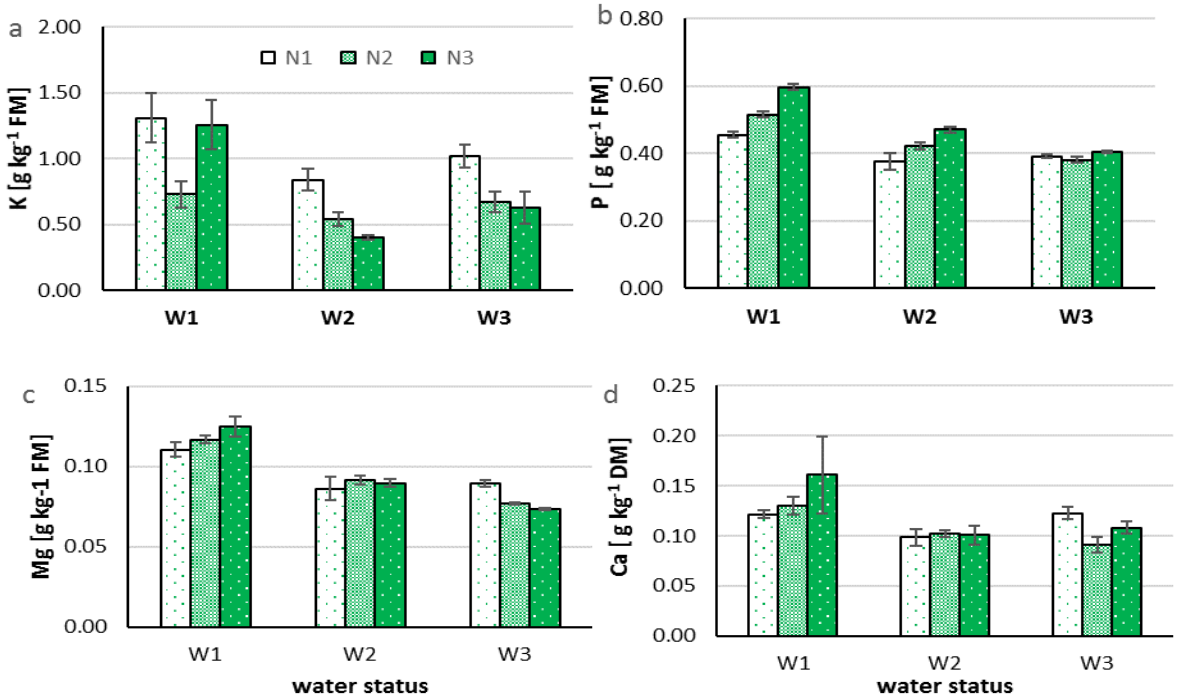

Fig. 4 : Effect of nitrogen status and drought stress on macronutrient concentration in tomato fruits. Vertical lines indicate standard errors of means (n=4)

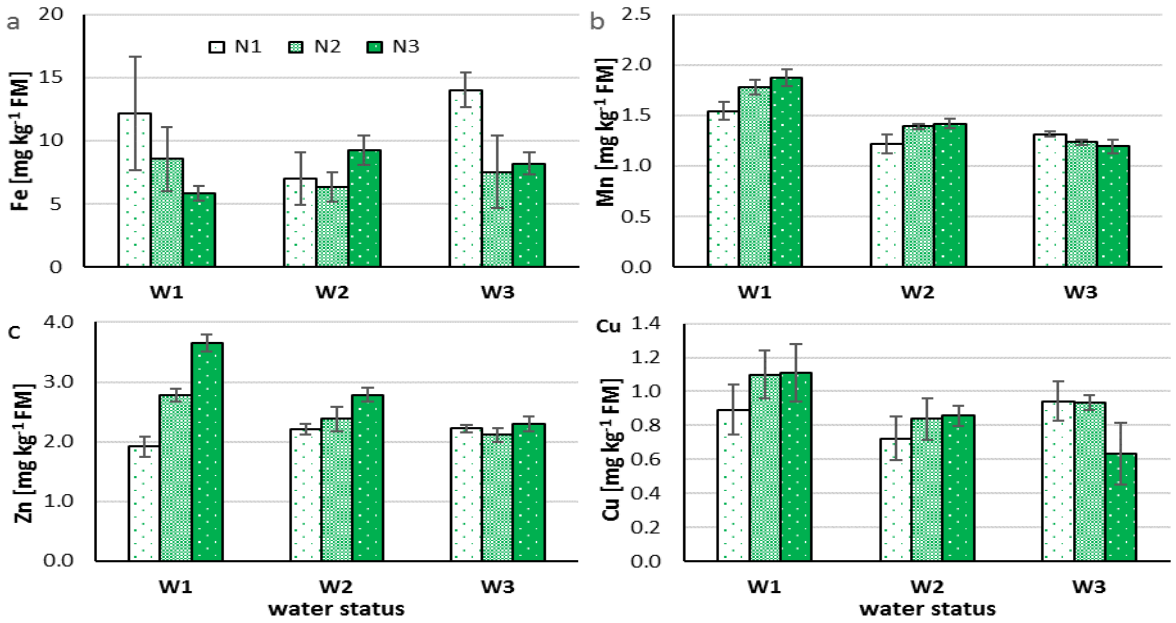

Fig. 5 : Effect of nitrogen status and drought stress on macronutrient concentration in tomato fruits. Vertical lines indicate standard errors of means (n=4).

Table 5 : Lycopene, β-Carotene, chlorophyll a and b in fruits as affected by drought stress and nitrogen status in tomato plants Different letters within a column indicate significant (Tukey-Kramer's test, P<0.05) differences of organ mass among nutrient treatments

Treatments		Vitamin C	lycopene	β-Carotene	Total phenols	R S A%
		(mg kg^{-1} FM)			(mg 100 g^{-1} FM)	
W1	N1	94.9 a	39.3 ab	21.5 abc	44.8 bc	34.3 b
	N2	91.8 a	42.1 a	28.0 a	46.7 ab	37.1 ab
	N3	66.3 c	32.1 d	25.5 ab	53.6 a	42.9 a
W2	N1	99.4 a	36.5 bcd	16.24 c	40.8 bc	33.2 b
	N2	92.6 a	36.3 bcd	16.84 bc	41.6 bc	34.0 b
	N3	72.1 bc	37.2 bc	21.14 abc	43.7 bc	37.8 ab
W3	N1	82.8 abc	36.7 bc	16.4 c	38.9 c	35.5 ab
	N2	86.7 ab	36.8 bc	19.4 abc	40.6 bc	34.6 ab
	N3	34.5 d	33.1 cd	15.2c	43.5 bc	35.6 ab

Effect of water and N status on nutritional values

Soluble sugar, titratable acidity TA and particularly sugar to TA ratio represents taste value of tomato fruits. Soluble sugar concentration in fruits increased significantly under drought stress, N status had no significant effect on soluble sugars concentration in fruits. There was no interaction effect between drought and N status with regard to soluble sugars concentration in tomato fruits (Fig. 3 a). Titratable acidity (TA) is the total amount of acid in the solution as determined by titration using a standard solution of sodium hydroxide (titrant). Severe drought stress was associated with higher TA concentration in fruits; whereas, N stress resulted in decreased TA concentration in tomato fruits. Regarding the interaction between drought and N status, the effect of N status on TA appeared under severe drought stress only. However, under moderate drought or well watering, N status had no significant effect on TA concentration in fruits and recorded the lowest TA concentration compared to N status under severe drought stress (Fig. 3b). Low water status seemed generally, tend to increase sugar in tomato fruits (Wang *et al*., 2011), mostly because drought increased the activities of sucrose synthase and sucrose phosphate synthase, which enlarged the gradient of sucrose between leaves and fruits (Qi *et al*., 2003). Sugar to TA ratio represents taste value of tomato fruits. In comparison to N status, drought stress was associated with higher sugar to TA ratio in fruits, where N status had no significant effect in Sugar to TA ratio. In terms of interaction, Treatment W1N1 gave the highest Sugar to TA value (Fig. 3 c).

N and water status had significant effects on most mineral in tomato fruits. Low water status resulted in increased K, P Mg, Ca Mn and Zn concentration in tomato fruits. Also, low N status enhanced K concentration in fruits whereas, P and Zn concentration in fruits were increased with increasing N status within plants. However, N status had no significant effect on Mg Ca and Mn concentration in fruits (Fig. 4 and Fig. 5). In terms of the interaction between water and N status.

The increment of K concentration due to low N status was cleared under medium and high-water status while, the increase of P, Mn and Zn concentration due to high N status appeared with reducing water status. Water and N status had no significant effect on Fe and Cu concentration in tomato fruits (Fig. 4, Fig. 5). High nutrient concentration in fruit was associated with low water status due to growth restriction resulted from water stress particularly nutrient concertation was calculated based on fresh mass. N status had no effect on nutrient under medium or high-water status, in exception with K which was reduced in medium and high N supplied plants. Under low water status, Zn increased with enhancing N status. It is well documented that N supply increased Zn in fruit because Zn needs amino acid as a carrier for its translocation from leaves to fruits (Kutman *et al*., 2011)

Lycopene, the predominant carotenoid in tomatoes, exhibits the highest antioxidant activity and singlet oxygen quenching ability of all dietary carotenoids (Dimascio *et al*., 1989). Tomato can provide an important proportion (85%) of antioxidants in the human diet through carotenoids and phenolic compounds (Aoun *et al*., 2013; Liu, 2013). Tomato is a good source of vitamins (A, C, K, E and B complex) and minerals (USDA National Nutrient Database for Standard Reference, 2016). More specifically, tomato is the third source of vitamin C in our diet and the fourth for vitamin A (due to its content in β-carotene and to the large amount consumed) (Bhowmik et al., 2012).

Water and N status had a significant effect on ascorbic acid and lycopene concentration in fruits (Table5). High water and N status resulted in reduced ascorbic acid and lycopene concentration in tomato fruits. Concerning the interaction between water and N status. High N status was associated with lower ascorbic acid concentration in fruits at different water status particularly highest water status. Whereas high N status was associated with lower lycopene concentration in fruits at low and high-water status. Under medium water status, N status had no significant effect on lycopene concentration in tomato fruits (Table 5). In previous studies, fruit quality shown as, sugar/acid ratio, and ascorbic acid content in pear-jujube fruits were all enhanced as a result of deficit irrigation (Cui *et al*., 2008). Low water status was proved to increase ascorbic acid content in tomato fruits [Patanè C, Cosentino 2010, Patanè *et al*., 2011 and Hui *et al*., 2017). But no significant evidence showed that N could improve ascorbic acid (Hui *et al*., 2017). Previous studies indicated that drought stress increased TA in tomato fruits, and high nitrogen amount improved TA in fruits. Low water status resulted in increased β-carotene concentration in fruits, whereas N status had no significant effect on β-carotene concentration in fruits. As to the interaction effects of water and N status on β-carotene in fruits, in contrast medium and high-water status, low water status was associated with higher β-carotene concentration irrespective of N status; W3N3 treatment recorded the lowest β-carotene concentration in fruits. In comparison to, high and medium water status, low water status was associated with higher phenols concentration in fruits; also, high N status enhanced phenols concentration in fruits. The interaction of water and N status on phenols revealed significant differences among N status treatments only under low water status. Water status had no significant effect on RSA% (radical scavenging activity) in fruits, whereas, in comparison to low and medium N status, high N status was associated with higher RSA %. In terms of the interaction between water and N status, effect of N status, it was clear that under the low water status, high N status recorded the highest RSA% in fruits

Conclusion

Water and nitrogen levels in plant seem to control plant performance and affect the various attributes of the resulting yield. Maintaining certain levels of water and nitrogen supply to tomato plants throughout the growth period may affect either positively or negatively fruit traits. Water status influenced the dry mass % in fruits whereas, N status effected the DM % in leaves. Soluble sugar content in fruits increased under drought stress, N status had no effect on soluble sugars content in fruits. High water and N status resulted in reduced ascorbic acid and lycopene concentration in tomato fruits. N and water status had effects on most macro and micro nutrients in tomato fruits. Low water status resulted in increased K, P, Mg, Ca, Mn and Zn content in tomato fruits. Also, low N status enhanced K content in fruits whereas, P and Zn content in fruits were increased with increasing N status within plants. Keeping a balanced combination of water and nitrogen levels during the different stages of growth may end up with tomatoes of high nutritional value.

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