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Tomato plant growth, leaf nutrient concentrations and fruit quality under nitrogen foliar applications

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Abstract: Tomato is a typical plant that has distinct response to different nitrogen forms in hydroponic culture. In addition, it is a well known susceptible plant to ammonium nutrition in hydroponic culture. However, its response to foliar application of nitrogen sources and N-forms has not been well investigated. In the present study, the growth, productivity and fruit quality of tomato was investigated under foliar application of nitrogen from different sources. Ammonium sulfate, urea and calcium nitrate with constant concentration of 100 mM N were weekly sprayed during four months under hydroponic culture system. A water spray treatment was considered as control. The results showed that vegetative growth parameters were significantly affected by N sources in different patterns. The factors such as plant height, leaf area, number of lateral shoots and shoot fresh and dry weight, as well as leaf nitrate reductase activity was significantly reduced by foliar application of ammonium sulfate and to less extent by urea, while there was improvement of these traits by foliar application of calcium nitrate compared to control. However, ammonium sulfate treated plants had the highest leaf SPAD value and leaf N concentrations. Plant fruiting pattern was also influenced by treatments, as ammonium sulfate spray reduced the fruit yield, and fruit vitamin C content, while it increased fruit TSS and titratable acidity. The highest value of yield and vitamin C was recorded in calcium nitrate sprayed plants.

1. Introduction

Application of different fertilizers play important role in agricultural production of food commodities. Supply of adequate essential nutrients can significantly improve plant growth, quality and their nutritional values (Marschner, 2011). Different sources of each nutrient element can be applied as fertilizer to meet plant's need of that special element. Generally for most nutrients, there is little difference among effects of various sources; however, regarding nitrogen there is significant different effect of N form and sources on many vegetative and reproductive traits of plants (Souri and Roemheld, 2009).

Nitrogen fertilizers have important role in improving crop productivity; however low use efficiency rate of N fertilizers threatens sustainable plant production (Souri, 2010). On market, various nitrogen sources exist for application under field and hydroponic culture. Urea, ammonium sulfate and ammonium nitrate are the main nitrogen fertilizers for soil application, while calcium nitrate and potassium nitrate are the major nitrogen sources commonly are used in hydroponic systems (Marschner, 2011). Nitrogen forms (ammonium *vs.* nitrate) can have significant effect on morphology and physiology of plants particularly under hydroponic culture (Souri and Roemheld, 2009). In addition, ammonium instead of nitrate (Smoleń and Sady, 2009; Souri 2010; Marschner, 2011) and foliar complementation (Kolota and Osinska, 1999; Dehnavard *et al.*, 2017) supply of nitrogen can significantly improve N fertilizing efficiency in cropping systems.

There may be several potential benefits of providing nitrogen to greenhouse crops via the foliage. These include: reduced nitrogen losses through denitrification and leaching, the ability to supply nitrogen when root activity is impaired e.g. in soil or water saline conditions, and luxury supply of plants with nitrogen. In cereals and some agronomic crops, late foliar application of urea generally results in higher grain protein and N content (Fageria *et al.*, 2009). The best quality parameters of plant growth and productivity of cabbage was reported when foliar versus soil application of fertilizers was applied (Atanasova *et al.*, 2007).

Tomato is one of the major vegetable crops that is cultivated in many parts of the world and consumed in many dishes. The application rate of N fertilizers in tomato culture is generally high (Souri and Roemheld, 2009), with N efficiency of about 30-50% (Zotarelli *et al.*, 2009; Souri, 2010). In many greenhouses due to continuous cultivation and fertilization, soil salinity level is generally much higher than the threshold. If some levels of required N could be applied on plants as foliar spray, it can improve N use efficiency with less soil salinity buildup and less environmental side effects. In literature the tomato responses to continuous foliar spray of nitrogen sources have not well been established. Therefore, the aim of this study was to evaluate response of tomato plants to foliar application of various nitrogen forms and sources under greenhouse and hydroponic culture.

2. Materials and Methods

Experimental set up

This study was conducted during 2012 under greenhouse conditions at Faculty of Agriculture, Trabiat Modares Uni., Tehran-Iran. The experiment was done in hydroponic system with four treatments and four replications arranged in completely randomized design. Tomato seeds (*Lycopersicon esculentum* var. Money Maker) were germinated in quartz sands and after germination (in four-leaf stage) two homogeneous seedlings were transferred to pots containing a mixture of cocopeat and perlite in ratio of 3:1 (v/v). One week later one of them was removed and one week later foliar treatments were applied on plants. The nutrient solution composition was prepared following Hoagland formula (Dehnavard *et al.*, 2017). Black plastic pots as replication, with a volume of nearly 12 liter were used for plants cultivation. For first two weeks after seedling transplanting, plants were supplied with one daily application of 100-200 ml of nutrient solution. Thereafter, pots were supplied two times per day with nutrient solution of a final quantity of 250-1500 mL until end of experiment. The amount of applied solution increased with plant size and reached the amount of 1.5 liter per day at full plant size.

Treatments were foliar spray of three nitrogen sources of ammonium sulfate (AS), urea, calcium nitrate (CN) and a no spray control. All three N sources were applied in constant concentration of 100 mM N (equals to 1400 mg L^{-1} N). Sprays were done on weekly basis during 4 months of active growth period from 25 March (first foliar spray) until the end of July 2012. Distilled water was sprayed in control plants. Spraying treatments were done in the early morning, one hour after sun rise.

Measurements

During plant growth period for four months, various vegetative traits as well as fruit harvesting records were collected. The final harvest of plants was done at the end of July. Chlorophyll index was measured two times by SPAD meter (model 502 Plus, Illinois, USA), each time with 30 readings on 3 different areas for 10 randomly selected leaves per pot that the average was presented as leaf SPAD value. SPAD readings were done at the middle of experiment and before final harvest, at 10 o'clock in the morning. Plant height, number of lateral shoots,

shoots fresh and dry weight were measured at final harvest. Cumulative harvest of fruits was recorded as final yield. Plant leaf area was measured by leaf area meter and calculated as average area of a single leaf.

Fruits after harvesting were transferred to laboratory for further quality assessment. Fruit firmness was measured by penetrometer (Model Wagner) after removing fruit skin using a blade. Fruit total soluble solids (TSS), titratable acidity (TA) and pH were determined in fruit juice squeezed by a squeezer. Fruit TSS percentage was measured by a portable refractometer (Atago, Tokyo, Japan). Fruit pH was determined using a portable pH meter, and titratable acidity was determined with titration of 5 mL of fruit juice with NaOH 0.1 N until end pH of 8.1.

For determination of vitamin C (L-ascorbic acid), 50 g of fresh fruit tissue was crushed in a porcelain mortar in vicinity of 20 mL metaphosphoric acid 6%, and then the juice transferred into a 50 ml tube, then centrifuged at 4000 rpm for 10 min. Five mL of the supernatant transferred into an Erlenmeyer flask, and received 20 mL of metaphosphoric acid 3%. Then titration of the extract was done by di-chloro phenol indophenols until appearance of a rosa color, which the amount of vitamin C (mg 100^{-1} g FW) was calculated accordingly and based on a standard curve of L-ascorbic acid concentrations.

From each treatment and replicates 3 fruits were kept in room temperature (25±2°C) for one week, thereafter their weight loss percentage was calculated. Total nitrogen of leaves was determined using kejeldahl method and the activity of leaf nitrate reductase enzyme (NR) was determined after grinding and homogenizing of leaf materials in a mortar containing liquid nitrogen. Nitrate reductase was extracted in a buffer consisting of 100 mM HEPES (pH 7.5), 1 mM EDTA, 7mM cystein, 3% polyvinyl polypyrolidone (PVPP), 10 Mm leupeptin, and 1 mM phenyl methyl sulfonyl fluoride (PMSF). After preparation of extracts sulfanilamide (0.5%) and N-(1-naphthyl)-ethylenediamine dihydrochloride (0.01%) in 1.5 M hydrochloric acid (HCl) were used for color development and the amount of NO_2^- was determined spectrophotometrically at 540 nm and then nitrate reductase activity was calculated accordingly.

Statistical analysis

Excel software was used for calculating means and standard deviations and data were analyzed by SPSS software. Comparison of means was performed at 5% by Duncan's multiple range test.

3. Results

The results of present study showed that plant vegetative growth parameters were significantly affected by nitrogen sources. Plant height was significantly higher in calcium nitrate treated plants compared to ammonium sulfate and urea treated plants (Table 1). The significant largest area of a single leaf and the highest number of lateral shoots were recorded in those plants which were treated with calcium nitrate, while the significant lowest records were in ammonium sulfate treated plants (Table 1). SPAD value as a chlorophyll concentration index of plants were highest in ammonium sulfate treated plants (Table 1), and there was no significant effects among other treatments.

Plant shoot fresh and dry weights were significantly affected by foliar spray of nitrogen sources. The significant highest shoot fresh and dry weight was obtained from plants treated with calcium nitrate and control plants. The significant lowest shoot fresh

Table 1 - Mean values of plant height, leaf area, number of lateral shoots and leaf SPAD of tomato plants. Plants were grown in Hoagland nutrient solution for 17 weeks

Foliar spray treatment	Plant height (m)	Leaf area (cm^2)	Lateral shoots (no.)	SPAD value
Control (d-water)	1.98±0.16 ab	74.95±6.2 b	59.25±3.5 b	37.025±1.6 b
Ammonium sulfate	1.365±0.22 c	66.60±6.0 c	43.25±5.6 c	46.925±3.8 a
Urea	1.753±0.17 b	71.25±5.6 bc	51.00±9.7 bc	39.825±1.8 b
Calcium nitrate	2.225±0.22 a	80.60±2.7 a	70.50±5.2 a	39.275±1.8 b

All three N sources were applied in constant concentration of 100 mM N.
SPAD readings were done two times at the middle of experiment and before final harvest, and the average was presented.
Data are average of 4 replications ± SD. In each column means with a common letter have no significant difference at 5% of Duncan test.

and dry weight was in plants treated with ammonium sulfate and urea (Table 2). Determination of leaf nitrogen concentration (Table 2) revealed that plants treated with ammonium sulfate and urea had significantly higher amounts compared to control and calcium nitrate treated plants. Foliar spray of nitrogen sources showed significant effect on leaf nitrate reductase enzyme activity (Table 2). Nitrate reductase is the key enzyme in nitrate assimilation that its activity depends on several factors including nitrogen and nitrate status of plant tissues. The significant highest activity of this enzyme was in leaf of plants treated with calcium nitrate followed by control plants and those treated with urea. The significant lowest nitrate reductase activity was in ammonium sulfate treated plants.

Fruiting pattern of plants was also influenced by sprays of nitrogen sources and forms (Table 3 and 4). Number of fruits per plant was highest in calcium nitrate treated plants; however, they had no significant difference with control and urea treated plants. Those plants which were treated with ammonium sulfate produced significant lowest number of fruits (Table 3). The amounts of fruit yield per plant was significantly higher in calcium nitrate treated plants (Table 3), followed by control, urea and ammonium sulfate treated plants. Fruit firmness was not affected by foliar spray of nitrogen sources; however the percentage of fruit weight loss was significantly influenced by N foliar treatments. The highest weight loss, during one week keeping fruits at room temperature, was in fruits of those plants which were treat-

Table 2 - Mean values of shoot fresh and dry weight, leaf N concentration and nitrate reductase activity of tomato plants

Foliar spray treatment	Shoot FW (g)	Shoot DW (g)	Leaf N concentration (%)	Leaf nitrate reductase activity (µ mol NO_2 g FW h)
Control (d-water)	1581±122 ab	13.7±1.6 a	2.3±0.17 b	0.71±0.1 b
Ammonium sulfate	1213±59 c	11.5±0.8 b	3.2±0.30 a	0.17±0.01 d
Urea	1469.5±79 b	11.4±1.14 b	2.9±0.32 a	0.30±0.09 c
Calcium nitrate	1758.3±186 a	14.2±1.7 a	2.5±0.13 b	0.90±0.06 a

Plants were grown in Hoagland nutrient solution for 17 weeks.
All three N sources were applied in constant concentration of 100 mM N.
Data are average of 4 replications ± SD. In each column means with a common letter have no significant difference at 5% of Duncan test.

Table 3 - Mean values of fruit number, fruit yield, fruit firmness and fruit postharvest weight loss in tomato

Foliar spray treatment	Number of fruits $plant^{-1}$	Fruit yield (g $plant^{-1}$)	Fruit firmness (kg cm^2)	Fruit weight loss (%)
Control (d-water)	19.7±2.7 a	2210.7±253 b	1.24±0.16 a	5.15±0.83 b
Ammonium sulfate	17.2±2.6 b	1824.5±218 c	1.32±0.10 a	12.25±2.04 a
Urea	19.0±2.2 a	1896.0±230 c	1.23±0.21 a	6.35±0.79 b
Calcium nitrate	20.2±2.2 a	2994.5±306 a	1.38±0.11 a	5.40±1.01 b

Plants were grown in Hoagland nutrient solution for 17 weeks.
All three N sources were applied in constant concentration of 100 mM N.
Fruit weight loss was measured after one week in room temperature of 25±°C
Data are average of 4 replications ± SD. In each column means with a common letter have no significant diff erence at 5% of Duncan test.

Table 4 - Mean values of fruit TSS, fruit TA, fruit pH, and fruit L-ascorbic acid content in tomato under different fertilization

Foliar spray treatment	Fruit TSS (%)	Fruit TA (%)	Fruit pH	Fruit L-ascorbic acid (mg 100 g FW^{-1})
Control (d-water)	5.50±0.16 ab	3.75±0.13 b	3.68±0.1 a	36.8±4.1 a
Ammonium sulfate	5.98±0.27 a	4.57±0.22 a	3.95±0.3 a	31.0±2.1 b
Urea	5.48±0.17 ab	3.85±0.21 b	3.90±0.2 a	33.5±2.9 ab
Calcium nitrate	5.35±0.18 b	3.60±0.18 b	3.78±0.2 a	37.1±3.4 a

Plants were grown in Hoagland nutrient solution for 17 weeks.
All three N sources were applied in constant concentration of 100 mM N.
Data are average of 4 replications ± SD. In each column means with a common letter have no significant difference at 5% of Duncan test.

ed with foliar spray of ammonium sulfate (Table 3).

Fruit TSS was also highest in ammonium sulfate treated plants and the significant lowest fruit TSS was in calcium nitrate treated plants (Table 4). Similarly, fruit titrateable acidity values (TA) unchanged in urea and calcium nitrate treated plants compared to control, however sprays of ammonium sulfate resulted in significant higher amounts of fruit TA (Table 4). Fruit juice pH was not affected by foliar sprays of nitrogen sources (Table 4). The significant highest L-ascorbic acid concentration was in calcium nitrate treated plants and control plants, while the significant lowest L-ascorbic acid was in ammonium sulfate treated plants (Table 4).

4. Discussion and Conclusions

The results showed that many growth and productivity traits of tomato plants were significantly affected by spray of N sources. Plant biomass production and its different parameters including plant height, leaf area, number of lateral shoots, fresh and dry weight were significantly reduced by both ammonium sulfate and to less extent by urea treatments, while calcium nitrate sprays resulted in improvement of all these growth parameters compared to AS and urea treatments.

Reduction in growth parameters of tomato due to foliar application of various concentration of ammonium sulfate have been reported by Dehnavard *et al.* (2017). On the other hand, tomato is a distinct sensitive plant to ammonium nutrition particularly under hydroponic culture (Loqué and von Wirén, 2004; Souri and Roemheld, 2009). In present study, despite plants were fed by nitrate in nutrient solution; however foliar application of ammonium forms of nitrogen (ammonium sulfate and urea) resulted in reduced growth of plants. Foliar absorption of nitrogen cannot be restricted by root medium N status, as there is always plant affinity to absorb nitrogen (Marschner, 2011; Dehnavard *et al.*, 2017). Foliar spray of ammonium sulfate in concentration of 100 and 200 mM with weekly application was resulted in significant growth restriction and less biomass production of tomato plants (Dehnavard *et al.*, 2017), while sprays of 50 mM improved tomato plant growth parameters, probably due to the fact that applied ammonium concentration and corresponding absorption was not in stressful level, but rather favored better photosynthesis and plant growth. Daily foliar application of urea as the sole N source for tomato seedlings improved seedlings growth (Nicouloud and Bloom, 1996). Despite the tomato tissue concentrations of ammonium increases significantly in 12-24 hour after foliar urea application (Nicouloud and Bloom, 1996), however from various studies it seems that plants can tolerate urea sprays better than ammonium sulfate (Souri and Roemheld, 2009). Metabolism of malate and excretion of protons play important role in maintaining pH during ammonium assimilation in the shoot following ammonium sprays (Peuke *et al.*, 1998). Urea in frequent applications and higher levels may have toxicity to plants (Bowman and Paul, 1992). However, although foliar spray of urea is common in some crops, but its physiological effects varies with season, cultivar and concentration (Bowman and Paul, 1992; Fageria *et al.*, 2009). In addition, it has been reported that foliar spray of urea compared to ammonium and nitrate may has less damage to leaves (Bowman and Paul, 1992). The absorption rate of urea is generally higher than calcium nitrate and ammonium sulfate in foliar spray (Bowman and Paul, 1992; Fageria *et al.*, 2009). However, within the tissues urea breakdowns to ammonium ions that similar to ammonium uptake can result in some toxicities (but with lesser extent) and restricted plant growth traits.

SPAD values and leaf N concentration in ammonium sulfate treated plants were significantly higher compared to control and calcium nitrate sprayed plants. There are few studies reporting the effects of foliar application of nitrogen sources on vegetable crops. Urea has been mainly used in one or limited applications with no negative side effect on plant growth (Fageria *et al.*, 2009; Zhang *et al.*, 2009). Higher leaf SPAD value by ammonium sprays could be mainly due to higher chlorophyll concentration induced by restricted leaf expansion and higher N concentrations (Souri and Roemheld, 2009; Dehnavard *et al.*, 2017). The effect of foliar applications of ammonium sulfate and urea on chlorophyll readings in this study is in agreement with the results of foliar ammonium application on tomato (Dehnavard *et al.*, 2017), and urea spray on broccoli (Yildirim *et al.*, 2007) and onion (Charbaji *et al.*, 2008). Ammonium spray probably by restriction of leaf area expansion has resulted in higher leaf N concentrations. In addition, ammonium absorption can take place by many nutrient specific and unspecific transporters, which results in less plant cell control over ammonium uptake and transport within the tissues (Souri and Roemheld, 2009).

It seems that chlorophyll biosynthesis is less sensitive to ammonium rather than other leaf parameters such as leaf cell expansion and cell division, root and shoots growth and protein biosynthesis. It has been shown that foliar application of urea or soil application of a stabilized ammonium fertilizer (ENTEC) can significantly increase N concentrations of leaves and root in carrot (Smoleń and Sady, 2009) and tomato (Souri and Roemheld, 2009) with less nitrate content. Three foliar sprays of urea with 3 days interval on onion plants showed that bulb fresh and dry weight were increased by urea levels to 5000 mg L^{-1} without any damage to leaves (Charbaji, *et al.*, 2008). The best quality parameters of dry weight, total soluble sugars, vitamin C and low nitrate content in the cabbage leaves were achieved by foliar versus soil application of fertilizers *(*Atanasova *et al.*, 2007).

Foliar application of ammonium and then urea significantly reduced nitrate reductase activity of tomato leaves (Table 2). This can be a negative factor when nitrate is actively taken up by plant roots and due to low activity of this enzyme most of nitrate accumulate in vacuoles resulting in higher nitrate accumulation in leaves and probably in fruits.

Tomato plant yield was significantly reduced by ammonium sprays, while it was increased by calcium nitrate treatment. This ammonium effect could be due to restricted vegetative growth, reduced hydraulic conductance, phloem translocation and less fruit set, while calcium nitrate had no effect on fruit number but it increased the average fruit weight resulted in higher yield compared to control. Foliar nitrogen application generally increase plant yield. The maximum increase in marketable yield in cabbage, onion, and cucumber using supplementary foliar N fertilization was 20.3%, 10.8% and 7.3%, respectively (Kolata and Osinska, 1999). Foliar fertilization significantly decreased the level of cucumber leaf infestation by downy mildew disease (Kolata and Osinska, 1999).

Changes in fruit quality traits are generally observed due to foliar N applications. In this study increase in fruit TSS by ammonium spray can be due to higher chlorophyll content of leaves and higher photosynthetic rates. It is probably feasible that foliar sprays of ammonium induced stress signals leading plant to have more fruit sugars and TSS content. Guvenc *et al.* (1995) reported that foliar urea application improved some quality (Vitamin C and Titratable acidity) and growth properties of tomato. In onion plants foliar spray of urea increased N, P, K and Ca concentration of leaves that resulted in higher photosynthesis and sugar production; however, other nutrients were not affected (Charbaji *et al.*, 2008). Similar results in carrot were found by foliar application of nitrogen sources (Smoleń and Sady, 2009).

In present study, ammonium spray reduced vitamin C content of fruits. This can be due to stress conditions induced by foliar ammonium spray and less hydraulic conductance of plant tissues (Souri and Roemheld, 2009). Changes in quality parameters of tomato by foliar nitrogen sprays are in agreement with similar studies on other vegetable crops (Guvenc *et al.*, 1995; Chaurasia *et al.*, 2005; Yildirim *et al.*, 2007; Dehnavard *et al.*, 2014), which showed that increasing nitrogen application reduced the vitamin C content. In addition, foliar application of nitrogen compounds can significantly improve plants tolerate to heat stress (Zhao *et al.*, 2008). Foliar N application can improve plant growth parameters of dry weight, relative water content and nitrate reductase activity under moderate water stress particularly with drought sensitive varieties (Zhang *et al.*, 2009).

In this experiment, foliar application of ammonium sulfate and to less extent urea reduced normal plant growth and some quality traits, while calcium nitrate generally improved tomato growth and some quality factors. However, calcium nitrate can increase nitrate content of fruits that is not suitable, and therefore is not recommended in repeated applications. Spray of ammonium sulfate increased fruit TSS and titrateable acidity while it decreased vitamin C and fruit postharvest freshness. So, restricted growth through lower height and less lateral shoots in ammonium foliar sprayed plants may suggest benefits regarding labor requirement for plant pruning-training and management under greenhouse production of tomatoes. In addition, by foliar N application (particularly Ammonium sulfate and urea) less soil salinity and less nitrate accumulation occur. Nevertheless, it is revealed that tomato still show sensitivity to ammonium nutrition through the foliage similar to root ammonium nutrition via nutrient solution.

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