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Growth, root morphology and leaf physiology of watermelon as affected by various rates and forms of nitrogen in the hydroponic system

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Abstract

The aim of this study was to determine the effects of different rates and forms of nitrogen (N) on shoot growth and root morphological and leaf physiological responses of watermelon (cv. Crimson Tide F1) under hydroponic growth condition. The nutrient solution experiment was conducted between January - March in 2018 by using an aerated Deep Water Culture (DWC) technique in a fully automated climate room placed in the Plant Physiology Laboratory of Erciyes University, Faculty of Agriculture, Kayseri in Turkey. Plants were tested under two N-Rates (N1: 1000 and N2: 2000 µM N) and three different N-Forms (Am-N: $NH4^+$, Nit-N: $NO3^-$, 50% mixture of both N-Forms Mix-N: NH4+NO3) by growing in 8 L pots filled continuously aerated nutrient solution (modified Hoagland). The experiment was conducted with a completely randomized block design with four replications. From each pot two plants were harvested 42 days after treatment (DAT) by separating into stem, leaf and root fractions. The results indicated that shoot growth, root morphological and leaf physiological responses were significantly (p<0.001) affected by N-Rate, N-Form and N-Rate x N-Form interaction. The lowest performance under sole Am-N supply was achieved, since it severely reduced shoot and root growth and leaf area development as compared to sole Nit-N and Mix-N treatments. Irrespective of N rates, best growth performance in shoot growth was achieved under Mix-N supply, while root growth significantly improved under sole Nit-N supply. All these clearly indicate that the application of sole ammonium (1000 µM N) is detrimentally toxic for hydroponically grown watermelon plants. On the other hand, a 50% mixed of ammonium with nitrate even at a higher dose (N2: 2000 µM ammonium N) can be more advantageous for the growth and development of watermelon plants grown in the hydroponic system. Furthermore, our study showed that the effects of N-Form (Nit-N and Mix-N) on the improvement of shoot growth, root morphology and leaf physiological development and photosynthesis were significantly higher than the effects of N-Rate. Therefore, the application of nitrogen fertilizers in the form of Mix-N could be a useful N management strategy for growth and yield of watermelon plants under hydroponic conditions.

Keywords: Nitrogen, Watermelon, N-form, Ammonium toxicity, Photosynthesis

Introduction

In crop production nitrogen (N) is the most common and widely used fertilizer nutrient. Due to be an important resource and essential input for crop growth and yield although, the available N is more often a limiting factor influencing plant growth than any other nutrient in both high-input and low-input agriculture systems (Grindlay, 1997). Plants may prefer the ammonium (NH_4^+) or the nitrate (NO_3^-) as the N source in the soil. There are several chemicals, physical and biotic soil factors determine which of the two forms prevails (Kinzel, 1983). Under favorable soil conditions NO_3 is usually the major N-Form in the soil solution and is therefore, supposed to be the most important N-source for crop growth and yield. Because, under favorable soil conditions, NH_4^+ is readily converted to

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NO_3^- by soil microorganisms through the nitrification process. However, this may be different when adverse soil conditions, in particular low oxygen and low pH, hamper the activity of nitrifying bacteria. For instance, agricultural practices such as the application of NH4 fertilizers or urea along with nitrification inhibitors or the creation of soil zones with high NH4 concentrations by specific application techniques (CULTAN technique: Sommer, 1994) may result in an enhanced NH4 supply. The N-Form and N-concentration have pronounced effects on plant growth (Wilcox et al., 1985). The response of plant species or cultivars to NH4 or NO3 nutrition varies widely. Some shows better growth with NH4, others with NO3. Beneficial effects of enhanced NH4 supply on yield (Huffman, 1989), specific yield components (Wiesler, 1997) and yield responses among genotypes (Below, 1987) were clearly demonstrated. However, the question remains unanswered as to which form of nitrogen, NO3 or NH4, or which combinations of these forms, is superior for obtaining maximum crop productivity. Moreover, in the literature, there is not enough information or conducted studies on the physiology of horticultural crops related to N-rates or N-forms in hydroponic growth systems. Therefore, the aim of this study was to assess the effects of different rates and forms of nitrogen on shoot growth and root morphological and leaf physiological responses of watermelon under hydroponic growth conditions.

Materials and Methods

Plant Material

In the present study, to obtain a high germination rate and also homogenous seedlings for hydroponic growth system, a well-known commercial watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] cultivar (Crimson Tide F1) was used as plant material.

Experimental Site and Plant Growth Conditions

An experiment was conducted between January - March in 2018 by using an aerated Deep Water Culture (DWC) technique in a fully automated climate room placed in the Plant Physiology Laboratory of Erciyes University, Agriculture Faculty, Kayseri in Turkey. During the vegetation period, the room temperatures (day/night) were 25/22 °C while the relative humidity was almost 65-70%. The supplied light intensity was almost 350 µmol m^{-2} S^{-1} photon flux of 16/8 h of light/dark photoperiod regimes. The watermelon seeds were sown in a mixture of peat (pH: 6.0-6.5) and perlite contained media with a ratio of 2:1 (v:v) for two weeks in the plastic multi-pots. The seedlings almost developed two to three true leaves, were carefully freed from peat-perlite medium by root washing and then transferred into 8 L plastic pots. Two plants were grown in each pot filled with 8 L nutrient solution (modified Hoagland). The solution was continuously aerated by an air pump to supply sufficient dissolved oxygen (8.0 mg/ L). The experiment was conducted with completely randomized block design with four replications. In the hydroponic experiment the total vegetation period from transplanting into 8 L plastic pots up to the final harvest was almost 42 days.

In this study, the basic nutrient solution was prepared regarding to Hoagland (modified) formulation. During hydroponic study only distilled water with analytical grade (99% pure) chemicals contained were used. In this hydroponic experiment, ammonium sulfate ($(NH_4)_2SO_4$) and clacium nitrate ($Ca(NO_3)_2$) were used as the N sources in two different N-Rates (N1: 1000 and N2: 2000 µM N) and three different N-Forms (sole nitrate: Nit-N, sole ammonium: Am-N and 50% mixture of both N-Forms: Mix-N). The pH of nutrient solution was maintained neutral by adding $CaCO_3$ and to hamper the nitrification process, a nitrification inhibitor (DMPP: 3,4-dimethylpyrazole phosphate) was applied to the solution. Furthermore, basic nutrient solution had the following composition (µM): K_2SO_4 (500); KH_2PO_4 (250); $CaSO_4$ (1000); $MgSO_4$ (325); NaCl (50); H_3BO_3 (8.0); $MnSO_4$ (0.4); $ZnSO_4$ (0.4); $CuSO_4$ (0.4); $MoNa_2O_4$ (0.4); Fe-EDDHA (80). All nutrients were replaced when the N concentration of the nutrient solution in the 2.0 mM N rate pots fell below 1.0 mM, as measured daily with nitrate test strips (Merck, Darmstadt, Germany) by using a NitracheckTM reflectometer. Distilled water was added every two days to replenish the water lost to evaporation, and the solution was changed weekly.

Harvest, Shoot- Root Fresh and Dry Weight, Root: Shoot Ratio Measurements

At the end of the experiment (42 DAT) watermelon plants were harvested by separating them into the leaf, stem and roots for the fresh weight determination. After measuring the fresh weights of each shoot and root fraction, samples were stored separately in paper bags and dried in a ventilated oven at 70 °C for 72 hours. Root: shoot ratio was calculated from the dry weight.

Root Morphological Measurements

In the hydroponic experiment, the plant root morphological parameters such as root length (m), root volume (cm^3) and root diameter (mm) of the plants were measured by using a special image analysis software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada) in combination with Epson Expression 11000XL scanner. From harvested fresh root samples of watermelon plants, almost 5.0 g sub-samples were taken. The samples were each (one after the other) placed in the scanner's tray.

Water was added and with the aid of a plastic forceps, the roots were homogenously spread across the tray; and the scanning and analysis have done from the WinRhizo system's interface on a computer connected to the scanner. The total plant root length and volume was then determined as the ratio of sub-sampled root fresh weight to the total root fresh weight.

Main Stem Length and Leaf Physiological Measurements

In the hydroponic experiment some of the leaf physiological parameters were determined destructively at harvest while some of them were measured non-destructively prior to the harvest. The main stem length was measured non-destructively prior to the harvest by using a ruler and it was recorded in centimeter (cm). The total leaf area of harvested plants was measured destructively with a leaf area measuring device (LI-COR LI-3100C, Inc., Lincoln, NE, USA). The measurements were recorded in centimeter square (cm^2).

On the other hand, the leaf chlorophyll index (SPAD) was determined non-destructively by using a portable chlorophyll (SPAD) meter (Minolta SPAD-502). During the growth period, SPAD readings were performed on 3th and 4th week of the vegetation period at the center of the leaves on the fully expanded youngest leaf of whole plants for each treatment.

The leaf-level CO_2 gas exchange (µmol CO_2 m^{-2} s^{-1}) measurements were done non-destructively in a controlled growth chamber by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). The leaf photosynthesis measurement was performed on the most recent fully expanded leaves, using four replicate leaves per treatment on 3th and 4th week of the vegetation period.

Shoot and Root Nitrogen Analysis

After grinding shoot (leaf) and root dry materials, almost 200 mg from each dry plant samples were taken to analyze the shoot and root N concentration (mg N g^{-1} d.w.) by using Kjeldahl Nitrogen Determination Method, introduced by Johan Kjeldahl in 1883 (Labconco, 1998). After the determination of shoot and root N concentration, the value was multiplied by total shoot or root dry matter in order to calculate the total shoot and root N content (N uptake) of a whole plant (mg N $plant^{-1}$).

Statistical Analysis

Statistical analysis of the nutrient solution experiment data was performed using the PROC GLM procedure of SAS Statistical Software (SAS for Windows 9.1. SAS Institute Inc., Cary, NC.). A two-factorial analysis of variance was performed to study the effects of N-Rate and N-Form and N-Rate x N-Form interactions on the plants. Levels of significance are represented by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ns means not significant (F-Test). Differences between the treatments were analyzed using Duncan's Multiple Test ($p < 0.05$).

Results and Discussion

Shoot and Root Biomass Production and Partitioning

Results obtained from hydroponic experiment indicated that shoot and root fresh weight, main stem length (Table 1), shoot and root dry weight and root:shoot ratio (Table 2) of watermelon plants were significantly (p <0.001) affected by N-Rate, N-Form and N-Rate x N-Form interaction. Plants under N2 supply showed usually a higher performance in shoot and root growth and biomass production than plants grown under N1 supply, but only when the nitrogen was applied in the form of sole nitrate (Nit-N) or a mixture of both N-Forms (Mix-N) (Table 1 and 2). On the other hand, a significant decline in shoot and root growth was recorded when the nitrogen was increased in the form of sole ammonium (Am-N) from N1 to N2 level. Increasing N supply from 1000 µM (N1) to 2000 µM (N2), led to an increase in shoot fresh weight by almost 19.9% and 23.2%, in root fresh weight by almost 35.6% and 36.1% and in main stem length by almost 26.2% and 16.4%, respectively, under supply of Nit-N and Mix-N (Table 1). In contrast, the reduction in shoot and root fresh weight and main stem length was almost by 31.2%, 24.9% and 28.1%, respectively, under the supply of sole Am-N.

Besides substantial nitrogen effects on the shoot and root growth of watermelon, also highly significant differences were found between N-Forms in shoot and root growth under N1 and N2 nitrogen levels (Table 1 and 2). As compared to both Nit-N and Mix-N forms, significantly lowest shoot and root fresh biomass and main stem length were recorded under the supply of sole Am-N at N1 and N2 levels (Table 1). Averaged over N-Rates, shoot fresh weight (SFW), root fresh weight (RFW) and main stem length (MSL) were increased almost by 167.3%, 190.3% and 93.5%, respectively at Nit-N, and almost by 196.5%, 165.6% and 114.2%, respectively at Mix-N, as compared to sole Am-N supply. Interestingly, this result clearly indicated that the effects of N-Forms (Nit-N and Mix-N) on the enhancement of SWF, RFW and MSL were substantially higher than the effects of N-Rates (Table 1 and 2).

In agreement with several studies (Wilcox et al., 1985; Sattelmacher et al., 1990; Schulte Auf'm Erley et al., 2007; Ulas et al., 2012; 2013; 2019), our results clearly demonstrated that nitrogen rates and forms have pronounced effects on the shoot and root growth of watermelon. Moreover, our results

Table 1. Shoot and root fresh weight and main stem length of watermelon as affected by different N-Rates (N1: 1000 and N2: 2000 µM) and N-Forms (Nit-N: NO_3^-, Am-N: NH_4^+ and 50% mixture of both N-Form; Mix-N) in hydroponic system

	Shoot fresh weight (g $plant^{-1}$)		Root fresh weight (g $plant^{-1}$)		Main stem length (cm $plant^{-1}$)	
N-Form	N1	N2	N1	N2	N1	N2
Am-N: NH_4^+	26.42 c	18.18 C	4.22 c	3.17 C	9.60 c	6.90 B
Nit-N: NO_3^-	54.20 b	65.00 B	9.10 a	12.34 A	13.93 b	18.00 A
Mix-N: NH_4+NO_3	59.27 a	73.00 A	8.30 b	11.30 B	16.33 a	19.00 A
F-Test						
N-Rate	***		***		***	
N-Form	***		***		***	
N-Rate x N-Form	***		***		***	

Values denoted by different letters (lower and upper case letters for N1 and N2, respectively) are significantly different between N-Forms within columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

are highly corroborating with the results of cereal crop experiments which indicated that the best growth was obtained when a mixture of both N-Forms (ammonium and nitrate) compared to sole nitrate was supplied to the plants (Hageman, 1984; Huffman, 1989). Furthermore, our results clearly showed that sole ammonium supply (Am-N) is also detrimental for the watermelon either grown under N1 or N2 nitrogen levels. This could be the result of supra-optimum ammonium (NH_4^+) uptake of plant roots which might be caused a growth reduction in this treatment.

Since ammonium-fed plants are not able to store excess nitrogen in the form of nitrate (NO_3^-), and hence the assimilation capacity of roots and accumulation capacity of shoot might be exceeded due to free ammonium/ammonia (Takács and Técsi, 1992).

In agreement with our results, similar growth inhibition was often observed when the plants (*Zea mays* L., Bennett et al. 1964; *Solanum lycopersicon*, Ganmore-Neumann and Kafkafi 1980; *Solanum lycopersicon*, Magalhaes and Wilcox 1983; *Phaseolis vulgaris*, Chaillou et al. 1986) were fed exclusively with sole ammonium in previous studies.

As similar as shoot and root fresh weight (Table 1), N-Rates and N-Forms were differed significantly (p <0.001) in shoot and root dry matter productions and their partitioning (root:shoot ratio) under both N1 and N2 nitrogen levels (Table 2). Although showing similar dry matter partitioning as Nit-N, the shoot and root dry matter accumulation were significantly lowest under sole Am-N as compared to both Nit-N and Mix-N forms under N1 and N2 supply. This clearly indicates that the root growth of ammonium-fed plants was relatively less reduced than shoot growth. Similar results have been observed by Atkinson (1985), De Viesser (1985) and Haynes and Goh (1978). The highest shoot dry matter was produced under Mix-N form while the highest root dry matter was produced under Nit-N form at both N1 and N2 nitrogen levels. This also clearly explaining why the dry mater partitioning between shoot and root was significantly higher at Nit-N than at Mix-N form at both N1 and N2 nitrogen levels. This might be due to a high assimilate allocation from shoot to roots under sole nitrate supply. Similar and confirmative results have been demonstrated in the studies of Feil (1994) and Ulas et al. (2013).

Table 2. Shoot and root dry weight and root:shoot ratio of watermelon as affected by different N-Rates (N1: 1000 and N2: 2000 µM) and N-Forms (Nit-N: NO_3^-, Am-N: NH_4^+ and 50% mixture of both N-Form; Mix-N) in hydroponic system

	Shoot dry weight (g plant^{-1})		Root dry weight (g plant^{-1})		Root: shoot ratio (g g^{-1})	
N-Form	N1	N2	N1	N2	N1	N2
Am-N: NH_4^+	2.42 c	1.52 C	0.27 c	0.18 C	0.11 b	0.12 A
Nit-N: NO_3^-	4.89 b	6.34 B	0.58 a	0.77 A	0.12 a	0.12 A
Mix-N: NH_4+NO_3	5.96 a	7.15 A	0.52 b	0.66 B	0.09 c	0.09 B
F-Test						
N-Rate	***		***		***	
N-Form	***		***		***	
N-Rate x N-Form	***		***		***	

Values denoted by different letters (lower and upper case letters for N1 and N2, respectively) are significantly different between N-Forms within columns at p < 0.05. ns, non-significant. *p < 0.05, **p < 0.01 and ***p < 0.001

Shoot and Root N Concentration and Total Plant N Uptake

Results indicated that highly significant (p <0.001) differences between N-Rates and N-Forms were found in shoot and root N concentrations and in total plant N uptake of watermelon plants (Table 3). The growth response to supplied N, i.e., the interaction between N-Rate and N-Form was also significant but only in shoot N concentration and total plant N uptake. Plants grown either under sole ammonium (Am-N), sole nitrate (Nit-N) or a mixture of both N-Forms (Mix-N) exhibited a significant increase in shoot (13.3% by Am-N, 13.6% by Nit-N and 11.7% by Mix-N) and root (31.5% by Am-N, 26.6% by Nit-N and 20.8% by Mix-N) N concentrations when the nitrogen was increased from N1 to N2 level. Interestingly, the highest shoot N concentrations at both N levels were found under sole Am-N, while the lowest was shown under sole Nit-N supply. However, the highest shoot N concertation under sole Am-N supply is contrasting with the lowest shoot fresh (Table 1) and dry (Table 2) matter production of the same watermelon plants. This can be explained by the detrimental effects of ammonium toxicity that occurred in watermelon plants grown under both 1000 µM (N1) and 2000 µM (N2) nitrogen levels supplied as the form of sole ammonium (Am-N). This might be due to a low leaf area formation which usually leads to an increase in the amount of N accumulated per unit of leaf area (Hirasawa and Hsiao, 1999). Similar and corroborative results were demonstrated by the study of Ulas et al. (2013) when the oilseed rape plants are grown under hydroponic sole ammonium supply at a rate of 1000 µM N. Moreover, our watermelon plants showed an intermediate shoot N concentration under Mix-N supply at both N1 and N2 levels (Table 3). On the other hand, the highest root N concentrations were found under Mix-N supply, while the lowest and similar root N concentrations were recorded under sole Am-N and Nit-N supply at both N1 and N2 levels.

Table 3. Shoot and root nitrogen concentration and total plant nitrogen uptake of watermelon as affected by different N-Rates (N1: 1000 and N2: 2000 µM) and N-Forms (Nit-N: NO_3^-, Am-N: NH_4^+ and 50% mixture of both N-Form; Mix-N) in hydroponic system

	Shoot N concentration (mg g dw.$^{-1}$)		Root N concentration (mg g dw.$^{-1}$)		Plant total N uptake (mg plant^{-1})	
N-Form	N1	N2	N1	N2	N1	N2
Am-N: NH_4^+	36.77 a	41.67 A	18.14 b	23.75 B	93.72 c	67.76 C
Nit-N: NO_3^-	29.49 c	33.47 C	19.17 b	24.33 B	155.43 b	230.90 B
Mix-N: NH_4+NO_3	31.62 b	35.28 B	22.55 a	27.29 A	200.06 a	270.30 A
F-Test						
N-Rate	***		***		***	
N-Form	***		***		***	
N-Rate x N-Form	*		n.s		***	

Values denoted by different letters (lower and upper case letters for N1 and N2, respectively) are significantly different between N-Forms within columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

Leaf Physiological Development and Photosynthetic Activity of Leaves

In this study, the total leaf area, leaf chlorophyll index (SPAD) and photosynthetic activity of leaves were significantly (p <0.001) affected by N-Rate, N-Form and N-Rate x N-Form interaction (Table 4). As similar as shoot and root biomass production (Table 1 and 2), the plants grown under N2 supply showed usually a higher performance in leaf area formation, SPAD value and photosynthesis than plants grown under N1 supply, but only when the nitrogen was applied in the form of sole nitrate (Nit-N) or a mixture of both N-Forms (Mix-N) (Table 4). These results clearly explaining which factors are primarily contributing to the shoot and root growth of watermelon plants under increased N supply of both N-Forms. Increasing N supply from N1 to N2 level, led to an increase in total leaf area by almost 37.1% and 22.8%, in SPAD value by almost 21.4% and 21.3% and in photosynthesis by almost 48.0% and 46.4%, respectively, under supply of Nit-N and Mix-N (Table 4). However, the opposite is also true, since significant reductions in total leaf area and photosynthesis were recorded when the nitrogen was increased in the form of sole ammonium (Am-N) from N1 to N2 level.

Table 4. Total leaf area, leaf chlorophyll index (SPAD) and photosynthesis of watermelon as affected by different N-Rates (N1: 1000 and N2: 2000 µM) and N-Forms (Nit-N: NO_3^-, Am-N: NH_4^+ and 50% mixture of both N-Form; Mix-N) in hydroponic system

	Total leaf area (cm^2 plant^{-1})		Leaf chlorophyll index (SPAD)		Photosynthesis (µmol m^{-2} s^{-1})	
N-Form	N1	N2	N1	N2	N1	N2
Am-N: NH_4^+	303.74 c	229.81 C	50.61 a	52.28 C	6.33 c	4.77 C
Nit-N: NO_3^-	738.91 b	1012.74 B	48.58 b	59.00 A	9.19 b	13.58 B
Mix-N: NH_4+NO_3	914.36 a	1122.18 A	45.54 c	55.18 B	10.60 a	15.52 A
F-Test						
N-Rate	***		***		***	
N-Form	***		***		***	
N-Rate x N-Form	***		***		***	

Values denoted by different letters (lower and upper case letters for N1 and N2, respectively) are significantly different between N-Forms within columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

The reduction in total leaf area and photosynthesis was almost by 24.4% and 24.2%, respectively, under the supply of sole Am-N. All these results are contrasting with the generally positive effects of nitrogen on plant growth and development.

However, similar negative effects of sole ammonium supply were demonstrated in several experiments that were conducted on various plant species (Bennett et al. 1964; Ganmore-Neumann and Kafkafi 1980; Magalhaes and Wilcox 1983; Chaillou et al. 1986, Ulas et al., 2013). However, a small increase in SPAD value by almost 3.4% under sole Am-N was recorded when the nitrogen was increased from N1 to N2 level. This is highly corroborating with the result of high shoot nitrogen concertation under sole Am-N supply (Table 3).

Highly significant differences were found between N-Forms in total leaf area, SPAD value and photosynthetic activity of leaves under both N rates (Table 4). As compared to both Nit-N and Mix-N forms, significantly lowest total leaf area and photosynthesis were recorded under the supply of sole Am-N at N1 and N2 levels. Averaged over N-Rates, total leaf area and photosynthesis were increased almost by 228.9% and 105.4%, respectively at Nit-N, and almost by 281.7%, and 135.7%, respectively at Mix-N, as compared to sole Am-N supply. Our results indicated clearly again that the effects of N-Forms (Nit-N and Mix-N) on the enhancement of leaf area and photosynthetic activity of leaves were substantially higher than the effects of N-Rates on both parameters.

Root Morphological Development and Root Architecture

The results indicated that total root length, total root volume and average root diameter of watermelon plants were significantly ($P<0.001$) affected by N-Rate, N-Form and N-Rate x N-Form interaction (Table 5). Highly significant increases in total root length and root volume, but oppositely significant reductions in average root diameter were recorded when the nitrogen was increased from N1 to N2 level in the form of sole nitrate (Nit-N) or a mixture of both N-Forms (Mix-N). On the other hand, significant reductions in total root length and root volume, but oppositely significant increases in average root diameter were recorded when the nitrogen was increased in the form of sole ammonium (Am-N) from N1 to N2 level. All these indicate that there is a negative relationship between total root length and average root diameter, irrespective of the N-rates.

Increasing N supply from 1000 µM (N1) to 2000 µM (N2), led to an increase in total root length by almost 38.9% and 48.1%, in root volume by almost 27.5% and 36.7%, respectively, while the average root diameter was declined almost by 4.2% and 4.0%, respectively, under supply of Nit-N and Mix-N (Table 5). In contrast, the reductions in total root length and root volume were almost by 30.4%, and 23.1%, respectively, while the average root diameter was increased almost by 3.7% under the supply of sole Am-N. Similar to shoot fresh and dry weight (Table1 and 2) sole ammonium supply also reduced root dry weight (Table 2), total root length and volume (Table 5). However, ammonium-fed plants showed a higher root:shoot ratio than ammonium-nitrate-fed plants (Table 2) indicating that the root growth of ammonium-fed plants was relatively less reduced than shoot growth. Similar results have been observed by Atkinson (1985), De Viesser (1985) and Haynes and Goh (1978).

The reduced growth of ammonium-fed plants might also have been caused by enhanced C demand for ammonium assimilation followed by deprivation of nonstructural carbohydrates exclusively in the roots (Blacquiere et al., 1987; Chaillou et al., 1991) or both, in the roots and shoots (Raab and Terry, 1995). The high demand on intermediates from the TCA cycle for ammonium assimilation and the need to stabilize cytosolic pH by decarboxylation of organic acids (Marschner, 1995) may also cause a deprivation of carboxylates in ammonium fed plants.

Table 5. Total root length, volume and root diameter of watermelon as affected by different N-Rates (N1: 1000 and N2: 2000 µM) and N-Forms (Nit-N: NO_3^-, Am-N: NH_4^+ and 50% mixture of both N-Form; Mix-N) in hydroponic system

	Total root length (m plant^{-1})		Total root volume (cm^3 plant^{-1})		Average root diameter (mm)	
N-Form	N1	N2	N1	N2	N1	N2
Am-N: NH_4^+	9.50 c	6.61 C	2.12 c	1.63 C	0.27 a	0.28 A
Nit-N: NO_3^-	21.30 a	29.58 A	3.85 a	4.91 A	0.24 c	0.23 B
Mix-N: NH_4+NO_3	16.69 b	24.72 B	3.27 b	4.47 B	0.25 b	0.24 B
F-Test						
N-Rate	***		***		n.s	
N-Form	***		***		***	
N-Rate x N-Form	***		***		**	

Values denoted by different letters (lower and upper case letters for N1 and N2, respectively) are significantly different between N-Forms within columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

The N-Forms differed significantly in total root length, root volume and average root diameter under both N rates (Table 5). As compared to both Nit-N and Mix-N forms, significantly lowest total root length and root volume were recorded under the supply of sole Am-N at N1 and N2 levels. Averaged over N-Rates, total root length and root volume were increased almost by 215.9% and 134.2%, respectively at Nit-N, and almost by 157.0%, and 106.9%, respectively at Mix-N, as compared to sole Am-N supply. Moreover, the average root diameter was reduced almost by 13.91% and 10.3%, respectively at Nit-N and Mix-N forms, as compared to sole Am-N supply. Our results indicated clearly again that the effects of N-Forms (Nit-N and Mix-N) on the enhancement of total root length and root volume were substantially higher than the effects of N-Rates on both parameters.

Conclusion

Usually, nitrogen concentration has pronounced effects on plant growth and development. However, the effects of different N-forms (ammonium or nitrate) on the response of different plant species or cultivars varies widely. Some showing better growth with nitrate while some oppositely with ammonium. Most of the studies revealed that best growth performance was often obtained when a mixture of both N-forms was supplied. Our study indicated that shoot growth, root morphological and leaf physiological responses were significantly (p<0.001) affected by N-Rate, N-Form and N-Rate x N-Form interaction. A lowest performance under sole Am-N supply was achieved, since it severely reduced shoot and root growth and leaf area development as compared to sole Nit-N and Mix-N treatments. Irrespective of N rates, best growth performance in shoot growth was achieved under Mix-N supply, while root growth significantly improved under sole Nit-N supply. All these clearly indicate that the application of sole ammonium (1000 µM N) is detrimentally toxic for hydroponically grown watermelon plants. On the other hand, a 50% mixed of ammonium with nitrate even at a higher dose (N2: 2000 µM ammonium N) can be more advantageous for the growth and development of watermelon plants grown in the hydroponic system. Furthermore, our study showed that the effects of N-Form (Nit-N and Mix-N) on the improvement of shoot growth, root morphology and leaf physiological development and photosynthesis were significantly higher than the effects of N-Rate. Therefore, the application of nitrogen fertilizers in the form of Mix-N could be a useful N management strategy for growth and yield of watermelon plants under hydroponic conditions.

Compliance with Ethical Standards

Conflict of interest
The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution
The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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