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Interactive Effects of Nitrogen Form and Oxygen Concentration on Growth and Nutritional Status of Eggplant in Hydroponics

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ABSTRACT

A greenhouse study was carried out to determine the effect of nitrogen forms and different O_2 levels on growth and mineral nutrient concentrations of eggplant. The experimental design was a completely randomized factorial experiment with two factors, namely: (i) Two nitrogen forms ($Ca(NO_3)_2$ and $(NH_4)_2SO_4$) and (ii) Three O_2 levels of the nutrient solutions (1±0.3, 2±0.3, 3±0.3, and 4±0.3 mg L^{-1} O_2). The results showed that ammonium application reduced all measured parameters of vegetative growth, whereas high oxygen levels increased the vegetative growth. Comparing with nitrate-N, ammonium application increased the concentrations of NPK and Zn in leaves and Zn and Cu in roots, while it decreased the concentration of Mg, Ca, Cu, Mn, and Na in leaves and Ca, Mg, Mn, and Na in roots. High levels of O_2 increased N, Mg, Ca, Cu, and Mn content of leaves, as well as Mn and Na content in roots, while it decreased the concentration of K in leaves and P and Zn in roots. According to the results, the increase in O_2 amount of the nutrient solutions partly alleviated ammonium toxicity in eggplant. Therefore, in floating hydroponic cultures, O_2 level and its distribution should be controlled and must not be lower than 4 mg L^{-1}.

Keywords: Ammonium, Nitrate-N, Nutrient solution aeration, Soilless culture, *Solanium melongena*.

INTRODUCTION

Nitrogen is a constituent of many plant cell components, including amino acids and nucleic acids. Therefore, nitrogen deficiency restricts the growth of the vegetative organs (Barker and Breyson, 2006). It is available in various inorganic forms such as nitrate, ammonium, and nitrogen molecules, and organic forms such as urea and amino acids, which can be changed to forms available for absorption by the plant (Marschner, 1995). Ammonium (NH_4^+) and nitrate (NO_3^-) are used as nitrogen source for the plant, although the plant response to a specific form of nitrogen varies from species to species (Britto and Krounzucker, 2002). At high concentrations, NH_4^+ toxicity can often be occurred for different species (Britto and Krounzucker, 2002; Roosta *et al*., 2009).

Oxygen deficiency is a common phenomenon and may occur in some hydroponic systems, especially floating culture, and in poorly-drained soils after a short period of heavy rain. Inhibitions of root cell division and decrease in root elongation have been found in plants

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hydroponically grown at lower dissolved O_2 concentrations (Atwell *et al*., 1985). Furthermore, decrease in dissolved O_2 concentration decrease leaf water potential and stomatal conductance (Else *et al*., 1995).

Because the lack of O_2 affects the energy status of the plant, root physiological functions such as respiration and water uptake are depressed (Drew, 1988; Chun and Takakura, 1994). Lack of O_2 may negatively affect the plant metabolism including nitrogen uptake and assimilation (Greenway and Gibbs, 2003; Morard *et al*., 2004). Under oxygen deficiency condition, the rates of NO_3^- and NH_4^+ uptake have been found to be very low (Brix *et al*., 1994). Therefore, plants growing in O_2 deficient conditions often exhibit N deficiency symptoms along with O_2 stress symptoms, because N uptake is inhibited by oxygen deficiency (Greenway and Gibbs, 2003; Morard *et al*., 2004).

Little information is available about the interaction between inorganic N form and dissolved O_2 concentrations and how it influences plant growth and nutrient concentration. In this study, we aimed to study hydroponically grown eggplant and measure some growth parameters and nutrient concentrations in response to different concentrations of dissolved O_2 and two forms of inorganic N.

MATERIALS AND METHODS

Plant Material and Culture Conditions

This study was performed with eggplant (*Solanum melongena* L. *cv* Long purple). The experiment was carried out as a factorial combination and completely randomized basic design with three replications. The first factor was N in two forms (including calcium nitrate and ammonium sulfate both at the N concentration of 5 mM) and the second factor was O_2 level of nutrient solution at 4 levels including: 1±0.3, 2±0.3, 3±0.3 and 4±0.3 mg L^{-1} O_2. Seeds were germinated in the pots containing perlite medium. The seedlings were transferred to pots containing 4 L of aerated nutrient solution. Four plants were grown together. The nutrient solution contained 0.2 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.3 mM $MgSO_4.7H_2O$, and 0.1 mM NaCl. Micro-nutrients were 20 µM Fe-NaEDTA, 7 µM $MnSO_4$.H2O, 0.7 µM $ZnCl_2$, 0.8 µM $CuSO_4.5H_2O$, 2 µM H_3BO_3, and 0.8 µM $Na_2MoO_4.2H_2O$ with either 5 mM N as Ca $(NO_3)_2.4H_2O$ or as $(NH_4)_2SO_4$ (Roosta and Schjoerring, 2007). Except N form, nutrient solution was the same in all treatments. For Ca adjustment in NH_4^+ containing solution, $CaCl_2$ was used, so that Ca concentration was equal in both treatments. The pH was controlled at 6.5 to 7 throughout the growth period using Calcium Carbonate ($CaCO_3$) as a buffer. Solutions were changed completely every week in the first 5 weeks and subsequently every 4^{th} day. The plants were grown in a greenhouse with 13 hours light (26±3ºC) and 11 hours darkness (23±3ºC). Three of the plants were harvested after 4 weeks and one plant remained in each pot. Plants after 9 weeks of initial treatment were harvested.

Oxygen Measurement and Adjustment

The atmospheric O_2 needed for the treatment was provided by an air pump with 2W power. Air distribution between pots was carried out with the use of medical serum set. Oxygen levels were controlled using portable O_2 meter (OXi 315, WTW Co., Germany) according to Chorianopoulou and Bouranis (2004).

Vegetative Growth Parameters

The height of the plants was measured on the harvest day by a ruler. Leaf number, stem number, fresh mass of leaves, roots, and stems were measured after harvesting the plants. The leaf area was determined using leaf area meter (model C1 202, USA). The organs of plants were put in the oven for 72 hours at 72ºC, and then their dry masses were determined.

Nutrient Elements

Oven dried (72°C for 48 hours) samples of roots and shoots were weighed separately and ground to pass a 40-mesh sieve. The ground plant samples were dry-ashed at 500°C for 4 hours; the ashes were dissolved in 10 mL HCl (2N) and made the volume to 100 mL with distilled water. Concentration of Mg, Ca, Mn, Zn, and Cu was determined by atomic absorption spectrometry (Version 1/33 GBC Avanta, Au). K and Na concentrations were determined by flame photometry (JENWAY, PFP7, Germany) and P concentration in the digest was determined by the Olsen *et al*. (1954) method using spectrophotometry. Kjeldahl method was used to measure the total N.

Statistical Analysis

Analysis of variances were performed by using the SAS software (SAS Institute, Cary, NC), if ANOVA determined that the effects of the treatments were significant ($P< 0.05$ for F-test), then the treatment means were separated by LSD test.

RESULTS AND DISCUSSION

Vegetative Growth

Interactive effect of N-source and O_2 supply on fresh mass of shoot and leaves were significant (Table 1). Both NO_3^-- and NH_4^+-fed plants had the greatest shoot and leaf fresh mass at 4 mg L^{-1} O_2 concentration and the smallest at concentration of 1 mg L^{-1} O_2. Growth inhibition by low O_2 may be due to the rise of toxic gases such as CO_2 in the root zone (Araki, 2006). Also, ethanol can be formed in plants under O_2 deficiency which is toxic in high concentrations (Marschner, 1995). The lack of oxygen in root tissues blocks mitochondrial respiration and thus reduces the uptake of some nutrients required for plant growth (Linkermer *et al*., 1998; Sullivan *et al*., 2001). The NH_4^+-fed plants were more affected by high O_2 levels, as their shoot and leaf fresh masses were increased more than 2 and 3 times in 4 mg L^{-1} O_2 in nutrient solution compared to 1 mg L^{-1} O_2, respectively. This issue was reported by Jampeetong and Brix (2009) in the *Salvinia natans*, which was probably due to the decrease in toxic NH_4^+ by rising O_2 concentration in the nutrient solution. High O_2 is needed for respiration and, consequently, carbon skeleton provision for NH_4^+ assimilation (Roosta and Schjoerring, 2008a).

Nitrogen form and O_2 level had significant interactive effects on leaf area and stem number (Table 2). Both NO_3^-- and NH_4^+-fed plants had the greatest values of leaf area and stem number at 4 mg L^{-1} O_2 concentration. It is reported that the Cytokinin (CK) production by root and its transportation from roots to leaves (von

Table 1. Interactive effects of N sources and O_2 supply on the shoot and leaf fresh masses of eggplant.[a]

N form	O_2 levels (mg L^{-1})			
(5 mM)	1±0.3	2±0.3	3±0.3	4±0.3
	Shoot fresh mass (g $plant^{-1}$)			
NH_4^+	99.3 e	121.1 de	174.6 cd	275.2 b
NO_3^-	212.4 bc	274.0 b	397.8 b	414.9 a
	Leaf fresh mass (g $plant^{-1}$)			
NH_4^+	41.9 e	56.6 de	86.9 cd	175.2 b
NO_3^-	107.4 c	152.1 b	219.6 a	248.6 a

[a] Means in each column or row followed by the same letter(s) are not significantly different, LSD test ($P\leq 0.05$).

Table 2. Interactive effects of N sources and O_2 supply on leaf area and stem number of eggplant. [a]

N form (5 mM)	O_2 levels (mg L^{-1}) 1±0.3	2±0.3	3±0.3	4±0.3
	Leaf area (cm^2 $plant^{-1}$)			
NH_4^+	1923 f	1496 g	2895 e	6208 b
NO_3^-	3265 d	4373 c	6132 b	7210 a
	Stem number ($plant^{-1}$)			
NH_4^+	7.33 d	13.00 c	17.33 b	22.33 a
NO_3^-	16.00 bc	18.00 b	22.67 a	26.00 a

[a] Means in each column or row followed by the same letter are not significantly different, LSD test ($P \leq 0.05$).

Wiren *et al*., 2000) and depressed root water uptake at low dissolved O_2 concentrations results in leaf turgor loss which cause decrease in leaf expansion (Yoshida *et al*., 1997). A decrease in the number of lateral stems during the O_2 deficiency condition and NH_4^+ presence has already been reported by Linkermer *et al.* (1998). It is reported that O_2 reduction decreased the CK production in the plant (Basra and Basra, 1997). Cytokinin induced lateral bud growth by decrease of apical dominance. Reduction in the number of stems in the current study can be due to the decrease of CK in plants.

Shoot and leaf fresh masses, leaf area, and stem number of NH_4^+-fed plants were lower than NO_3^--fed plants at the same O_2 concentration in nutrient solutions (Table 1). This may be related to the decrease in some nutrient uptake, lowering pH of medium, hormonal imbalance, ethylene evolution, futile trans-membrane NH_4^+ cycling, carbon skeleton depletion in root (Britto and Keronzucker, 2002; Thomas and Sodek, 2005; Roosta and Schjoerring, 2007; 2008a, b), and the photosynthesis reduction in NH_4^+-fed plants (Takacs and Tecsi, 1992). Reduction of fresh and dry mass in the NH_4^+-fed plants has already been reported in many species including cucumber (Roosta *et al*., 2009), lettuce (Hay and Porter, 2006), onion (Gameily *et al*., 1991), and soybean (Thomas and Sodek, 2005).

Both variables (N form and O_2 level) were effective on plant height and leaf number, although analysis of variances showed that their interactive effect was not significant (Data not shown).

Nutrient Elements

The concentrations of Mg, Ca, Mn, and Na in root and shoot of the eggplants were found to be significantly affected by the interaction between N form and oxygen levels ($P \leq 0.01$).

Mg concentration was higher in leaves of NO_3^--fed plants as compared to those of NH_4^+-fed plants. Both NO_3^-- and NH_4^+- fed plants had the highest leaf Mg concentration at 4 mg L^{-1} O_2 level and the lowest at level of 1 mg L^{-1} O_2 (Table 3). Decreased Mg concentration in the NH_4^+-fed plants could be the result of a competition with NH_4^+, as reported in cucumber plants by Roosta and Schjoerring (2007). Decrease in the content of Mg during O_2 deficiency has already been reported by Board (2008). Uptake of Mg into plant roots occurred by both passive process with high Mg concentration and active process with low Mg concentration in nutrient solution (Morard *et al*., 2004). Active uptake of Mg is dependent on oxygen levels, thus, the active process may be stopped at low level of O_2, and uptake was merely a passive process.

Calcium concentration in leaves, but not in roots, increased with elevating O_2 concentrations for both NH_4^+- and NO_3^--fed plants, however, NO_3^-- fed plants had higher concentrations of Ca in the leaves and roots as compared to those of NH_4^+-

Table 3. Interactive effects of N sources and O_2 supply on leaf and root Mg, Ca, Mn and Na in eggplant.[a]

N form (5 mM)	O_2 levels (mg L^{-1}) 1±0.3	2±0.3	3±0.3	4±0.3
		Leaf Mg (%DM)		
NH_4^+	0.34 e	0.34 e	0.39 de	0.49 cd
NO_3^-	0.41 de	0.54 c	0.70 b	0.86 a
		Root Mg (%DM)		
NH_4^+	0.158 c	0.172 c	0.222 bc	0.336 ab
NO_3^-	0.331 ab	0.385 a	0.422 a	0.384 a
		Leaf Ca (% DM)		
NH_4^+	0.49 f	0.47 f	0.82 f	1.31 e
NO_3^-	1.85 d	2.6 c	3.31 b	3.74 a
		Root Ca (% DM)		
NH_4^+	0.30 b	0.33 b	0.39 b	0.32 b
NO_3^-	2.85 a	3.66 a	2.35 a	2.44 a
		Leaf Mn (mg kg^{-1} DM)		
NH_4^+	83.0 d	92.5 d	110.0 cd	168.5 a
NO_3^-	100.5 cd	129.5 bc	128.5 bc	142.0ab
		Root Mn (mg kg^{-1} DM)		
NH_4^+	18.77 b	17.60 b	21.22 b	27.06 a
NO_3^-	20.08 b	21.60 b	20.13 b	17.10 b
		Leaf Na (mg kg^{-1} DM)		
NH_4^+	0.09 b	0.11 b	0.14 b	0.15 b
NO_3^-	0.50 a	0.53 a	0.56 a	0.52 a
		Root Na (mg kg^{-1} DM)		
NH_4^+	1.87 c	0.82 d	0.85 d	0.91 d
NO_3^-	1.23bc	1.43 ab	1.63 a	1.48 a

[a] Means in each column or row followed by the same letter(s) are not significantly different, LSD test ($P \leq 0.01$).

fed plants at the same O2 concentrations (Table 3). Our results about the decrease of Ca concentration in NH4+-fed plants agree with the findings of Tabatabaei *et al*. (2006) and Kotsiras *et al*. (2002). Reduced Ca concentration might have been due to either the reduced uptake of Ca (such as an antagonist effect) or the reduced translocation in the xylem (Marschner, 1995). A reduction in Ca content at the O_2 deficiency agrees with the findings of Board (2008). Although Ca uptake is a passive process and needs no energy, lack of O_2 conditions decreases the water uptake. The reduction in water uptake influences the transport of Ca in plant and thus results in decreasing Ca concentration in the plant organs.

Both NO_3^-- and NH_4^+-fed plants had the highest leaf Mn concentration at 4 mg L^{-1} O_2 level and the lowest at level of 1 mg L^{-1} O_2. NH_4^+-fed plants had greater root Mn concentration than NO_3^-- fed plants at 4 mg L^{-1} O_2 level but not at the other O_2 concentrations (Table 3). Board (2008) reported that Mn concentrations in soybean leaf were lost due to the lack of O_2, which is consistent with our results.

In contrary to NO_3^--fed plants, NH_4^+-supplied plants generally showed a trend toward decreasing root-Na concentrations with increasing O_2 level (Table 3). Decreased Na concentration in the root of NH_4^+-fed plants may be due to the competition between these two elements for uptake by roots.

The interaction between N forms and O_2 levels was not found to be significant for leaf and root concentrations of N, P, K, Cu, and Zn (data not shown). The main effects of N forms and O_2 levels on these nutrients are shown in Tables 4 and 5, respectively. The concentration of N was higher in leaves of plants fed with NH_4^+ than those fed with NO_3^- (Table 4). The highest O_2 level caused a significant increase in N concentration (Table 5). Increased N uptake in NH_4^+-fed plants has been reported in cucumber (Roosta and Schjoerring, 2007), and *Salvinia natans* (Jampeetong and Brix, 2009). On the other hand, decreased N concentration during the lack of O_2 has been reported in maize (Lizaso *et al*., 2001), and soybean (Sullivan *et al*., 2001; Board, 2008). Application of NH_4^+ increased P concentration in leaf, but did not affect the P concentration in root (Table 4). Increased P concentration in NH_4^+-fed plants has been reported by Roosta and Schjoerring (2007). Plants fed with NH_4^+ contain less low-molecular mass anions (NO_3^-, carboxylates) and, consequently, have less negative charges to balance. Thus, they may uptake more phosphorus for provision of anion equivalents and charge balance (Roosta and Schjoerring, 2007; Zhang *et al.*, 2005). The highest O_2 level caused a significant decrease in P concentration in leaf and root (Table 5).

Potassium concentration was higher in leaves of plants fed with NH_4^+ than those fed with NO_3^- (Table 4). On the other hand, high O_2 level in nutrient solution decreased K concentration in the leaf, but not in the root (Table 5). The lower K concentration in leaves of NO_3^- grown plants in the current experiment could be due to the dilution effects as a result of higher growth.

Table 4. Effect of N form in the nutrient solution on nutrients concentrations in leaf and root of eggplant.[a]

Nutrient element	N form (5 mM)	
	NH_4^+	NO_3^-
Leaf N (% DM)	4.64 a	3.30 b
Leaf P (% DM)	0.043 a	0.021 b
Root P (% DM)	0.36 a	0.367 a
Leaf K (% DM)	4.54 a	2.02 b
Root K (% DM)	0.973a	0.849a
Leaf Zn (mg kg^{-1} DM)	35.00 a	10.00 b
Root Zn (mg kg^{-1} DM)	139.5 a	103.1 b
Leaf Cu (mg kg^{-1} DM)	58.25 b	63.38 a
Root Cu (mg kg^{-1} DM)	167.5 a	125.8 b

[a] Means with the different letter in each row are significantly different, LSD test ($P \leq 0.01$).

Table 5. Effect of O_2 levels in the nutrient solution on nutrients concentrations in leaf and root of eggplant.[a]

Element	O_2 Levels (mg L^{-1})			
	1±0.3	2±0.3	3±0.3	4±0.3
Leaf N (% DM)	3.90 b[†]	3.80 b	3.80 b	4.20 a
Leaf P (% DM)	0.42 a	0.44 a	0.33 ab	0.26 b
Root P (% DM)	1.39 ab	1.56 a	1.47 a	0.35 b
Leaf K (% DM)	4.03 a	3.80 a	2.72 b	2.57 b
Root K (% DM)	1.02 a	1.12 a	0.874 a	0.628 a
Leaf Cu (mg kg^{-1})	57.25 b	57.50 b	62.25 ab	66.25 a
Root Cu (mg kg $^{-1}$)	136.0 b	163.5 a	156.5 a	130.0 b
Leaf Zn (mg kg-1)	21.5 a	19.8 a	22.8 a	26 a
Root Zn (mg kg^{-1})	147.72 a	147.75 a	121.00 a	68.75 b

[a] Means with the same letters in each row are not significantly different, LSD test ($P \leq 0.01$).

Increasing the concentration of K in NH_4^+ grown plants might be also due to the plant demands for increase in NH_4^+ assimilation via elevating of glutamine synthetase activity. Increase of glutamine synthetase activity by K has already been reported (Roosta and Schjoerring, 2008b). Low-level O_2 increased K concentration in the leaves. These results were the same as the results achieved by Sullivan *et al.* (2001) on soybean.

The results of this research showed that NH_4^+-fed plants had higher concentrations of Zn in the leaves and roots as compared to those of NO_3^--fed plants (Table 4). Increasing the concentration of Zn in plants fed with NH_4^+ has been reported in lettuce (Roosta and Schjoerring, 2007), spinach (Assimakopoulou, 2006), and azalea (Clark and *et al*., 2003). At the highest level of O_2 i.e. 4 mg L^{-1}, Zn concentration in the root decreased compared to the other O_2 concentrations (Table 5).

Compared to NO_3^--fed plants, the NH_4^+-fed plants had lower Cu concentration in the leaf and higher Cu in the root (Table 4). This is in agreement with the findings of Roosta and Schjoerring (2007) and Clark *et al.* (2003) who reported NH_4^+ application decreased Cu concentration in the leaves of cucumber and azalea, respectively. Leaf Cu concentration generally showed a trend toward increasing with increasing O_2 concentrations, while root Cu concentration varied widely across O_2 treatments (Table 5). Chorianopoulou and Bouranis (2004) observed that short-term lack of O_2 increases the Cu concentration in leaves and roots of water cress.

CONCLUSIONS

This study showed that overall growth of eggplant was significantly decreased by NH_4^+, whereas high levels of oxygen increased the vegetative growth. Both NO_3^-- and NH_4^+-fed plants had the greatest shoot and leaf fresh mass at 4 mg L^{-1} O_2 concentration and the smallest at concentration of 1 mg L^{-1} O_2. NH_4^+ application decreased the concentration of Mg, Ca, Cu, and Mn in plants, compared to NO_3^-. On the other hand, the increase in O_2 amount of nutrient solutions partly alleviated ammonium toxicity by increasing Mg, Ca, Cu, and Mn concentration in plants. Therefore, in floating hydroponic cultures, O_2 level and its distribution should be controlled and must not be lower than 4 mg L^{-1}.

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اثرات متقابل شکل نیتروژن و غلظت اکسیژن بر رشد و وضعیت تغذیه‌ای بادمجان در سیستم هیدروپونیک

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چکیده

تحقیقی گلخانه‌ای برای تعیین اثر شکل‌های نیتروژن و سطوح مختلف اکسیژن بر رشد و غلظت عناصر غذایی در بادمجان انجام شد. آزمایش بصورت فاکتوریل و در قالب طرح کاملاً تصادفی با دو فاکتور شکل نیتروژن ($Ca(NO_3)_2$ و $(NH_4)_2SO_4$) و سطوح اکسیژن (۰/۳±۱، ۰/۳±۲، ۰/۳±۳ و ۰/۳±۴ میلی‌گرم بر لیتر اکسیژن) انجام شد. نتایج نشان داد که کاربرد آمونیوم همه پارامترهای رشد رویشی اندازه‌گیری شده را کاهش داد، درصورتیکه سطوح بالای اکسیژن آنها را افزایش داد. کاربرد آمونیوم غلظت نیتروژن، فسفر، پتاسیم و روی را در برگها و روی و مس را در ریشه‌ها افزایش داد، درصورتیکه غلظت منیزیوم، کلسیم، مس، منگنز و سدیم را در برگ‌ها و کلسیم، منیزیوم، منگنز و سدیم را در ریشه‌ها کاهش داد. سطوح بالای اکسیژن میزان نیتروژن، منیزیوم، کلسیم، مس و منگنز را در برگ‌ها و میزان منگنز و سدیم را در ریشه‌ها افزایش داد، درصورتیکه غلظت پتاسیم برگ‌ها و فسفر و روی ریشه‌ها را کاهش داد. بر طبق نتایج بدست آمده افزایش میزان اکسیژن در محلول غذایی تا حدودی سمیت آمونیوم را در گیاه بادمجان کاهش داد. بنابراین، در کشت هیدروپونیک شناور، سطح اکسیژن و توزیع آن باید کنترل شده و میزان آن نباید پایین‌تر از ۴ میلی‌گرم بر لیتر باشد.