Supplementary Potassium Sustains Fruit Yield in Bell Pepper under High Ammonium Nutrition

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Abstract. The uptake of nitrogen (N) in nitrate or ammonium (NH_4^+) form affects physiological and metabolic processes and toxicity may develop in plants receiving high concentrations of NH_4^+ . The objective of the present study was to delineate the response of bell pepper plants to varying proportions of NH_4^+ combined with increasing concentrations of potassium (K) in the nutrient solution. Bell pepper plants were tolerant to moderate proportions of NH_4^+ (25% or less or 50% or less); however, higher proportions resulted in growth reduction. The application of higher K concentrations in the nutrient solution did not ameliorate the growth on vegetative plant parts; however, when K was increased to 9 mM, the yield was sustained even when 50% of total N was in the NH_4^+ form. Decreased shoot:root ratio and harvest index indicated that biomass accumulation was affected more in the shoot than in the root and in the fruit than in the shoot, respectively. There was a lower concentration of NH_4^+ in the roots compared with leaves, suggesting that the higher K concentration that resulted from the increased K in the nutrient solution was associated with NH_4^+ translocation through the xylem. A decrease in calcium and magnesium detected in leaves suggests an antagonistic relationship with NH_4^+ and K in the nutrient solution, which was correlated with the acidification of the growing medium. Higher yields when K was at 9 mM may be the result of the high photosynthetic rate and stomatal conductance (g_s) detected in plants fertigated with 25% of total N as NH_4^+ and the higher leaf water potential when the proportion of NH_4^+ was 50%. The biochemical composition of fruits was affected because both high NH_4^+ and increased K resulted in higher ethylene production, lipid peroxidation, superoxide dismutase activity, and carotenoids.

Nitrogen is a key component of most organic compounds found in plants, including proteins, amino acids, nucleic acids, and secondary metabolites (Marschner, 1995)

and accounts for $\approx 3\%$ of plant biomass on a dry mass basis. Plant N demand is often higher than that of other nutrients and N deficiency symptoms are frequently reported, resulting in reduced growth and marketability of plant material.

Plant N requirements are species-dependent and may vary within plant parts and the developmental phase. At optimum supplementation, N stimulates growth, delays senescence,

modifies plant morphology, increases protein synthesis, and chloroplast formation as well as the concentration of chlorophylls, carotenoids, vitamins, and lipids in leaves (Marschner, 1995). However, suboptimal or excess N levels may result in reduced growth.

Nitrate (NO_3^-) and NH_4^+ are the main sources of inorganic N absorbed by roots of higher plants. The uptake of either NO_3^- or NH_4^+ affects physiological and metabolic processes as a result of the difference in ion charge (Gerendás et al., 1997) and the proportion of carbohydrates used for plant growth or N metabolism. In that, NH_4^+ assimilation demands lower energy inputs because its oxidative state eliminates the need for reduction (Marschner, 1995). However, toxicity may develop in plants that receive high concentrations/proportions of NH_4^+ because it is reduced to ammonia (NH_3), which is deleterious for plant growth even at low concentrations (Gerendás et al., 1997). Ammonium toxicity has been attributed to reduced proton extrusion at low external pH, acidification of the cytosol, and growing media (Walch-Liu et al., 2000), reduced content of sugars in the roots, and induced deficiency of cations, particularly K (Marschner, 1995).

Potassium is a highly abundant cation in plants and has a significant effect on N nutrition. Potassium affects NO_3^- uptake by being a counteranion for NO_3^- translocation from the root to the shoot (Marschner, 1995). Potassium is also related to NH_4^+ because they share several similarities: charge type, diameter of hydrated ion, and effect on the electrical potential of cell membrane. High K concentrations, within the luxury consumption zone, have also been reported to have an antagonistic effect on the uptake of other cations such as calcium (Ca), magnesium (Mg), sodium, and NH_4^+ (Marschner, 1995). The objective of the present study was to delineate the response of *Capsicum annuum* L. cv. Dársena (bell pepper) plants, a species widely cultivated in protected agricultural and soilless systems in México, to varying proportions of NH_4^+ combined with increasing concentrations of K in the nutrient solution.

Materials and Methods

Cultural conditions and plant material.

The study was conducted under greenhouse conditions in north México (lat. $25^\circ 27' \text{ N}$, long. $101^\circ 02' \text{ W}$, 1610 m above sea level) starting in the Fall to Winter of 2009 and ending in spring to summer of 2010. Average maximum/minimum temperatures were $22/10^\circ \text{ C}$ for the fall to winter and $28/15^\circ \text{ C}$ for the spring to summer. Minimum/maximum relative humidity for the experimental duration averaged 45%/75%. Average photosynthetically active radiation (PAR) measured at solar noon was $460 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for the fall to winter and $501 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for the spring to summer.

Thirty-three-day old bell pepper plants (15 cm height) were transplanted (18 Sept. 2009) into rigid plastic 20.3-cm pots, one

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plant per container, and filled with 12 L sphagnum peatmoss (Sun Gro Horticulture Inc., Bellevue, WA) (pH 6.3). Plants were trellised with two stems and density was maintained at three one-plant containers per m² for the experiment duration. Senescent leaves were removed periodically throughout the study period.

Nutrient solutions. Nutrient solutions with 13 mM total N were prepared with varying proportions of NH₄⁺: 0% (control), 25%, 50%, and 75% (the remainder was completed with N in the NO₃⁻ form) and three concentrations of K: 6 (control), 9, and 12 mM. Phosphorus (P), Ca, Mg, and micronutrients were supplied at Hoagland's nutrient solution concentration (Hoagland and Arnon, 1938). The pH ranged from 5.5 to 6.0 and electrical conductivity from 2.2 to 3.3 dS·m⁻¹. An automated fertigation system was used for application of the nutrient solutions, consisting of a drip irrigation system (144 L·h⁻¹) and one emitter per pot dispensing 1 L·h⁻¹. During the vegetative phase (fall to winter), three 2-h irrigations were applied per week. In the reproductive phase (spring to summer), ≈3.5-h irrigations were applied on a daily

basis. Leaching fraction was maintained at ≈30% throughout the experiment.

Assessment of plant growth. At experiment termination, a sample of the growing media was taken at 10-cm depth from each container. Media samples were air-dried, thoroughly mixed with distilled water (1:2 v/v), allowed to equilibrate (60 min), and then filtered (Whatman Grade 1; 11 μm). Filtrate pH was determined (Model 430, WinLab potentiometer; Barloworld Scientific, U.K.). Plants were harvested and separated into roots, leaves, stems, and fruits. Vegetative plant parts were bagged and placed in an oven at 70 °C for 72 h before measuring dry mass (DM). Total yield was measured by harvesting fruits that met market quality when ripe (≈50% of fruit exterior had turned red) throughout the study period.

Photosynthetic parameters, transpiration rate, and leaf water potential. Photosynthetic rate, g_S, transpiration rate (LI-6200; LI-COR, Inc., Lincoln, NE), and water potential (Ψ_w) (Scholander Pressure Chamber; Soil Moisture Equipment Corp., Santa Barbara, CA) of young fully developed leaves (perpendicular to solar radiation) were measured at approximately

solar noon (at four different times, at 2-week intervals throughout the experiment) during the reproductive phase. Average PAR, air CO₂ concentration, and air temperature during the measurements of photosynthetic parameters were 475 μmol·m⁻²·s⁻¹, 362 ppm, and 26.5 °C, respectively. Such environmental conditions for measurements of photosynthetic parameters and leaf Ψ_w were maintained constant in all the samplings performed; therefore, statistical analysis of data was conducted on the average of the measurements for each replication.

Nutrient status. Concentration of N forms (NO₃⁻-N, NH₄⁺-N, and total N) and K were determined in young roots and leaves sampled during the summer. Samples were rinsed (twice) in distilled water, bagged, and dried (75 °C for 72 h); dried material was ground to pass a 40-mesh screen (Tekmar, Model A-10; Cole-Parmer Instrument, Chicago, IL). Nitrate and NH₄⁺ concentration were determined following Cataldo's (Cataldo et al., 1975) and Nessler's method (Krug et al., 1979). Total N was determined with the Kjeldhal procedure. Phosphorus, Ca, and Mg were determined in leaf tissues digested

Table 1. Growing medium pH and leaf growth parameters in bell pepper (*Capsicum annuum* L.) in response to increasing proportion of ammonium (NH₄⁺) and potassium (K) concentration in the nutrient solution.^z

NH ₄ ⁺ proportion (%)	Medium pH			Leaf area (cm ²)			Leaf dry mass (g/plant)			Specific leaf area (mg·cm ⁻²)		
	K concn (mM)			K concn (mM)			K concn (mM)			K concn (mM)		
	6	9	12	6	9	12	6	9	12	6	9	12
0	6.20	6.35	6.33	10,410	9,626	9,345	126	131	132	12.1	13.7	14.1
25	5.75	5.75	5.80	9,550	9,195	9,111	156	135	127	16.7	14.8	14.0
50	5.10	5.08	5.00	6,965	7,994	7,737	130	135	117	19.5	17.1	15.3
75	4.83	4.83	4.80	5,330	4,910	4,648	111	102	105	21.2	24.0	23.6
Trend ^y	C*	Q*	C*	L***	L***	Q*	Q*	Q**	L*	L**	L*	Q*
Main effects												
NH ₄ ⁺	P < 0.001			P < 0.001			P < 0.001			P < 0.001		
K	NS			NS			NS			NS		
Interaction	NS			NS			NS			NS		

^zTotal nitrogen in the nutrient solution was maintained at 13 mM and completed with nitrogen in the nitrate form.

^yL, Q, and C = linear, quadratic, and cubic effects, respectively.

NS, *, **, *** Nonsignificant and significant at P < 0.05, < 0.01, and < 0.001, respectively.

Table 2. Shoot and root growth parameters and harvest index in bell pepper (*Capsicum annuum* L.) in response to increasing proportion of ammonium (NH₄⁺) and potassium (K) concentration in the nutrient solution.^z

NH ₄ ⁺ proportion (%)	Shoot dry mass (g/plant)			Root dry mass (g/plant)			Shoot:root ratio (g·g ⁻¹)			Harvest index ^y (g·g ⁻¹)		
	K (mM)			K (mM)			K (mM)			K (mM)		
	6	9	12	6	9	12	6	9	12	6	9	12
0	382	407	427	34.5	46.0	34.3	11.7	8.9	12.9	5.68	5.36	5.04
25	453	438	386	49.7	51.2	56.4	9.2	9.1	7.1	4.93	5.67	4.63
50	365	396	347	52.5	44.4	43.8	7.0	9.0	7.0	4.89	5.50	4.58
75	313	298	267	46.9	42.1	31.3	6.7	7.2	8.6	5.08	4.38	4.15
Trend ^x	L*Q*	L**Q*	L***	L*Q*	NS	Q**	L**	NS	L**Q**	NS	L**Q**	L*
Main effects												
NH ₄ ⁺	P < 0.001			P < 0.001			P < 0.001			P < 0.001		
K	NS			NS			NS			P < 0.002		
Interaction	NS			NS			P < 0.02			NS		

^zTotal nitrogen in the nutrient solution was maintained at 13 mM and completed with nitrogen in the nitrate form.

^yFruit yield/shoot dry mass.

^xL, Q, and C = linear, quadratic, and cubic effects, respectively.

NS, *, **, *** Nonsignificant and significant at P < 0.05, < 0.01, and < 0.001, respectively.

(2:1 mixture of $\text{H}_2\text{SO}_4\text{:HClO}_4$ and 2 mL of 30% H_2O_2) with an inductively coupled plasma emission spectrometer (Model Liberty; VARIAN, Santa Clara, CA).

Lipid peroxidation, antioxidant activity, respiration, ethylene production, and carotenoids in fruits. In selected treatments (control, 0% NH_4^+ + 9 mM K, 25% NH_4^+ + 9 mM K, and 50% NH_4^+ + 9 mM K), three fruits per replication were sampled at experiment termination. Lipid peroxidation was measured by quantifying the concentration of malondialdehyde using the thiobarbituric acid method (Kuk et al., 2003). Superoxide dismutase (SOD) and ascorbate peroxidase activity were determined according to Bonnet and Veisseire (2000) and Kuk et al. (2003), respectively, and the results expressed as units, where one (1) enzyme activity unit corresponds to an increase in 0.001 in absorbance per minute. Fruit ethylene and CO_2 release were measured by maintaining a fruit in a tightly closed container for 1 h at room temperature ($\approx 24^\circ\text{C}$), after which a sample of headspace gas was drawn and measured (Varian 3400; Varian Associates, Walnut Creek, CA). Concentration of total carotenoids was determined using the spectrophotometric methods described by Lichtenthaler (1989).

Statistical analysis. Four replicates of each nutrient solution were distributed in a complete randomized block factorial design. There was one plant per container and three containers as a single replicate. Plant responses to the NH_4^+ proportion and K concentrations were analyzed with SAS (Version 8.0; SAS Institute, Cary, NC) using analysis of variance (ANOVA) and linear, quadratic, and cubic trend analysis. Lipid peroxidation, antioxidant activity, respiration, ethylene production, and carotenoids in fruits were analyzed with ANOVA and means comparison with Tukey's procedure ($P < 0.05$).

Results

Substrate pH in plants fertigated with 0% NH_4^+ (Table 1) was comparable to that of the initial pH at the start of the experiment; however, increasing the proportion of NH_4^+ in the nutrient solution increased substrate acidification. Averaged across K concentrations, increasing the proportion of NH_4^+ reduced leaf area (Table 1), leaf DM (Table 1), shoot DM (Table 2), and the shoot:root ratio (Table 2) when NH_4^+ was 50% or greater, 75%, 50% or greater, and 25% or greater, respectively, whereas root DM (Table 2) increased when NH_4^+ was 25% to 50%. Specific leaf area (Table 1) exhibited a contrasting response because a significant increase was observed when plants were fertigated with solutions containing the highest proportion of NH_4^+ . Regardless of the proportion of NH_4^+ , K had no effect on substrate pH or growth parameters (Tables 1 and 2).

Similar to other growth parameters, fruit yield decreased when the proportion of NH_4^+ increased; however, the effect of NH_4^+ was

dependent on the concentration of K (Fig. 1). At 6 mM K, yield was unaffected by NH_4^+ at 25%; however, higher NH_4^+ proportions resulted in a reduction in yield at this K concentration. Increasing K to 9 mM resulted in increased tolerance to NH_4^+ as yield increased

when NH_4^+ was 25%, and it was comparable to that of the control plants when NH_4^+ was 50% (Fig. 1). Potassium at 12 mM was associated with a linear decrease in yield as NH_4^+ increased. Harvest index was in general decreased by increasing NH_4^+ , except when it

Fig. 1. Fruit yield of bell pepper (*Capsicum annuum* L.) plants in response to increasing proportion of ammonium (NH_4^+) and potassium (K) concentration in the nutrient solution. Total nitrogen in the nutrient solution was maintained at 13 mM and completed with nitrogen in nitrate form. Each point represents the average of four replications with three plants each and bars are the SEM ($n = 4$). Letters L and Q are the linear and quadratic trends that were significant at $**P < 0.01$ or $***P < 0.001$, respectively.

Fig. 2. (A–D) Physiological responses of bell pepper (*Capsicum annuum* L.) plants as affected by increasing proportion of ammonium (NH_4^+) and potassium (K) concentration in the nutrient solution. Total nitrogen in the nutrient solution was maintained at 13 mM and completed with nitrogen in nitrate form. Each point represents the average of four replications with three plants each and bars are the SEM ($n = 4$). Letters L, Q, and C are the linear, quadratic, and cubic trends that were significant at $*P < 0.05$, $**P < 0.01$, or $***P < 0.001$, respectively.

was at 25% and 50% and combined with 9 mM K (Table 2).

Photosynthetic rate decreased at high proportions of NH_4^+ (50% or greater) (Fig. 2A). However, like with fruit yield (Fig. 1), K modified the photosynthetic response to NH_4^+ (Fig. 2A). Increasing K to 9 mM resulted in the highest photosynthetic rate when NH_4^+ was at 25%. Potassium at 6 mM was associated with a decrease in photosynthetic rate when NH_4^+ was 25% or greater, whereas at 9 and 12 mM K, photosynthetic rate decreased when NH_4^+ was 50% or greater. Osmotic potential exhibited a similar response to photosynthetic rate (Fig. 2B). Transpiration rate in plants irrigated with solutions containing 9 or 12 mM K was higher when NH_4^+ was 25% to 50% or 25% or greater, respectively (Fig. 2C), and leaf ψ_w decreased when NH_4^+ was 50% or greater; however, this decrease was less pronounced when the solution contained supplementary K at 9 and 12 mM (Fig. 2D).

Ammonium concentration was higher in leaves than in roots and increased as NH_4^+ increased in the nutrient solution (Fig. 3A–B). Leaf NH_4^+ was higher when K was at 9 mM, whereas in roots, there was no K effect. Root NO_3^- concentration was higher than in leaves and was unaffected by K in the nutrient solution (Fig. 3C–D). Increasing NH_4^+ in the nutrient solution resulted in increased leaf NO_3^- regardless of K concentration, whereas in the roots, there was a decrease. In general, total N increased in leaves and roots as NH_4^+ in the nutrient solution increased regardless of K concentration (Fig. 3E–F).

Plants irrigated exclusively with NO_3^- -N had a higher leaf (Fig. 4A) and root K (Fig. 4B) concentration when the nutrient solution contained 9 to 12 mM or 12 mM K, respectively. Increasing NH_4^+ caused a decrease in leaf K when in the nutrient solution K was at 6 and 9 mM; however, when the nutrient solution contained 12 mM K, a decrease in leaf K was detected when NH_4^+ was at 75%. Nonetheless, when the nutrient solution contained 9 mM K, leaf K concentration was similar to that of plants in the control solution regardless of NH_4^+ proportion (Fig. 4A). Plants irrigated with nutrient solutions containing either 6 or 12 mM K exhibited decreased root K when NH_4^+ was 50% or greater or 75%, respectively; however, plants irrigated with 9 mM of K showed no NH_4^+ effect (Fig. 4B). Plants irrigated with 0% NH_4^+ had decreased leaf P (Fig. 5A), Ca (Fig. 5B), and Mg (Fig. 5C) as K increased in the nutrient solution, whereas increasing NH_4^+ was associated with a decrease in leaf P, Ca, and Mg.

Compared with plants irrigated with no NH_4^+ and 6 mM K (control solution), increasing K from 6 to 9 mM resulted in increased ethylene release and carotenoid concentration in fruit tissues (Table 3). Increased NH_4^+ in the nutrient solution increased ethylene production, had no effect on carotenoid concentration, and decreased ascorbate peroxidase activity. Fruit CO_2 production was significantly reduced when K was increased from 6 to 9 mM and no NH_4^+ was included in the

Fig. 3. (A–F) Leaf (left) and root (right) ammonium (NH_4^+), nitrate (NO_3^-), and total nitrogen (N) concentration in bell pepper (*Capsicum annuum* L.) plants as affected by increasing proportion of NH_4^+ and potassium (K) concentration in the nutrient solution. Total nitrogen in the nutrient solution was maintained at 13 mM and completed with nitrogen in nitrate form. Each point represents the average of four replications with three plants each and bars are the SEM ($n = 4$). Letters L, Q, and C are the linear, quadratic, and cubic trends that were significant at * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$, respectively.

nutrient solution (Table 3). Increasing NH_4^+ in the nutrient solution resulted in a significant increase in CO_2 production. Superoxide dismutase activity was unaffected by increasing K in the nutrient solution; however, when NH_4^+ was 50% of total N, a significant increase in the enzymatic activity was detected (Table 3). Lipid peroxidation activity was slightly decreased by increased K in nutrient solutions with 0 NH_4^+ or NH_4^+ at 25%; however, further increase in NH_4^+ resulted in increased peroxidation activity (Table 3).

Discussion

Bell pepper plants were tolerant to moderate proportions of NH_4^+ as leaf area and shoot DM were unaffected when NH_4^+ was 25% or less, and leaf DM was unaffected when NH_4^+ was 50% or less; however, higher

proportions of NH_4^+ in the nutrient solution resulted in growth reduction. Fruit production exhibited a similar tendency because NH_4^+ at 25% or less did not affect yield; however, NH_4^+ at 50% or greater was detrimental. The negative effect of high proportions of NH_4^+ on the growth of bell pepper plants was associated with substrate acidification, as reported by Walch-Liu et al. (2000). In our study, when the substrate pH was less than 5.1, a linear decrease was detected for all growth parameters regardless of K concentration. Rhizosphere acidification is the result of NH_4^+ root uptake through a proton-cation antiporter, resulting in H^+ extrusion (Marschner, 1995).

The application of higher K concentrations in the nutrient solution did not ameliorate the growth of vegetative plant parts; however, when K was increased to 9 mM,

Fig. 4. (A–B) Leaf and root potassium (K) concentration in bell pepper (*Capsicum annuum* L.) plants as affected by increasing proportion of ammonium (NH_4^+) and K concentration in the nutrient solution. Total nitrogen in the nutrient solution was maintained at 13 mM and completed with nitrogen in nitrate form. Each point represents the average of four replications with three plants each and bars are the SEM ($n = 4$). Letters L, Q, and C are the linear, quadratic, and cubic trends that were significant at $*P < 0.05$, $**P < 0.01$, or $***P < 0.001$.

bell peppers plants exhibited increased tolerance to NH_4^+ as yield was sustained at 50% of total N in the NH_4^+ form. Thus, increased K in the nutrient solution protected plants from the toxic effects of NH_4^+ , as reported in cucumber (*Cucumis sativus* L.) (Roosta and Schjoerring, 2008; Szczerba et al., 2006).

Increasing K in the nutrient solution was associated with a 15% increase in fruit yield when NH_4^+ was 25% of total N and K was 9 mM, which is in agreement with results reported in tomato (*Solanum lycopersicum* L.) in which 10% of total N in NH_4^+ form was associated with a 15% increase in fruit yield (Siddiqi et al., 2002). This enhanced yield when both N forms are supplemented has been attributed to the lower energy cost of NH_4^+ assimilation and its proportion is not high enough to impose toxic effects on plant growth.

Decreased shoot:root ratio and harvest index indicate that biomass accumulation was more affected in the shoot than in the root and in the fruit than in the shoot, respectively, suggesting that NH_4^+ promoted preferential biomass allocation such that root > shoot > fruit in this study. This preferential biomass allocation was corroborated by the fact that NH_4^+ at 25% to 50% resulted in a higher root DM. Furthermore, increased specific leaf area with high NH_4^+ suggests that leaf expansion was more affected than leaf biomass accumulation, which was probably associated with the reduction in leaf ψ_w . Decreased leaf area and biomass allocation may be connected to the lower K concentration in leaves and roots with high NH_4^+ proportions in the nutrient solution as a result of competition between both cations for uptake sites (Hoopen et al., 2010). The lower leaf ψ_w suggests an impairment of the roles of K in osmoregulation and transport of water (Clarkson et al., 2000) and photoassimilates (Cakmak et al., 1994), affecting the source–sink relationships within the plant.

Increasing K in the nutrient solution to 9 mM resulted in increased leaf and root K

when compared with control plants at 6 mM. This would explain the increased harvest index resulting from an enhanced water movement and biomass allocation to the fruit, instead of to the shoot, when NH_4^+ was at 25% to 50%. Furthermore, the shoot:root ratio was unaffected by K in the nutrient solution regardless of increased root K concentration at higher K supplementation. We suggest that the higher K in the roots may have increased the translocation of photoassimilates from the shoot to the root, allowing root DM to increase. Decreased root biomass accumulation at the highest K and NH_4^+ supplemented in the nutrient solution may be the result of the unexpected decrease in root K concentration, resulting in less translocation of photoassimilates (Ganmore-Newmann and Kafkafi, 1983) or in an “uncontrolled” rate of passive NH_4^+ uptake, which depleted root reserves, as suggested by the increase in NH_4^+ concentration in root tissues in our study.

In plants exclusively fed with NO_3^- (0% NH_4^+), the presence of NH_4^+ in plant tissues was the result of NO_3^- reduction to NH_4^+ in the roots, which was then transported to the leaves. The lower concentration of NH_4^+ in the roots compared with leaves suggests that the higher K concentration that resulted from the increased K in the nutrient solution, especially when K was at 9 mM, was associated with NH_4^+ translocation through the xylem as a result of enhanced water transport.

High proportions of NH_4^+ resulted in decreased root NO_3^- and increased root and leaf NH_4^+ . However, the linear increase in leaf NO_3^- regardless of the decreased NO_3^- availability suggests that: 1) roots efficiently absorbed and transported NO_3^- to the leaves; and/or 2) the activity of the nitrate reductase was impaired as a result of a high NH_4^+ proportion, as reported by Claussen and Lenz (1999).

Plants irrigated with 6 mM K exhibited increasing total N in the leaves as NH_4^+ in the nutrient solution was increased; however, the increase in total N in plants with 9 or 12 mM K

Fig. 5. (A–C) Leaf phosphorus (P), calcium (Ca), and magnesium (Mg) concentration in bell pepper (*Capsicum annuum* L.) plants as affected by increasing proportion of ammonium (NH_4^+) and potassium (K) concentration in the nutrient solution. Total nitrogen in the nutrient solution was maintained at 13 mM and completed with nitrogen in nitrate form. Each point represents the average of four replications with three plants each and bars are the SEM ($n = 4$). Letters L, Q, and C are the linear, quadratic, and cubic trends that were significant at $*P < 0.05$, $**P < 0.01$, or $***P < 0.001$, respectively.

was less pronounced. Furthermore, the higher total N concentration was not associated with higher yield, suggesting that most of the N accumulation was the result of excessive NH_4^+ uptake, which when reduced to NH_3 is toxic and/or affected intracellular pH (Marschner, 1995). Increasing external K to 9 mM did not result in increased total N in the leaves; however, it resulted in increased fruit yield even at high proportions of NH_4^+ (25% to 50% of total N). Similar results have been reported in cucumber by Roosta and Schjoerring (2008). We suggest that increased K ameliorated the response of bell peppers by competing with NH_4^+ , which is supported by the highest leaf and root K concentrations

Table 3. Biochemical and physiological responses of bell pepper (*Capsicum annuum* L.) fruits to increasing proportion of ammonium (NH_4^+) and potassium (K) concentration in the nutrient solution (all units on a fresh weight basis).^z

NH_4^+ (%)	K concn (mM)	Ethylene ^y ($\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$)	Fruit respiration ($\text{mL CO}_2/\text{kg}^{-1}\cdot\text{h}^{-1}$)	Lipid peroxidation ($\mu\text{mol}\cdot\text{g}^{-1}$)	Carotenoids ($\mu\text{g}\cdot\text{g}^{-1}$)	Ascorbate peroxidase ($\text{U}\cdot\text{g}^{-1}$)	Superoxide dismutase ($\text{U}\cdot\text{g}^{-1}$)
0	6	25.4 b	15.0 a	48.1	38.3 b	86.1	10.1
0	9	42.2 a	10.7 b	40.9	58.0 a	105.6	8.2
ANOVA		$P < 0.019$	$P < 0.050$	NS	$P = 0.035$	NS	NS
0	9	42.2 b	10.7 b	40.9 ab	58.0	105.6 a	8.2 b
25	9	38.4 b	14.4 a	36.1 b	43.3	53.6 b	10.9 ab
50	9	51.8 a	14.7 a	48.6 a	45.8	63.0 b	19.9 a
ANOVA		$P < 0.021$	$P < 0.001$	$P = 0.043$	NS	$P < 0.002$	$P < 0.022$

^zTotal nitrogen in the nutrient solution was maintained at 13 mM and completed with nitrogen in the nitrate form.

^yMeans with the same letter are nonsignificant (NS) according to Tukey's test.

ANOVA = analysis of variance.

when 0% NH_4^+ was used in the nutrient solution. Similar results have been reported in barley (Szczerba et al., 2006). In our study, when compared with the control solution, plants irrigated with solutions containing 9 mM K exhibited similar leaf K when NH_4^+ in the nutrient solution was 25% to 75%, whereas in the root, K concentration was unaffected by NH_4^+ . We suggest that the potential K deficiency resulting from NH_4^+ competition was ameliorated by the supplemental addition of external K. The K- NH_4^+ antagonism has been suggested to be the result of direct competition for uptake sites in the plasma membranes (Hoopen et al., 2010) and/or an inhibitory action of NH_4^+ on the high-affinity K carriers (Balkos et al., 2010).

Decreased leaf Ca and Mg in our study suggest that there is an antagonistic relation with NH_4^+ and K in the nutrient solution. Kawasaki (1995) reported that in rice (*Oryza sativa* L.), barley, maize (*Zea mays* L.), tomato, and cucumber, there was a lower Ca content when plants were fertigated with NH_4^+ . Similarly, Clark et al. (2003) reported that in *Rhododendron canescens* (Michaux) and *R. austrinum* (Small) (azalea), NO_3^- nutrition was associated with increased accumulation of Ca. The inhibitory effect on Ca and Mg has been suggested to be the result of cation-cation competition between K and NH_4^+ (Marschner, 1995); however, in the present study, decreased leaf Ca and Mg concentration at high proportions of NH_4^+ was associated with the acidification of the substrate as demonstrated by the correlation with the decrease in leaf Ca ($\text{Ca} = 123\text{pH} - 424$, $R^2 = 0.522$, $n = 48$) and Mg ($\text{Mg} = 66.3\text{pH} - 168$, $R^2 = 0.546$, $n = 48$) concentration.

Higher yields of bell peppers when NH_4^+ was at 25% to 50% and K at 9 mM may be the result of high photosynthetic rate and g_s detected in plants fertigated with 25% of total N as NH_4^+ and the higher leaf ψ_w when the proportion of NH_4^+ was 50%. The higher photosynthetic rate may have been the result of the higher g_s , which allowed gas exchange at normal/unstressed rates, photoenergy-saving (Guo et al., 2007), lower energy cost of N assimilation (Marschner, 1995), higher concentration, activity, and regeneration rate

of Rubisco (Guo et al., 2007). The slight decrease in leaf ψ_w and high g_s in our study were probably the result of the comparatively limited decrease in leaf K that resulted from the competition with increasing NH_4^+ in the nutrient solution. Supplementary K in the nutrient solution resulted in optimal/unstressed leaf K, which, when transported from the leaves, allowed maximum cell expansion and yield, thus maintaining high yields despite the high proportions of NH_4^+ .

In the present study, NH_4^+ and K at the highest concentrations were not connected with increased photosynthetic rate, which was probably the result of competition with other ions (such as Ca, Mg, and P), that negatively affected photosynthesis and other metabolic processes. The decreased Mg concentration in leaves may also be responsible for the lower photosynthetic rate in plants with high NH_4^+ and K concentrations in the nutrient solution, because it is a component of the chlorophyll molecule and has an important role in the activity of the Mg-chelatase, the enzyme that catalyzes biosynthesis of chlorophyll (Walker and Weinstein, 1991). Magnesium deficiency is also associated with photosynthesis decline because it is a component of Rubisco, which in turn enhances the generation of reactive oxygen species (Cakmak and Marschner, 1992).

The biochemical composition of bell pepper fruit was affected by the proportion of NH_4^+ and K in the nutrient solution. Other negative effects of high NH_4^+ that have been reported include ethylene synthesis (Britto and Kronzucker, 2002). Our results indicate that both high NH_4^+ and increased K (9 mM) resulted in higher ethylene production in fruit, which may have affected their development because they were of larger diameter and length when compared with other treatments (data not shown). Furthermore, moderate levels of NH_4^+ (25%) were associated with a lower ethylene production in fruit when compared with that from plants irrigated with solutions containing 50% NH_4^+ . Ethylene synthesis has been associated with abiotic stress such as the acidic pH in the substrate resulting from irrigation with nutrient solutions high in NH_4^+ . Along with the acidic pH, excessive NH_4^+ resulted in other

abiotic stresses, including modification of nutrient uptake and water status, as demonstrated by the decreased leaf ψ_w and Ca and Mg concentrations in our study.

Lipid peroxidation activity in fruit was reduced by increasing K from 6 to 9 mM when 0% NH_4^+ was provided; however, increasing NH_4^+ to 50% of total N was associated with an increase in peroxidation activity. Our results are in agreement with Gerendás et al. (1997), who reported that high concentrations of NH_4^+ cause damage to the cell membrane integrity and antioxidant system. The increase in lipid peroxidation suggests that excess NH_4^+ stimulated the production of reactive oxygen species (ROS), which in turn affected cell membrane integrity (Bhattacharjee, 2005). Enhanced uptake of loosely bound iron may be responsible for initiating lipid peroxidation as a result of high NH_4^+ , inducing an acidic pH (Bhattacharjee, 2005). Decreased CO_2 fixation associated with decreased photosynthetic rate observed under high NH_4^+ may also be responsible for the activation of O_2 . Plant responses to increased production of ROS include the activation of the antioxidative system, including SOD and ascorbate peroxidase (Kuzniak and Skłodowska, 2001). In our study, high proportions of NH_4^+ (25% to 50%) increased the activity of SOD and carotenoids, suggesting that bell pepper fruit exhibited increased antioxidant capacity in response to NH_4^+ stress.

Conclusions

Excessive NH_4^+ nutrition resulted in decreased plant growth and yield. Potassium did not ameliorate the plant response to excess NH_4^+ in the vegetative plant parts; however, increasing K in the nutrient solution to 9 mM allowed bell pepper plants to tolerate up to 50% of total N as NH_4^+ because fruit yield was unaffected. Ammonium at high proportions modified biomass allocation, giving preference to root over shoot and shoot over fruit. An antagonistic relationship between NH_4^+ and K was observed and between NH_4^+ and K with Ca, Mg, and P. We suggest that supplementary K enhanced plant tolerance to excessive proportions of NH_4^+ by: 1) reducing root uptake rates of NH_4^+ , probably through competition for uptake sites; 2) maintaining water relations by sustaining optimal levels of K in plant tissues; 3) enhancing photosynthesis rate and g_s ; and 4) modifying biomass allocation to the fruit. Excessive NH_4^+ affected fruit quality by enhancing lipid peroxidation activity and ethylene synthesis; however, fruits exhibited an increased activity of SOD and carotenoid synthesis, both of which are components of the antioxidant system.

Literature Cited

- Balkos, K.D., D.T. Britto, and H.J. Kronzucker. 2010. Optimization of ammonium acquisition and metabolism by potassium in rice (*Oryza sativa* L. cv. IR-72). *Plant Cell Environ.* 33: 23–34.

- Bhattacharjee, S. 2005. Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plants. *Curr. Sci.* 89:1113–1121.
- Bonnet, M.O.C. and P. Veisseire. 2000. Effects of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perenne* L. cv Apollo). *J. Expt. Bot.* 51:945–953.
- Britto, D. and H. Kronzucker. 2002. NH_4^+ toxicity in higher plants: A critical review. *J. Plant Physiol.* 159:567–584.
- Cakmak, I., C. Hengeler, and H. Marschner. 1994. Changes in phloem export of sucrose in leaves in response to phosphorus, potassium and magnesium deficiency in bean plants. *J. Expt. Bot.* 45:1251–1257.
- Cakmak, I. and H. Marschner. 1992. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. *Plant Physiol.* 98:1222–1227.
- Cataldo, D.A., M. Horoon, L.E. Schrader, and V.L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil Sci. Plant Anal.* 6:71–86.
- Clark, M.B., H.A. Mills, C.D. Robacker, and J.G. Latimer. 2003. Influence of nitrate:ammonium ratios on growth and elemental concentration in two azalea cultivars. *J. Plant Nutr.* 26:2503–2520.
- Clarkson, D.T., M. Carvajal, T. Henzler, R.N. Waterhouse, A.J. Smyth, D.T. Cooke, and E. Steudle. 2000. Root hydraulic conductance: Diurnal aquaporin expression and the effects of nutrient stress. *J. Expt. Bot.* 51:61–70.
- Claussen, W. and F. Lenz. 1999. Effect of ammonium or nitrate nutrition on net photosynthesis growth and activity of the enzymes nitrate reductase and glutamine synthetase in blueberry, raspberry and strawberry. *Plant Soil* 208:95–102.
- Ganmore-Newmann, R. and U. Kafafi. 1983. The effect of root temperature and $\text{NO}_3^-/\text{NH}_4^+$ ratio on strawberry plants. I. Growth, flowering and root development. *Agron. J.* 75:941–947.
- Gerendás, J., Z. Zhu, R. Bendixen, R. Ratcliffe, and B. Sattelmacher. 1997. Physiological and biochemical processes related to ammonium toxicity in higher plants. *J. Plant Nutr. Soil Sci.* 160:239–251.
- Guo, S., Y. Zhou, Q. Shen, and F. Zhang. 2007. Effect of ammonium and nitrate nutrition on some physiological processes in higher plants—g, photosynthesis, photorespiration, and water relations. *Plant Biol.* 9:21–29.
- Hoagland, D.R. and D.J. Arnon. 1938. The water culture method for growing plants without soil. University of California, Berkeley, CA. *Agr. Expt. Sta. Circ.* 347. p. 1–39.
- Hoopen, F., T.A. Cuin, P. Pédas, J.N. Hegelund, S. Shabala, J.K. Schjoerring, and T.P. Jahn. 2010. Competition between uptake of ammonium and potassium in barley and Arabidopsis roots: Molecular mechanisms and physiological consequences. *J. Expt. Bot.* 61:2303–2315.
- Kawasaki, T. 1995. Metabolism and physiology of calcium and magnesium, p. 412–419. In: Matsuo, T., K. Kumazawa, R. Ishii, K. Ishihara, and H. Hirata (eds.). *Science of the rice plant*. Food and Agricultural Policy Research Center, Tokyo, Japan.
- Krug, F.J., J. Ruzicka, and E.H. Hansen. 1979. Determination of ammonia in low concentrations with Nessler's reagent by flow injection analysis. *Analyst (Lond.)* 104:47–54.
- Kuk, Y., J.S. Shin, N.R. Burgos, T.E. Hwang, O. Han, B.H. Cho, S. Jung, and J.O. Guh. 2003. Antioxidative enzymes offer protection from chilling damage in rice plants. *Crop Sci.* 43:2109–2117.
- Kuzniak, E. and M. Skłodowska. 2001. Ascorbate, glutathione and related enzymes in chloroplasts of tomato leaves infected by *Botrytis cinerea*. *Plant Sci.* 160:723–731.
- Lichtenthaler, H.K. 1989. Chlorophylls and carotenoids: Pigments of photosynthetic membranes. *Methods Enzymol.* 148:350–382.
- Marschner, H. 1995. Mineral nutrition of higher plants. 2nd Ed. Academic Press, London, UK.
- Roosta, H.R. and J.K. Schjoerring. 2008. Effects of nitrate and potassium on ammonium toxicity in cucumber plants. *J. Plant Nutr.* 31:1270–1283.
- Siddiqi, M.Y., B. Malhotra, X. Min, and A.D.M. Glass. 2002. Effects of ammonium and inorganic carbon enrichment on growth and yield of a hydroponic tomato crop. *J. Plant Nutr. Soil Sci.* 165:191–197.
- Szczerba, M.W., D.T. Britto, and H.J. Kronzucker. 2006. Rapid, futile K^+ cycling and pool-size dynamics define low-affinity potassium transport in barley. *Plant Physiol.* 141:1494–1507.
- Walch-Liu, P., G. Neumann, F. Bangerth, and C. Engels. 2000. Rapid effects of nitrogen form on leaf morphogenesis in tobacco. *J. Expt. Bot.* 51:227–237.
- Walker, C.J. and J.D. Weinstein. 1991. Further Characterization of the magnesium chelatase in isolated developing cucumber chloroplasts. *Plant Physiol.* 95:1189–1196.