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MODELING GROWTH AND ION CONCENTRATION OF LILIUM IN RESPONSE TO NITROGEN:POTASSIUM:CALCIUM MIXTURE SOLUTIONS

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□ *Nutrient solution composition plays an important role in root uptake rate due to interactions among nutrients and internal regulation. Studies to determine the optimum nutrient solution concentration are focused on individual ions, ignoring the adaptation mechanisms triggered by plants when growing in a varying external nutrient concentration. The objective of the present study was to determine the response in growth and tissue ion concentration of lilium cv. 'Navona' to nutrient mixtures of varying proportions of nitrogen (N), potassium (K^+), and calcium (Ca^{2+}) in solution using mixture experiments methodology in order to determine the optimum concentration. Bulbs of lilium were transplanted in plastic crates and drip-irrigated with the treatment solutions, which consisted of a mixture of N, K^+, and Ca^{2+} whose total concentration was 340 mg L^{-1} and minimum concentrations of each ion was 34 mg L^{-1}. Chlorophyll concentration (SPAD), shoot fresh weight (FW), leaf FW, and leaf area were measured 60 days after transplanting and ion analysis was performed on shoot tissues from selected treatments. Lilium exhibited a moderate demand for N and K^+ (136–170 mg L^{-1} N and 116–136 mg L^{-1} K^+) and a very low demand for Ca^{2+} (34–88 mg · L^{-1}). This low demand may be due to the remobilization of the nutrients stored in the bulbs. Integrating the predictions of the models estimated to produce $> 90\%$ of maximum growth, the optimum nutrient solution should contain Ca^{2+} at a concentration between 34 and 126 mg · L^{-1}, K^+ between 119 and 211 mg · L^{-1}, and N between 92 mg · L^{-1} and 211 mg · L^{-1}. Increasing external N concentration affected internal N concentration but not internal K^+ or Ca^{2+} concentrations, despite that the increase in external N was associated with a decrease in external K^+ and Ca^{2+}. Similar trends were observed for external K^+ and Ca^{2+} concentration. In conclusion, lilium was able to maintain a relatively constant K^+ and Ca^{2+} concentration regardless of the lower concentration in the nutrient solution when N was increased (similar response was observed for K^+ and Ca^{2+}) and it has a low Ca^{2+} demand and moderate N and K^+ supply.*

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INTRODUCTION

Nutrient uptake is regulated by growth rate and nutrient availability. Actively growing plants usually exhibit a high nutrient uptake rate in order to meet demands for optimum growth. Optimum growth is a result of high carbon fixation in the leaves, which in turn supplies the roots with carbohydrates and energy for nutrient uptake. Internal nutritional status may also regulate uptake rate by the synthesis of signal molecules that are transported from the shoot to the root. For instance, regulation of nitrogen (N) uptake is based on the size of the soluble organic N pool in the plant (Soussana et al., 2002) and through amino acids and other organic acids that are translocated to the roots (Tillard et al., 1998; Tinker and Nye, 2000), resulting in increased N uptake (Touraine et al., 1994) to maintain plant growth. Uptake rate is also affected by external nutrient activity since plants under a specific nutrient deprivation show increased uptake rate when the deficient nutrient is supplied in the soil solution, even in diluted concentrations (Tinker and Nye, 2000). Uptake regulation is possible due to two systems of nutrient acquisition, the low and high affinity systems, by which the internal nutrient concentration may be independent of external concentrations (Mengel and Kirkby, 2001).

Ion interactions in the external solution also play a role in nutrient uptake rate. Some cations, for example ammonium (NH_4^+) and potassium (K^+), compete for root cell membrane uptake sites due to the similarity of their ionic charge and ion diameter (Tinker and Nye, 2000) and K^+ uptake is increased by the presence of calcium (Ca^{2+}), probably due to improved membrane integrity (Marschner, 1995). Compared to nitrate (NO_3^-), NH_4^+ tends to inhibit the uptake of K^+ and Ca^{2+}, probably due to competition for negative charges (Mengel and Kirkby, 2001). Nonetheless, perhaps for simplicity, most studies performed to determine optimum external concentration in soilless production systems are focused on individual nutrient assays. In subirrigated New Guinea impatiens, maximum growth was reported when N was at 8 mM (Kent and Reed, 1996), while phosphorus (P) was at 0.75 to 0.96 mM for shoot dry weight and flower number, respectively (Whitcher et al., 2005), and K^+ at 2 mM (Blessington-Haley and Reed, 2004). However, as previously indicated, uptake rate is influenced by internal and external factors, so that the acquisition rate of a single nutrient may be adjusted in response to the external concentration of other ions. The design of experiments in which the concentration of two or more nutrients is varied results in overwhelmingly high numbers of treatments. Mixture experiment methodology is an excellent statistical tool when dealing with components, i.e., the nutrients, which can be mixed in a blend, i.e. the nutrient solution (Cornell, 2002). This methodology can be applied to nutritional studies since the solutions would represent a mixture of two or three nutrients in which the total concentration of them is maintained constant, only varying their proportion, and therefore, the individual concentration of each ion. The response to the proportions of the nutrients can be modeled considering each nutrient individually (trace plots), in pairs (binary blends), or as a response surface in response to the single (one component), binary (two components), and tertiary blends (three components).

Asiatic lilium is one of the most important cut flower plants cultivated worldwide. Commercially, Asiatic lilium is grown in soil or soilless cultures, and usually nutrients are provided through fertigation. Nutritional studies with lilium are of interest because the plant stores nutrients and carbohydrates in the bulb, and this may affect the response to external nutrient concentration compared to non-geophyte plants. The objective of the present study was to determine the effect on growth and tissue nutrient concentration during the forcing phase of perlite-grown lilium cv. 'Navona' in response to mixtures of varying proportions of N, K^+, and Ca^{2+} in solution, using mixture experiments methodology, in order to determine the optimum proportion and concentration in a fertigation solution.

MATERIALS AND METHODS

The experiment was conducted under glasshouse conditions from 16 March to 15 May 2006. Average day and night temperature was 26.0°C and 15.0°C, respectively, and average maximum and minimum relative humidity was 70% and 40%, respectively. Bulbs of Asiatic lilium (*Lilium* L. hybrids) cv. 'Navona' (circumference = 12–14 cm) were immersed in a fungicide drench for 10 seconds and rinsed with plain water previous to transplant in plastic crates (36 × 65 × 50 cm). The crates had a 10 cm perlite layer on which 10 uniformly distributed bulbs were positioned. After placement of bulbs, roots were scattered and another layer of perlite was deposited to cover the bulbs plus 5 cm above the tip.

Plants were established for five days after which they were drip-irrigated with the treatment solutions. The treatment solutions were designed as a mixture experiment with three components (each ingredient present in the mixture and is expressed as a fraction) mixed in varying proportions (Cornell, 2002). Since the total concentration of the mixed components is maintained constant, the sum of all the fractions must be equal to one and the response of plants to the mixtures depends only on the proportions of the components. Treatment solutions consisted of a mixture of N, K^+, and Ca^{2+} whose total concentration (considered as 1) was 340 mg L^{-1}

TABLE 1 Mixtures of N : K^+ : Ca^{2+} designed using Design Expert version 6.0.4 and the respective concentration of each nutrient

	Nutrient concentration mg · L^{-1}		
N : K^+ : Ca^{2+} mixtures	N	K^+	Ca^{2+}
0.10 : 0.10 : 0.80	34.0	34.0	272.0
0.10 : 0.33 : 0.57	34.0	112.2	193.8
0.10 : 0.57 : 0.33	34.0	193.8	112.2
0.10 : 0.80 : 0.10	34.0	272.0	34.0
0.22 : 0.22 : 0.57	74.8	74.8	193.8
0.22 : 0.57 : 0.22	74.8	193.8	74.8
0.33 : 0.10 : 0.57	112.2	34.0	193.8
0.33 : 0.33 : 0.33	112.2	112.2	112.2
0.33 : 0.57 : 0.10	112.2	193.8	34.0
0.45 : 0.45 : 0.10	153.0	34.0	153.0
0.57 : 0.10 : 0.33	193.8	34.0	112.2
0.57 : 0.22 : 0.22	193.8	74.8	74.8
0.57 : 0.33 : 0.10	193.8	112.2	34.0
0.80 : 0.10 : 0.10	272.0	34.0	34.0

and the proportion at which they were incorporated into the mixture solutions was varied. The mixture solutions were designed using Design Expert version 6.0.4 (Stat Ease Inc., Minneapolis, MN, USA) to fit a special cubic model. Treatments with zero N, K^+, or Ca^{2+} in the mixtures were avoided by constraining the proportion to a minimum of 0.1 and a maximum of 0.8, that is, 34 mg L^{-1} minimum and 272 mg L^{-1} maximum. The resulting N:K^+:Ca^{2+} mixtures are shown in Table 1 along with the corresponding N, K^+, and Ca^{2+} concentrations. The response to the mixtures is represented in an equilateral triangle (Figure 1) in which each vertex corresponds to one component. The N, K^+, and Ca^{2+} coordinates begin with the mixture 0.1:0.45:0.45 and continue with the mixtures 0.33:0.33:0.33, 0.57:0.22:0.22, and 0.8:0.1:0.1 (Figure 1). In addition to N, K^+, and Ca^{2+}, all the nutrient solutions contained (in mg L^{-1}): 31 P, 48 magnesium (Mg^{2+}), 4.6 iron (Fe)—ethylenediaminetetraacetic acid (EDTA), 0.4 zinc (Zn), 0.3 molybdenum (Mo), 0.6 manganese (Mn), 0.1 boron (B), and 0.2 copper (Cu), and sulfur (S) (which varied between 16 and 26 mg · L^{-1}).

Plants were irrigated with the mixture solutions though a drip irrigation system designed to capture the leachate and re-use the solutions. Solution pH was maintained at 6.0 by additions of sulfuric acid (H_2SO_4) every other day and the solutions were renewed every 10 days. Municipal water (electrical conductivity = 0.76 dS m^{-1}) was used to prepare the mixture solutions and addition of fertilizer salts was adjusted according to the chemical composition of water to meet the concentrations and proportions under study. Each mixture treatment was replicated twice and was imposed five days after transplanting. Plants were harvested 60 days after transplanting when the first flower was blooming. Chlorophyll concentration was determined at

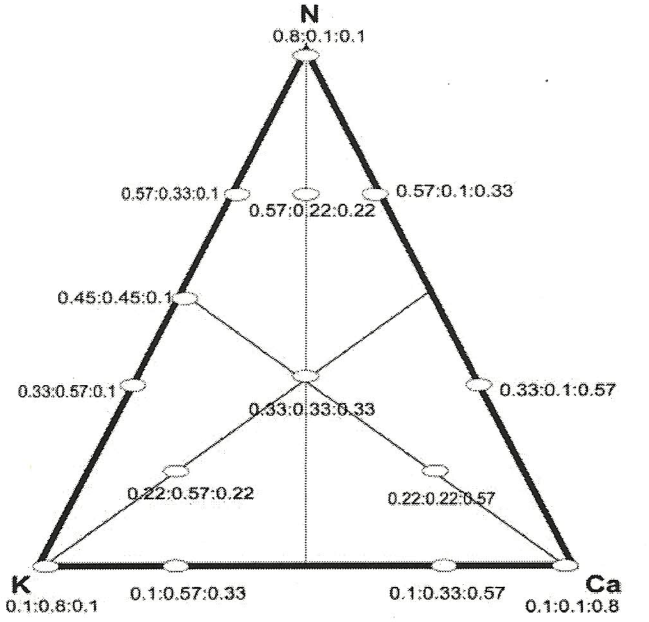

FIGURE 1 Design points corresponding to the mixtures of N : K^+ : Ca^{2+} evaluated in terms of proportion. Total concentration was 340 mg · L^{-1}. Fractions correspond to the N : K^+ : Ca^{2+} proportion in the mixture, in that order.

experiment termination with a SPAD meter (Model 501, Minolta Camera Co., LTD, Tokyo, Japan) from two leaves at the bottom, middle, and top portion of the stem. Shoot fresh weight (FW), leaf FW, and leaf area were also measured. Leaf area was measured with a Leaf Area Meter (LI-3100®; Li-Cor Inc., Lincoln, NE, USA). Shoots from selected treatments were dried for four days at 65°C and ground to pass a 40-mesh screen for tissue analysis, which included: N, K^+, Ca^{2+}, P, Mg^{2+}, B, Cu, Fe, Mn, Na, and Zn ($n = 3$). Shoot samples were digested in 4 mL of a mixture of sulfuric acid + perchloric acid (2:1) and 2 mL of 30% hydrogen peroxide. Nitrogen was determined by Kjedahl procedure and the reminding elements by inductively coupled plasma mass spectrometry. Data were analyzed with Design Expert to plot the response surface, calculate ANOVA and the models that best fit to the response. Model selection was based on significance, high R^2, and the model with fewer terms. Shoot nutrient concentration was statistically analyzed using SAS (SAS Institute, Cary, NC, USA) to obtain ANOVA and multiple comparison tests according to Tukey's procedure.

RESULTS

Shoot FW was significantly affected by the N : K^+ : Ca^{2+} mixtures (Figure 2). The N × K^+ interaction had a synergistic effect, despite that Ca^{2+} was at the lowest proportion (0.1), as indicated by the increased shoot FW when both were present in the mixtures (note the rise in the N × K^+

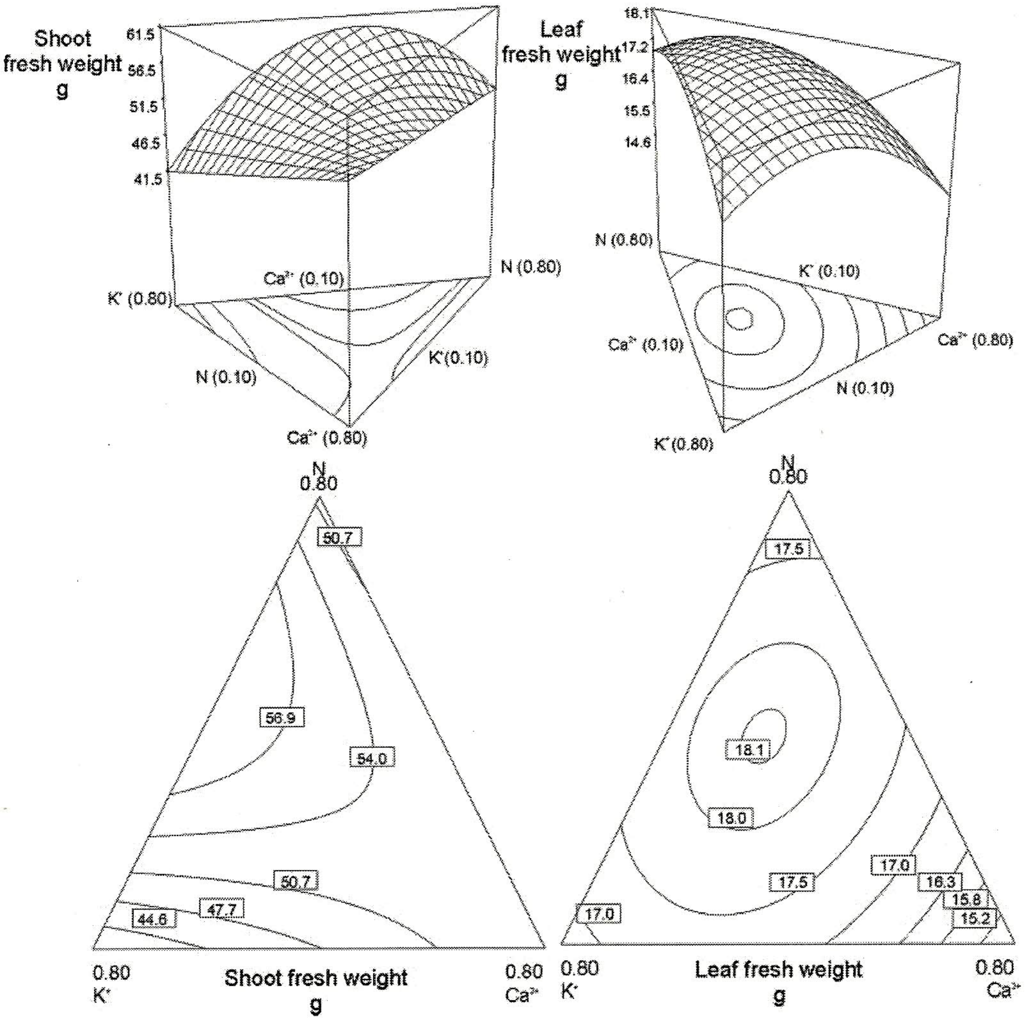

FIGURE 2 Response surface and counter-plots exhibiting the effect of $N : K^+ : Ca^{2+}$ mixtures in nutrient solutions used to irrigate lilium cv. Navona on shoot fresh weight (SFW) and leaf fresh weight (LFW). The models were: $SFW = 40.47N + 28.38K + 57.01Ca + 113.36N \times K$ ($P = 0.005$, $R^2 = 0.743$, Adj. $R^2 = 0.658$) and $LFW = 15.41N + 15.14K + 11.68Ca + 8.49N \times K + 14.51N \times Ca + 12.43K \times Ca$ ($P = 0.009$, $R^2 = 0.849$, Adj. $R^2 = 0.740$).

edge in Figure 2). Trace plot (Figure 3) shows that shoot FW was higher when the proportion of Ca^{2+} was low (<0.33) and K^+ and N proportions were high (<0.5 for K^+ and between 0.33 and 0.75 for N). The mixture that the model predicts to produce to highest shoot FW (60.0 g) was 0.5 : 0.4 : 0.1, which would contain 170 mg L^{-1} N, 130 mg $\cdot$ L^{-1} K^+, and 34 mg $\cdot$ L^{-1} Ca^{2+}. The highest 10% of maximum shoot FW is predicted to be produced when the mixtures contained N at proportions between 0.30 and 0.64 (102–218 mg $\cdot$ L^{-1} N), K^+ between 0.19 and 0.54 (65–184 mg $\cdot$ L^{-1} K^+), and Ca^{2+} between 0.11 and 0.39 (37—133 mg $\cdot$ L^{-1} Ca^{2+}).

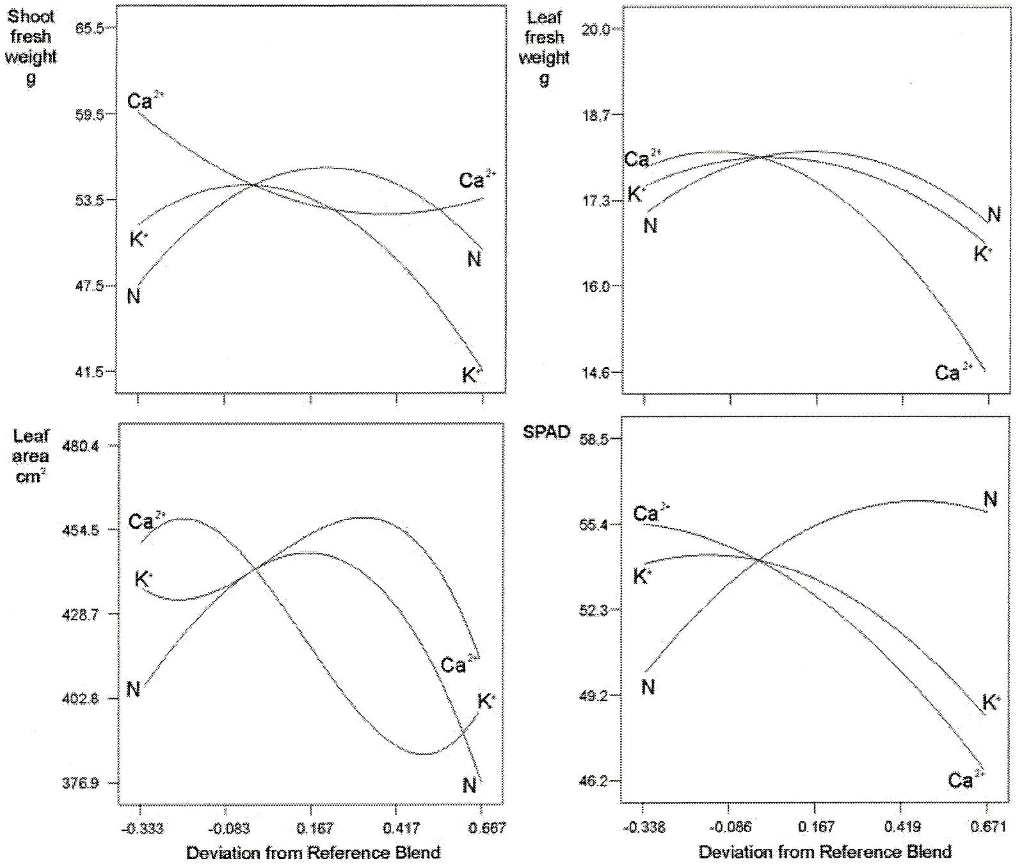

FIGURE 3 Trace plots showing the individual effect of N, K^+, and Ca^{2+} proportions in nutrient solutions used to irrigate lilium cv. Navona on shoot and leaf fresh weight, leaf area, and SPAD. The predictions are based on a reference blend which is 0.33:0.33:0.33.

Leaf FW was significantly affected by the $N:K^+:Ca^{2+}$ mixtures (Figure 2). In this case, the $N \times K^+$, $N \times Ca^{2+}$, and $K^+ \times Ca^{2+}$ interactions had a synergistic effect since shoot FW increased when both elements were present in the mixtures. Trace plot (Figure 3) shows that Ca^{2+} had a marked effect on leaf growth while N and K^+ effect was marginal. Leaf FW was higher when the proportion of Ca^{2+} was low (<0.33) while K^+ and N proportions were between 0.33 and 0.55. The mixture that the model predicts to produce the highest leaf FW (18.1 g) was 0.40:0.34:0.26, which would contain 136 mg L^{-1} N, 116 mg L^{-1} K^+, and 88 mg L^{-1} Ca^{2+}. The highest 10% of maximum leaf FW was predicted to be produced when the mixtures contained N at proportions between 0.23 and 0.79, K^+ between 0.10 and 0.42, and Ca^{2+} between 0.10 to 0.46 (78–269 mg L^{-1} N, 34–143 mg L^{-1} K^+, and 34–156 mg L^{-1} Ca^{2+}).

Leaf area was significantly affected by the $N : K^+ : Ca^{2+}$ mixtures (Figure 4). Nitrogen, K^+, and Ca^{2+} had a marked effect on leaf growth (Figure 3). Leaf area was higher when the proportion of Ca^{2+} was low (<0.33)

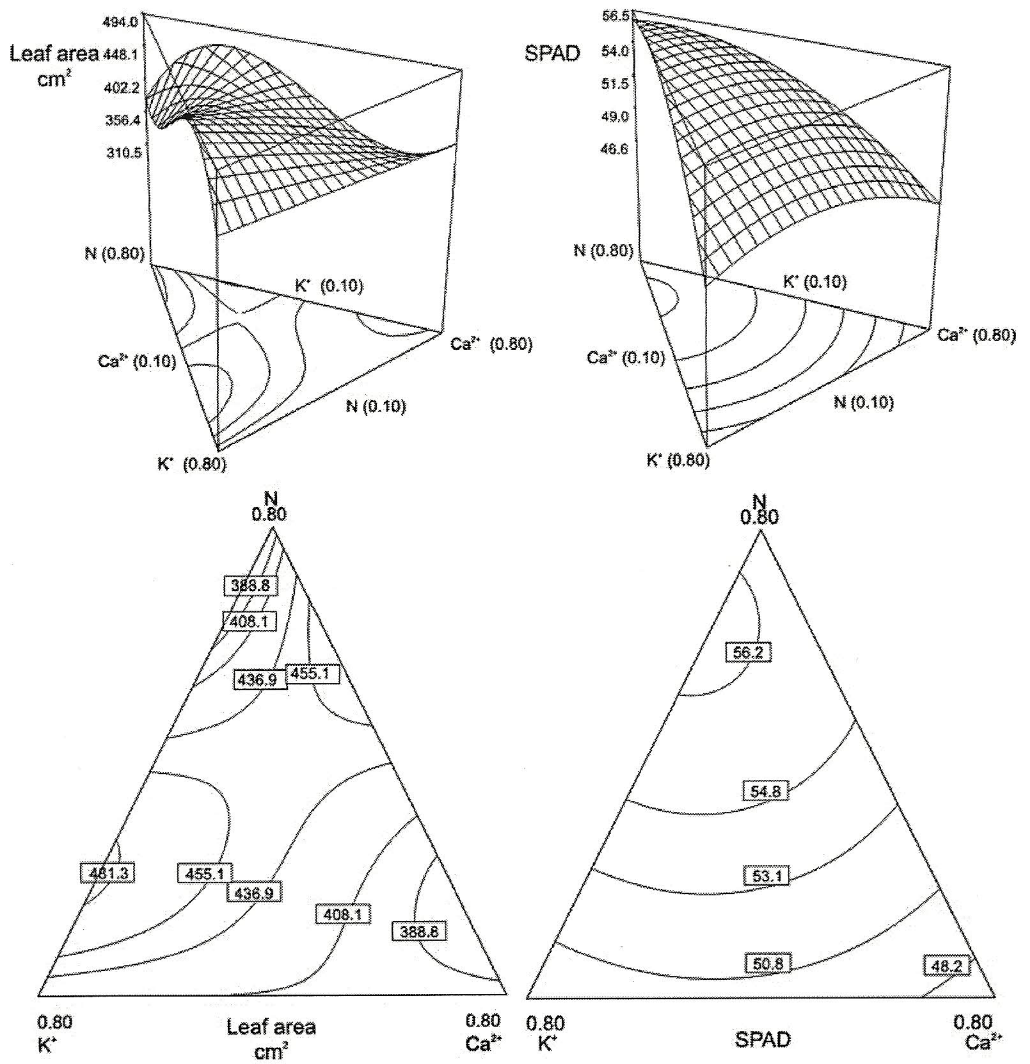

FIGURE 4 Response surface and counter-plots exhibiting the effect of N : K^+ : Ca^{2+} mixtures in nutrient solutions used to irrigate lilium cv. Navona on leaf area (LA) and SPAD. The models were: $LA = 250.3N + 277.0 + 505.5Ca + 603.0N \times K + 299.4N \times Ca—1170.2N \times K(N\text{-}K) + 1620.0N \times Ca(N\text{-}Ca)$ ($P = 0.011$, $R^2 = 0.892$, Adj. $R^2 = 0.784$) and $SPAD = 53.86N + 43.63K + 41.26Ca + 26.82N \times K + 23.29N \times Ca + 20.79K \times Ca$ ($P = 0.002$, $R^2 = 0.898$, Adj. $R^2 = 0.826$).

while K^+ was between 0.49 to 0.88 and N at 0.42 to 0.69. The mixture that the model predicts to produce the highest leaf area (485.4 cm^2) was 0.28:0.62:0.10, which would contain 95 mg · L^{-1} N, 211 mg L^{-1} K^+, and 34 mg L^{-1} Ca^{2+}. The highest 10% of maximum leaf area was predicted to be produced when the mixtures contain N at proportions between 0.28 and 0.60, K^+ between 0.10 and 0.62, and Ca^{2+} between 0.10 and 0.31 (95–204 mg L^{-1} N, 34–211 mg L^{-1} K^+, and 34–105 mg L^{-1} Ca^{2+}).

SPAD index was significantly affected by the N:K^+:Ca^{2+} mixtures (Figure 4). Trace plot (Figure 3) indicates that increasing Ca^{2+} proportion in the mixtures had a detrimental effect in SPAD, while increasing N resulted in increasing SPAD. Potassium was associated with high SPAD when the proportion was <0.33. The mixture that the model predicts to produce to highest SPAD (56.4) was 0.65: 0.25: 0.10, which would contain 221 mg L^{-1} N, 85 mg L^{-1} K^+, and 34 mg L^{-1} Ca^{2+}. The highest 10% SPAD was predicted to be produced when the mixtures contained N at proportions between 0.19 and 0.70, K^+ between 0.13 and 0.59, and Ca^{2+} between 0.11 to 0.56 (65–238 mg L^{-1} N, 44–201 mg L^{-1} K^+, and 37–190 mg L^{-1} Ca^{2+}).

Shoot concentration of P, K^+, Ca^{2+}, Mg^{2+}, S, Cu, Mn, and Zn were significantly affected by the N:K^+:Ca^{2+} mixtures (Table 2). The highest P, Mg^{2+}, and Mn concentrations were observed in plants irrigated with solutions containing N at proportions higher than 0.57, while highest S concentration was in plants irrigated with mixtures containing N at 0.33. Shoot Cu and Zn concentrations were significantly higher in plants irrigated with mixtures containing Ca^{2+} at 0.57 and N at proportions of 0.8, respectively. Shoot N, K^+, and Ca^{2+} concentration was directly affected by N:K^+:Ca^{2+} composition of the nutrient solution (Figure 5). Increasing N concentration in the nutrient solution was correlated with shoot N concentration ($r^2 = 0.641$) but not with shoot K^+ ($r^2 = 0.214$) or Ca^{2+} ($r^2 = 0.042$) concentration (Figure 5). However, N concentration in the nutrient solution exhibited a significant correlation with shoot concentration with most of the reminding nutrients analyzed; direct correlations were observed between N with P ($r^2 = 0.836$), Mg^{2+} ($r^2 = 0.609$), Fe ($r^2 = 0.855$), and Mn ($r^2 = 0.787$), while quadratic trends were observed with S ($r^2 = 0.907$), B ($r^2 = 0.990$), and Zn ($r^2 = 0.730$). Similarly, increasing K^+ concentration in the solution was directly correlated with shoot K^+ concentration ($r^2 = 0.640$) but not with shoot N ($r^2 = 0.113$) or Ca^{2+} ($r^2 = 0.448$) (Figure 5), while increasing Ca^{2+} in the nutrient solution was directly correlated with shoot Ca^{2+} concentration ($r^2 = 0.723$) but not with N ($r^2 = 0.216$) or K^+ ($r^2 = 0.093$) concentration (Figure 5).

DISCUSSION

Lilium is reported to require no fertilization under greenhouse conditions during the forcing phase (McKenzie, 1989) or to require, starting at shoot emergence (Aimone, 1986), a moderate fertilizer supply (De Hertogh and Le Nard, 1993). The moderate or no fertilizer requirement by lilium is explained by the high concentration of nutrients stored in the bulb (McKenzie, 1989) that may be remobilized when the shoot is actively growing.

In the present study, the fact that no significant models were estimated for shoot length, number of flowers, and flower diameter (data not shown), suggests that these parameters were not affected by the N, K^+, and Ca^{2+} composition of the nutrient solution. Thus, lilium can be considered a species

TABLE 2 Shoot nutrient concentration at experiment termination in Lilium cv. Navona irrigated with solutions of varying N : K^+ : Ca^{2+} proportions and concentrations. Shoot N concentration is in percent and the reminding elements are in mg · Kg^{-1}

Mixture solutions	Shoot nutrient concentration										
N : K^+ : Ca^{2+}	N	P^z	K^+	Ca^{2+}	Mg^{2+}	S	B	Cu	Fe	Mn	Zn
0.1 : 0.1 : 0.8	3.01	2719 cd	14716 d	6880 a	2501 bc	2531 c	367	49.5 c	114	46.1 c	82.5 c
0.1 : 0.8 : 0.1	3.01	2420 d	20316 a	3768 d	2181 d	3086 b	332	49.1 c	114	74.6 b	101.2 bc
0.22 : 0.22 : 0.57	3.50	2609 d	18116 bc	6770 ab	2454 c	3519 a	586	59.4 ab	128	78.5 b	109.8 bc
0.33 : 0.1 : 0.57	3.26	3122 bc	13427 d	7349 a	2667 bc	3395 ab	442	56.3 abc	143	72.5 b	129.5 ab
0.33 : 0.33 : 0.33	3.51	2646 d	19623 ab	5849 bc	2753 ab	3210 ab	642	51.7 bc	145	74.8 b	113.5 b
0.57 : 0.1 : 0.33	3.51	3621 a	14787 d	6562 abc	2989 a	2407 c	442	52.5 bc	196	115.8 a	122.5 b
0.57 : 0.22 : 0.22	4.51	3127 bc	17409 c	5717 c	2576 bc	2346 c	421	52.8 bc	146	122.9 a	112.7 b
0.8 : 0.1 : 0.1	4.26	3446 ab	15055 d	4326 d	2990 a	1173 d	375	61.0 a	162	129.6 a	155.3 a
ANOVA[y]	NS	**	***	***	***	***	NS	*	NS	***	**

[z] Means ($n = 3$) followed by the same letter are not significantly different according to Tukey's multiple comparison test.

[y] NS, *, **, *** non significant and significant at $P < 0.05$, 0.01, and 0.001, respectively.

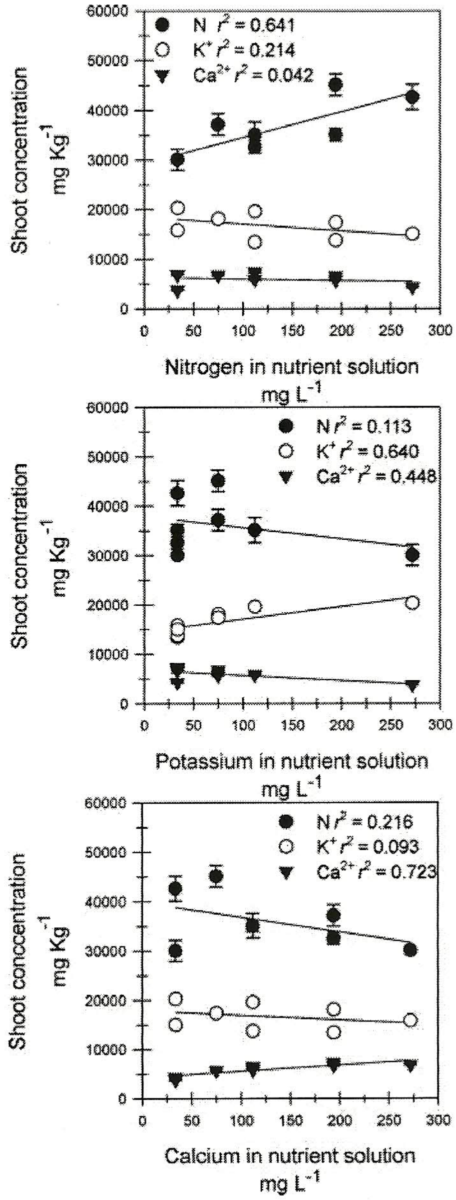

FIGURE 5 Effect of solution N (top), K^+ (middle), and Ca^{2+} (bottom) concentration on shoot N, K^+, and Ca^{2+} concentration.

that is adaptable to a wide range of nutrient concentrations; however, the significant models for other growth attributes indicate that lilium responded to the N, K^+, and Ca^{2+} composition of the external solution, indicating that the nutrients provided by the bulb are not sufficient to meet growth demands, as indicated by Ortega-Blu et al. (2006). Shoot FW and leaf FW were highest when plants were irrigated with solutions higher in N and K^+ proportions (0.40–0.50 and 0.34–0.40, respectively), while the demands for maximum leaf area and chlorophyll concentration (SPAD) were high in K^+ (0.65) and

N (0.62), respectively. Therefore, FW accumulation was mainly influenced by N and K^+, while leaf expansion was mostly influenced only by K^+. In the geophyte *Sandersonia aurantiaca*, Clark (1997) demonstrated that shoot FW was not affected by K^+ rates but biomass accumulation increased by 10% when N supply increased. Similarly, *Lilium longiflorum* cv 'Nellie White' is reported to exhibit a significant decrease in stem + leaf FW when fertilized with N-P-K^+ -deprived solutions, but individual nutrient deprivation caused no significant reduction in FW accumulation (Niedziela et al, 2008). This is in contrast with the results observed in the present study since lilium plants produced the highest FW when N, K^+, and Ca^{2+} concentrations in the nutrient solution were as low as 92, 112, and 34 $mg \cdot L^{-1}$, respectively, which may be probably due to the absolute deprivation of N-P-K^+ reported by Niedziela et al. (2008), while in our study no nutrient was at zero concentration.

The models indicate a small supply of Ca^{2+} requirement since the optimum proportion ranged between 0.1 and 0.26 (34 to 88 $mg \cdot L^{-1}$). The low demand for Ca^{2+} in lilium may be due to the relatively high concentration of this ion stored in the bulb (Dole and Wilkins, 1999), however, the storage of N and K^+ was not sufficient for growth maintenance and thus these nutrients must be supplied at higher rates in the irrigation solution. This is in agreement with reports recommending liquid fertilization of lilium based on KNO_3 but not on $Ca(NO_3)_2$ (McKenzie, 1989). Chang and Miller (2003) demonstrated that the lower and middle leaves of lilium rely on the Ca^{2+} supplied by bulb, while the upper leaves demanded more Ca^{2+} assimilated and transported from the roots. The authors also showed that if the bulb does not contain enough Ca^{2+} and this is not supplied externally, the plants will develop upper leaf necrosis, a typical symptom of Ca^{2+} deficiency.

According to our results, leaf area was associated with a high K^+ concentration in the nutrient solution, probably due to its role in water relations, turgor maintenance, and cell expansion (Mengel and Kirkby, 2001). Thus, an ample supply of K^+ is recommended for leaf development, which will contribute to plant quality since foliage is considered an important quality factor to lilium (McKenzie, 1989). Ortega-Blu et al. (2006) reported a total accumulation of N and K^+ that was 3.5 and 7.6 times higher than that of Ca^{2+}, respectively, in the shoots of lilium cv 'Navona'; nonetheless, the accumulation was only 0.7 and 2.6 times higher in the bulbs. This suggests that the demand for K^+ is much higher than that of N and Ca^{2+}, which is in agreement with the results observed in the present study. The higher SPAD associated with high N–low K^+ solutions was probably due to a concentration effect of chlorophyll in leaves with the smaller leaf area that resulted in plants irrigated with such a solution.

The correlation between shoot N concentration and most of the reminding nutrients may be related to the influence of N in plant growth since actively growing plants usually exhibit a high nutrient demand. Accordingly, a positive correlation between N and P and other micronutrients has been reported by Fageria (2001). The external concentration of N affected the internal N concentration but not the internal concentration of K^+ or Ca^{2+} despite that the increase in external N was associated to a decrease in K^+ and Ca^{2+} in the nutrient solution. Similarly, *Sandersonia aurantiaca* was reported to exhibit increased N and K^+ concentration in response to increased fertilization (Clark, 1997). Similar trends were observed for K^+ and Ca^{2+} in which external K^+ affected internal K^+ but not internal N or Ca^{2+}, whereas external Ca^{2+} affected internal Ca^{2+} concentration but not internal N or K^+. The increasing shoot N, K^+, or Ca^{2+} concentration in response to the respective external concentration is probably due to the passive transport associated with a higher nutrient availability in the external solution. However, the fact that increasing external N concentration (or K^+ or Ca^{2+}) did not affect shoot K^+ (or N or Ca^{2+}) and Ca^{2+} (or N or K^+) concentration suggests that plants are regulating the uptake of the nutrients in decreasing external concentrations in order to maintain a constant shoot concentration.

The integration of the predictions of each individual model allows the definition of a specific area in the contour plot that includes those solutions at which a threshold can be achieved. Considering as optimum solutions in which plants produced >90% of maximum shoot FW, leaf FW, leaf area, and SPAD, the area predicted for optimum growth is shown in Figure 6.

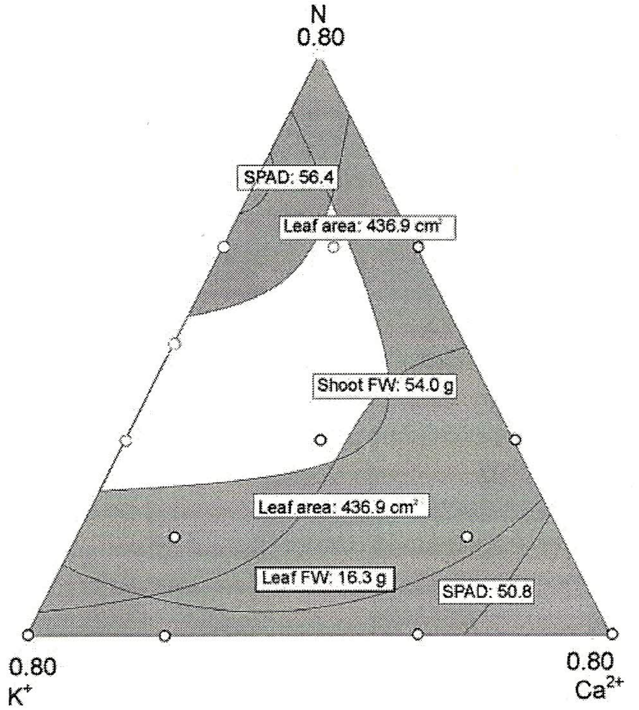

FIGURE 6 Contour plot showing the area (white) in which the combination of N, K^+, and Ca^{2+} will render >90% of maximum shoot fresh weight, leaf fresh weight, leaf area, and SPAD.

In this area, the proportion of Ca^{2+} was as low as 0.1 (34 mg L^{-1}) and as high as 0.37 (126 mg $\cdot L^{-1}$), K^+ as low as 0.35 (119 mg L^{-1}) and as high as 0.62 (211 mg L^{-1}), and N as low as 0.27 (92 mg L^{-1}) and as high as 0.62 (211 mg L^{-1}). The optimum growth predicted despite the wide range of optimum N, K^+, and Ca^{2+} concentrations was possible probably due to active nutrient uptake or because of an homeostatic plant response in which a high affinity system is activated for nutrient uptake in spite of marginal external concentrations (Marschner, 1995), allowing ion accumulation and growth. Subasinghe (2007) demonstrated that N and K^+ stress increased root affinity for NO_3^- and K^+ absorption in sugarcane.

Merhaut and Newman (2005) stressed the importance of decreasing NO_3^- leaching loss in nurseries and greenhouses of coastal California, where lilium is an important crop. Using commercial practices, the authors fertilized Oriental lilium plants with a solution containing 185 mg L^{-1} N, 275 mg L^{-1} K^+, and 160 mg L^{-1} Ca^{2+}. However, according to our data, acceptable growth of lilium can be attained with 92 mg $\cdot$ L^{-1} N. The models estimate that fertilizing with this N concentration and K^+ and Ca^{2+} at 214 and 34 mg L^{-1} (0.27: 0.63: 0.10), respectively, shoot FW will be 90% of that obtained by plants with maximum growth, leaf area will be 100%, and leaf FW will be 96%. Thus, N losses can be markedly reduced for lilium cut flower production with minimal effects on growth.

In conclusion, greenhouse-forced lilium plants were able to maintain a relatively constant K^+ and Ca^{2+}concentration regardless of the lower concentration in the nutrient solution when N was increased, and had a low Ca^{2+} demand, a moderate N demand, and a high K^+ demand. Adequate Ca^{2+} stored in the bulb must play an important role on these results. The information provided can be used to design fertigation solutions with a low concentration of N, K^+, and Ca^{2+} in order to reduce the negative impacts on the environment.

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