

Full Length Research Paper

Effects of calcium and humic acid treatment on the growth and nutrient uptake of Oriental lily

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Humic acid (HA) may facilitate plant growth by improving the nutrient uptake as well as through hormonal effects. Studies were conducted to investigate the effect of a mixture of calcium and HA on Oriental lily, which is sensitive to calcium deficiency. Two levels of Ca (3.5 and 7.0 meq/L) were combined with 500 mg/L HA and applied to the nutrient solution of the *Lilium* Oriental hybrid 'Sorbonne'. The plant growth, physiological response and the macro- and micro-nutrient contents of the leaves, stems and roots were measured. The results show that low Ca (3.5 meq/L) and HA significantly promoted plant growth and root development. The flowering period started eight days earlier as compared to the treatment CK and the active absorbing area of the root surface was 29.41% higher. Also, low Ca increased the leaf chlorophyll content, and the proline content dramatically increased when low Ca was combined with HA. The application of Ca effectively increased the potassium and iron content of the leaves and increased the phosphorus content of the stems. The nitrogen in the leaves and the zinc in the roots were remarkably enhanced by HA.

Key words: *Lilium* Oriental hybrid, calcium, humic acid, plant growth, nutrient uptake.

INTRODUCTION

Calcium plays an important role in plant growth and in many physiological activities of bulbous flowers (Pan and Dong, 1995). Ca deficiency may result in problems such as upper leaf necrosis in the *Lilium* Oriental hybrid 'Star Gazer' (Chang, 2002; Chang and Miller, 2003). A good fertilizer that accelerates Ca uptake enhances the quality of both cut flowers and lily bulbs (Choi et al., 2005). Humic acid (HA) is a promising natural resource that can be used as an alternative to synthetic fertilizers to increase crop production. It exerts either a direct effect, such as on enzymatic activities and membrane permeability (Chen et al., 1990; Pinton et al., 1992), or an indirect effect, mainly by changing the soil structure (Alianiello et al., 1991; Biondi et al., 1994). HA is also

proven to be effective in enhancing the mineral nutrient uptake (Pan and Dong, 1995). Given its powerful binding capacity, HA synergizes with Na, K, Mg, Zn, Ca, Fe, Cu and other various elements to overcome a particular element deficiency in the soil (Aiken et al., 1985).

Little information on the effects of a Ca-HA treatment on plant growth and nutrient uptake of lilies is available. Therefore, the present investigation was conducted to evaluate the optimal concentration of Ca bonded with HA based on the root absorption area, plant growth, physiological response and nutrient uptake assessments. The root absorption area is a parameter that indicates root development, and relevant reports for other crops can be found in many manuals of plant physiology in China (Miao et al., 2005; Hou et al., 2003; Zou et al., 2001).

The *Lilium* Oriental hybrid 'Sorbonne' was used as the experimental material because of the popularity of its cultivars and the Ca deficiency in cut lilies produced in Zhejiang Province, China. The current study hopes to contribute to the practical application of fertilizers in the commercial production of cut lilies.

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Abbreviations: HA, Humic acid; DAP, days after planting; DM, dry matter; MB, methylene blue.

MATERIALS AND METHODS

Plant materials, growth conditions and treatments

The *Lilium* Oriental hybrid "Sorbonne" from the mountainous region was planted in the spring of 2008 in Zhejiang University, Hangzhou (30°15' N, 120°10' E), China. Three lily bulbs (14 to 16 cm in circumference) were grown in a 7.57 L plastic pot filled with the mixed medium (peat and perlite, 2:1 by volume; sterilized in advance) in a plastic greenhouse. Fifteen replications per treatment were used in a completely randomized experimental design. The mean air temperatures in the greenhouse were $29 \pm 2/24 \pm 3^\circ\text{C}$ (day/night) and the relative humidity varied between 75.5 ± 5.2 and $50 \pm 8.5\%$. The average daily photosynthetic active radiation in the greenhouse was 420 ± 150 to $140.5 \pm 60 \text{ w/m}^2$ during the irrigation treatments, as measured using the data logger system.

Low and medium concentrations of Ca (3.5 and 7.0 meq/L) were applied to the nutrient solution together with 500 mg/L HA according to our previous study (Ali et al., 2008). The different treatments were as follows: A (3.5 meq/L Ca + 500 mg/L HA), B (3.5 meq/L Ca), C (7.0 meq/L Ca + 500 mg/L HA), D (7.0 meq/L Ca) and CK (no Ca and no HA). The treatments were prepared using distilled water, and the composition of the complete nutrient solution was as follows (macronutrients in meq/L, micronutrients in μM): K (5.84), Mg (2.2), NH_4 (1.1), NO_3 (11.2), SO_4 (2.54), H_2PO_4 (1.2), Fe (35), Mn (5), Zn (4), Cu (0.75) and B (30). Fe was added as Fe-EDTA. The electrical conductivity of the nutrient solution was 1.8 to 1.9, and the pH was adjusted to 5.6 in all treatments. Each fertilizer was added from a separate stock solution tank. Commercial HA prepared from leonardite [containing 61.2% C, 3.13 g/kg N DM (dry matter) and 2.89 g/kg P DM] was purchased from Hongyue Seed Company, China. The nutrient solution (250 ml/pot) was applied twice a week from the vegetative growth period to the end-blooming stage.

Root activity measurement

The absorbing root area in the end-blooming stage was investigated. The roots were carefully washed with tap water and given a final rinse with deionized water to retain all the roots. The roots were successively dipped into three beakers (numbered 1, 2 and 3) containing 0.2 mmol/L methylene blue (MB) solutions for 1.5 min each, ensuring that the MB solutions completely covered the roots. The solution was then dripped back into its corresponding beaker. Accurately, 1 ml of the MB solution was collected from each beaker, and the absorbance at 660 nm was measured using a spectrophotometer (DU-800, Beckman Coulter Fullerton, CA, USA). The amount of MB absorbed by the roots was determined using a calibration curve and recorded as X1, X2 and X3, corresponding to beakers 1 to 3, respectively (Zhang, 1990; Zou et al., 2001). The absorbing root area was calculated as follows:

1. Total absorbing area (m^2) = $(X1 + X2) \times 1.1 \text{ m}^2$
2. Active absorbing area (m^2) = $X3 \times 1.1 \text{ m}^2$
3. Active absorbing area (%) = $100\% \times \text{active absorbing area} (\text{m}^2) / \text{total absorbing area} (\text{m}^2)$
4. Surface area (m^2/cm^3) = $\text{total absorbing area} (\text{m}^2) / \text{root volume} (\text{cm}^3)$

Leaf chlorophyll and proline contents

The chlorophyll content was estimated using the spectrophotometric method described by Hiscox and Israelstam (1979). Chlorophyll was extracted from the leaf samples using a mixture of dimethylsulfoxide and 80% acetone. The absorbance was recorded at 645 and 663 nm using a spectrophotometer (DU-800, Beckman

Coulter, USA).

The free proline content in the leaves was measured and calculated as described by Bates et al. (1973). Fresh leaf samples (0.5 g) were ground and added to 10 ml of 3% aqueous sulfosalicylic acid and filtered through a Whatman No. 2 filter paper. Exactly 2 ml of the filtrate was mixed with 2 ml acid-ninhydrin and 2 ml glacial acetic acid in a test tube and placed in a water bath for 60 min at 100°C . The reaction was stopped using an ice bath. The reaction mixture was extracted with 4 ml toluene and cooled to room temperature. The absorbance of the toluene fraction aspirated from the liquid phase was measured at 520 nm using a spectrophotometer (DU-800, Beckman Coulter, USA). The proline concentration was determined as $\mu\text{mol proline g}^{-1} \text{ FW}$ using a calibration curve.

Macro- and micro-nutrient content extraction and determination

The leaves, stems and roots (0.5 g each) for each sample were weighed, thoroughly washed with tap water, and then quickly rinsed in deionized water. The samples were then incubated to constant weight at 65°C using an oven and then ground to determine their mineral composition. The total N determination in the leaf, stem and root samples was based on the Kjeldahl method (Ali et al., 2008). The extraction of P, K, Ca, Mg, Fe and Zn from the plant tissue material was performed using 1 N HCl after dry ashing at 550°C for 5 h. The K, Ca, Mg, Fe and Zn concentrations were obtained via inductively coupled plasma emission spectrometry (Shield Torch System, Agilent 7500a; Agilent Technologies, Santa Clara, CA, USA), whereas that of phosphorus was estimated via the vanadomolybdophosphoric acid colorimetric method at 460 nm (Eaton et al., 1995). The P colorimetric determinations were performed using a Shimadzu UV 2401 PC (Shimadzu, Torrance, CA, USA) spectrophotometer.

Three replications were performed for each experiment in a completely randomized experimental design.

Statistical analysis

The experiments were designed and analyzed as random single-factor ANOVA, followed by the least significant difference comparison of the means using the Tukey test system.

RESULTS AND DISCUSSION

Flowering period and rate

The combination of Ca and HA obviously accelerated the flowering period, which started eight days earlier when compared with the control (treatment CK) regardless of the Ca concentration (Table 1). The planting date was March 22, 2008. At the early stage of flowering [June 2, 70 days after planting (DAP)], all plants treated with Ca showed higher flowering rate when compared with the untreated plants, which produced no flowers. Moreover, the combination of Ca and 500 mg/L HA produced the highest flowering rate at 17.28% (7.0 meq/L Ca) and 15.58% (3.5 meq/L Ca). During subsequent period, the Ca-HA treatments significantly increased the flowering rate when compared with the Ca-only treatments. HA is apparently vital to the blooming rate when compared with

Table 1. Effect of Ca and HA on the flowering rate of lilies.

Treatment	Flowering rate (number of flowers/number of flowers and buds, %)			Total blooming period (day)
	Early-blooming stage* (70 DAP)	Full-blooming stage* (82 DAP)	End-blooming stage* (96 DAP)	
CK	0.00 ^c	24.24 ^c	83.33 ^b	22.8 ^c
A	15.58 ^a	55.84 ^a	93.02 ^a	33.2 ^a
B	4.88 ^{bc}	43.90 ^b	95.12 ^a	25.7 ^b
C	17.28 ^a	55.56 ^a	93.83 ^a	34.6 ^a
D	6.98 ^b	40.70 ^b	93.02 ^a	28.4 ^b

Values in the same column followed by the same letters are not significantly different based on the Tukey test at the $P < 0.05$ level. CK (no Ca and no HA); A. 3.5 meq/L Ca + 500 mg/L HA; B. 3.5 meq/L Ca; C. 7.0 meq/L Ca + 500 mg/L HA; D. 7.0 meq/L Ca. *Standards for lily blooming period division (Peng and Wang 2006): Early-blooming stage, approximately 5% of the total flower buds became full flowers, whereas 80% were white. Full-blooming stage, 80% of the total flower buds became full flowers. End-blooming stage, 80% of the flowers have started to fade and wither.

Table 2. Effect of Ca and HA on the absorbing area of the root surface.

Treatment	Total absorbing area (m ²)	Active absorbing area (m ²)	Active absorbing area/total absorbing area (%)
CK	23.31 ^b	10.16 ^c	43.54 ^c
A	28.79 ^a	17.76 ^a	61.68 ^a
B	23.77 ^b	12.78 ^{bc}	58.01 ^b
C	27.35 ^a	15.87 ^a	57.48 ^b
D	26.08 ^{ab}	14.69 ^{ab}	56.34 ^b

Values in the same column followed by the same letters are not significantly different based on the Tukey test at the $P < 0.05$ level. CK (no Ca and no HA); A. 3.5 meq/L Ca + 500 mg/L HA; B. 3.5 meq/L Ca; C. 7.0 meq/L Ca + 500 mg/L HA; D. 7.0 meq/L Ca.

the Ca-only solution at either the early-blooming or full-blooming stage (June 14, 82 DAP). No significant difference between treatments at the end-blooming stage (June 28, 96 DAP) was observed despite the presence of HA or Ca (Table 1). The total blooming period was extended in all plants treated with Ca. This result indicates that the combination of Ca and HA significantly induced the extension of the blooming period, which was 10.4 to 11.8 days longer than that of the treatment CK (Table 1).

Root development and absorbing area

Table 2 shows that treatment A noticeably promoted the root growth of Oriental lily. The total absorbing area of the root surface for this treatment reached 28.79 m², and the absorbing area accounted for 61.68% of the total absorbing area, which was the highest for all treatments and significantly higher than that of treatments B and CK (Table 2). The stem roots under treatment A were numerous and longer, whereas the stem roots in the treatment CK were few and scattered. Wang and Ma, (2006) proved that supplementing the Oriental lily with 3.26 mmol/L Ca produced dense stem roots with many lateral roots, whereas the biomass prior to the Ca treatment was

deficient during the whole growth period. In the study, HA enhanced the uptake of low concentrations of Ca which promoted the development of the stem roots.

Chlorophyll and proline contents

At the full-blooming stage, a significant increase in the chlorophyll content was observed between the treatments of 3.5 meq/L Ca with or without HA (treatment A and B) and the treatments of 7.0 meq/L Ca (treatment D). However, HA did not exhibit any significant effect on the chlorophyll content. At the end-blooming stage, the chlorophyll content declined in all treatments, probably because of plant senescence.

Treatment A significantly increased the proline content at the full blooming stage to 7.07 $\mu\text{g}\cdot\text{g}^{-1}$ FW, which was 10.5 times higher than that of the treatment CK. As the experiment continued, the proline content greatly increased by 8.37 times at most in each treatment when compared with the treatment CK; however, only a 15.13% increase was observed for treatment A. Nevertheless, this level was still the highest among all treatments. The 40°C regular high temperature in Zhejiang Province caused a heat stress to the plants even in the greenhouse. The stress resistance of the lily was

Table 3. Effect of Ca and HA on the chlorophyll and proline contents in the leaves.

Treatment	Total chlorophyll content (mg·g ⁻¹ FW)		Proline content (µg·g ⁻¹ FW)	
	Full-blooming stage	End-blooming stage	Full-blooming stage	End-blooming stage
CK	3.76 ^c	3.46 ^a	0.67 ^b	6.28 ^b
A	4.97 ^a	4.20 ^a	3.07 ^a	8.14 ^a
B	4.70 ^{ab}	4.06 ^a	2.83 ^b	7.34 ^{ab}
C	4.20 ^{bc}	4.04 ^a	0.95 ^b	6.89 ^b
D	4.02 ^c	3.87 ^a	0.72 ^b	6.62 ^b

Values in the same column followed by the same letters are not significantly different based on the Tukey test at the $P < 0.05$ level. CK (no Ca and no HA); A. 3.5meq/L Ca + 500mg/L HA; B. 3.5meq/L Ca; C. 7.0meq/L Ca + 500mg/L HA; D. 7.0meq/L Ca.

Table 4. Effect of Ca and HA on the macro- and micro-nutrient uptake of the leaves, stems and roots of Oriental lily.

Treatment	Plant macronutrients															Plant micronutrients					
	N (%)			P (%)			K (%)			Ca (%)			Mg (%)			Fe (% × 10 ³)			Zn (% × 10 ³)		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
CK	2.88	2.11	1.64	0.26	0.20	0.15	0.20	0.18	0.034	0.125	0.026	0.054	0.032	0.008	0.010	0.939	2.43	31.68	2.319	2.412	2.999
A	3.15	2.41	1.73	0.21	0.32	0.15	0.28	0.24	0.042	0.126	0.020	0.044	0.028	0.007	0.016	3.006	1.013	22.47	2.187	2.444	3.606
B	2.35	2.48	1.93	0.19	0.31	0.18	0.24	0.20	0.085	0.152	0.024	0.042	0.034	0.001	0.012	4.193	0.295	16.46	2.630	2.034	3.230
C	3.26	2.67	1.85	0.26	0.42	0.17	0.27	0.23	0.078	0.138	0.024	0.045	0.034	0.007	0.013	2.465	0.741	23.48	2.739	2.402	4.625
D	2.66	2.68	1.71	0.22	0.41	0.14	0.26	0.23	0.046	0.136	0.021	0.054	0.030	0.003	0.013	4.079	2.874	31.02	2.331	2.448	3.240

CK (no Ca and no HA); A. 3.5meq/L Ca + 500mg/L HA; B. 3.5meq/L Ca; C. 7.0meq/L Ca + 500mg/L HA; D. 7.0meq/L Ca.

enhanced by the low Ca and HA treatment by accelerating the Ca absorption (Table 3).

Nutrient uptake

The effect of the Ca-HA treatment on the macro-nutrient (N, P, K, Ca and Mg) and micronutrient (Fe and Zn) uptake of the leaves, stems and roots are shown in Table 4. Ca had no effect on the N content of the leaves or on the Zn content of the roots, whereas HA significantly boosted their levels. The Ca treatment effectively increased the P content in the stems by 110% at most. The K

accumulation in the leaves, stems and roots was improved to a certain extent after the Ca treatment, but the differences were not significant. The Fe content was markedly increased in the leaves and decreased in the roots under the Ca treatments regardless of the Ca concentration. However, HA played little role in these processes. The Fe content in the leaves was increased by the Ca-HA treatments to as much as 220%, which is much higher than previously reported results (Ali et al., 2008). They concluded that 500 mg/L HA can significantly enhance the Fe uptake in both the leaves and scapes of Gerbra by 49%. Adani et al. (1998) proved that the reduction of Fe³⁺ to Fe²⁺

by HA accounted for the higher Fe availability for plants in their experiments. Alikien et al. (1985) posited that a major benefit of HA in agricultural systems is its ability to form complexes with metal ions as well as aqueous complexes with micronutrients. In the current study, HA effectively improved the N uptake in the leaves and the Zn uptake in the roots.

Bohme and Thi (1997) reported that HA has beneficial effects on the nutrient uptake by plants and is particularly important to the transportation and availability of micronutrients. Hypotheses that account for the stimulatory effects of HA are numerous, the most convincing of which is the

“direct” action on the plant, which is hormonal in nature, together with an “indirect action” on the metabolism of microorganisms and the dynamics of soil nutrient uptake as well as substrate physical conditions through positive effects on the seed germination, seedling growth, root growth and shoot development (Chen et al., 1990). Zhang and Ervin (2004) showed that HA displays cytokinin activity. Pizzeghello et al. (2001) measured the auxin-like and gibberellin-like activities as well as the indole-3-acetic acid concentration in HA. Other hypothetical mechanisms include the enhanced uptake of metabolic ions and increases in cell permeability (Chen et al., 1990). As a consequence, the use of humic substances has often been proposed as a method of improving crop production.

Conclusion

The combination treatment of Ca and 500 mg/L HA exhibited significant effects on the plant growth and nutrient uptake of the Oriental lily. The 7.0 meq/L Ca and HA treatment enhanced the flowering period, and the 3.5 meq/L Ca and HA treatment resulted in high chlorophyll and proline contents. These findings indicate that this combination can be beneficial to root growth, increase the nutrient uptake and enhance the heat tolerance of the lily. The N content of the leaves and the Zn content of the roots were significantly increased by HA. These plant growth and nutrient uptake responses are probably due to the Ca accumulation in the scales and the hormone-like activity of HA. However, the effect of HA and Ca on the bulblet development of the lily needs further investigation.

REFERENCES

- Adani FP, Genevini PZ, Zocchi G (1998). The effect of humic acid on tomato plant growth and mineral nutrition. *J. Plant Nutr.* 21: 561–575.
- Ali N, Mohsen K, Mesbah B, Xia YP, Luo AC, Nematallah E (2008). Effect of Humic Acid on Plant Growth, Nutrient Uptake, and Postharvest Life of Gerbra. *J. Plant Nutr.* 31: 2155-2167.
- Aiken GR, McKnight DM, Wershaw RL, MacCarthy P (1985). Humic substances in soil, sediment, and water. Wiley-Interscience, New York, USA.
- Alianiello F, Benedetti A, Canali S, Rossi G (1991). Effects of NPK-humic acids on soil biological activity. III Int. Nordic Symp. on Humic Substances. *Finnish Humus News*, 3: p. 357.
- Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water stress studies. *Plant Soil.* 39: 205-207.
- Bohme M, Thi LH (1997). Influence of mineral and organic treatments in the rhizosphere on the growth of tomato plants. *Acta Horticult.* 450:161-168.
- Biondi FA, Figholia A, Indiatì R, Izza C (1994). Effects of fertilization with humic acids on soil and plant metabolism: a multidisciplinary approach. Note III: phosphorus dynamics and behaviour of some plant enzymatic activities. In *Humic Substances in the Global Environment and Implications on Human Health*, ed. Senesi N & Miano TM. Elsevier, New York, pp. 239-244.
- Chang YC (2002). Upper leaf necrosis on *Lilium* cv. Star Gazer-A calcium deficiency disorder. PhD diss. Cornell Univ., Ithaca, N.Y.
- Chang YC, Miller WB (2003). Growth and calcium partitioning in *Lilium* 'Star Gazer' in relation to leaf calcium deficiency. *J. Am. Soc. Hortic. Sci.* 128(6): 788-796.
- Chen Y, Aviad T (1990). Effects of humic substances on plant growth. Humic substances in soil and crop science: Selected readings, eds. MacCarthy P, Clapp CE, Malcolm RL and Bloom PR, Madison, WI: SSSA ASA. pp. 161-186.
- Choi JM, Lee KH, Lee EM (2005). Effect of calcium concentrations in fertilizer solution on growth of and nutrient uptake by Oriental hybrid lily 'Casa Blanca'. *Proceedings of the Ninth International Symposium on Flower Bulbs.* 673: 755-760.
- Eaton AD, Clesceri LS, Greenberg AE (1995). *Standard Methods for the Examination of Water and Wastewater.* 19th ed. Washington, D.C.: American Public Health Association.
- Hiscox JD, Israelstam GF (1979). A method for the extraction of chlorophyll from leaf tissues without maceration. *Can. Bot.* 57: 1332-1334.
- Hou D, Yue HZ, Liu MJ, Guo LX (2003). Effects of embryonic-root cutting on growth and development of cucumber seedlings. *Gansu Agric. Sci. Technol.* 8: 32-34. (in Chinese).
- Miao HX, Sun MG, Xia Y, Li GL, Zhang JF, Zhang LY (2005). Effects of salt stress on root activity of *Melia azedarach* L. seedlings. *J. Shandong Agric. Univ.* 36(1): 9-12.
- Pan RC, Dong YD (1995). *Plant physiology* (third edition). High education Press, Beijing, China. (in Chinese).
- Pizzeghello D, Nicolini G, Nardi S (2001). Hormone-like activity of humic substances in *Fagus sylvatica* forests. *New Phytologist*, 51: 647-657.
- Pinton R, Varanini V, Vizzoto G, Maggioni A (1992). Humic substances affect transport properties of tonoplast vesicles isolated from oat roots. *Plant Soil*, 142: 203-210.
- Peng GQ, Wang LH (2006). Quantitative variation of endogenous hormones in *Rosa rugosa* leaf during flower bud differentiation. *Bull. Bot. Res.* 26(2): 206-210. (in Chinese).
- Wang SL, Ma WQ (2006). Effects of calcium on the growth and quality of Oriental lily. *J. Agric. Univ. Hebei*, 29(3): 17-21. (in Chinese).
- Zou CQ, Li ZS, Li JY (2001). Study on difference in morphological and physiological characters of wheat varieties to potassium. *Plant Nutria. Fertilizer Sci.* 7(1):36-43. (in Chinese).
- Zhang XZ, Ervin EH (2004). Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. *Crop Sci.* 5: 1737-1745.
- Zhang ZL (1990). *The Experiment guide for plant Physiology* 2nd edition. High Education Press Beijing, China china. Chinese.