Effect of phosphorus on celery growth and nutrient uptake under different calcium and magnesium levels in substrate culture

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Abstract


A greenhouse pot experiment was performed to study the effect of phosphorus (P) on celery (Apium graveolens L.) growth, quality and nutrient uptake under different calcium (Ca) and magnesium (Mg) levels in substrate culture. Results showed that there were significant interactions between P and the level of Ca and Mg in the growing media. Celery above-ground fresh weight, total dry biomass, leaf area, and P, Ca and Mg concentrations in celery leaves significantly increased from 0 to 124 mg/l P application. The opposite trends were observed for root/shoot ratio, leaf chlorophyll, carotenoids, soluble protein, soluble sugar, vitamin C, and nitrogen and potassium concentration in celery leaves. Medium Ca and Mg level (Ca 320 and Mg 192 mg/l) significantly increased celery above-ground fresh weight, total dry biomass and leaf area compared to low (Ca 160 and Mg 96 mg/l) and high (Ca 640 and Mg 384 mg/l) levels. This study suggested that 124–248 mg/l P applications under medium Ca and Mg level were appropriate for celery nutritional requirements.

Keywords: Apium graveolens L.; quality; growing media; nutrient solution; optimization; fertilization

In intensive horticulture, the management of mineral nutrition is a key factor determining the yield and nutritional quality of vegetable crops (Kader 2008; Fallovo et al. 2009). In practice, excessive supplies of mineral nutrients often occur because growers seem to believe that high fertilizer inputs can result in high crop yields (Owen et al. 2008). However, excessive fertilizer inputs can cause nutrient salt accumulation in growing media. Too high salinity in growing media can inhibit the growth of vegetable crops and decrease the nutritional quality. Studies have shown that predominant salinity is not from sodium (Na) salt, but calcium (Ca) and magnesium (Mg) salts in intensive production system (Grattan, Grieve 1999; Lü, St 2004), which can result in decreased phosphorus (P) availability due to ionic strength, sorption process and low solubility of Ca and P minerals (Grattan, Grieve 1999). Therefore, it is of great significance to optimize P supply in different Ca and Mg environments for optimum plant growth.

Phosphorus is an essential element in plants, and deficiency can significantly limit plant growth. Thus, excessive P application frequently occurs in practice, especially in China, due to the lack of a formal fertilizer recommendation system and weakness of the local extension service. Many studies have indicated that excessive P application can result in plant toxicity leading to inhibition of growth, leaf chlorosis, and micronutrient deficiency (Marschner 1995; Burnett, Zhang 2008; Hawkin et al. 2008). Mean-

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while, P runoff poses a known environmental threat to natural waters, with high nutrient concentration leading to eutrophication of waterways (Burnett, Zhang 2008; Owen et al. 2008). In addition, Espinoza et al. (1993) found that previous P fertilizer recommendations for celery (Apium graveolens L.) were too high for the cultivars grown currently, but P is important at increasing total above-ground mass, marketable trimmed yield of celery and yield of the larger grade sizes.

Celery is a popular vegetable in the Yangtze River Basin of China, where in-ground greenhouse cultivation has gradually increased due to increasing demand for green vegetables. Meanwhile, the technique of fertigation has been rapidly adopted in the region. However, celery is very sensitive to nutritional disorders and growers frequently experience a wide variety of quality problems that can often be traced to nutrient deficiencies, excesses or imbalances (Tremblay et al. 1993). At present, there are no available data on optimum P fertilization under different Ca and Mg levels and their interaction effects on celery growth, quality and nutrient uptake. Therefore, the objectives of this study were to optimize P fertilization under different Ca and Mg levels and to characterize the effects of P supply, Ca and Mg level and their interactions on celery growth, quality and nutrient uptake in substrate culture.

MATERIAL AND METHODS

Growing media

River sand and oligotrophic peat (95:5, dry weight ratio) were thoroughly mixed as the growing medium. River sand was rinsed repeatedly with tap water until rinsed water was clear, and then air-dried before use. Oligotrophic peat, obtained from Wangqing of Jilin province, China, was sieved through a 5-mm mesh before use. Fifteen kg of the growing medium was placed into plastic pot (upper diameter 40 cm, bottom diameter 25 cm, height 30 cm) and equilibrated at a moisture capacity of 20% to 25% for 30 days. Organic matter content, field moisture capacity, pH and electrical conductivity (EC) of equilibrated growing medium were 3%, 25%, 6.1, and 0.04 mS/cm, respectively. Available nitrogen (N), P, potassium (K), Na, Ca Mg, iron (Fe), and manganese (Mn) concentrations in equilibrated growing medium were 8.69, 1.58, 121, 3.13, 83.2, 20.3, 18.0, and 1.13 mg/kg, respectively.

Nutrient solution

According to Sun et al. (2004) and Ao et al. (2008), 18 different nutrient solutions were prepared, which consisted of all combinations of six P concentrations (0, 31, 62, 124, 248, and 496 mg/l) with three levels of Ca and Mg [Ca 160 and Mg 96 mg/l (low), Ca 320 and Mg 192 mg/l (medium), and Ca 640 and Mg 384 mg/l (high)]. The sources of P, Ca and Mg were NaH$_2$PO$_4$, CaCl$_2$ and MgSO$_4$·7H$_2$O, respectively. Thus, Na, Cl and SO$_4$-S concentrations in different nutrient solutions had same changes with P, Ca and Mg, respectively. In all the nutrient solutions, N 112 and K 312 mg/l were applied as KNO$_3$. The concentrations of micronutrients were Fe 5.6, B 0.44, Mn 0.55, Zn 6.5 × 10$^{-2}$, Cu 1.92 × 10$^{-2}$, and Mo 9.6 × 10$^{-4}$ mg/l, which were applied as EDTA-Fe, H$_4$BO$_4$, MnSO$_4$·4H$_2$O, ZnSO$_4$·7H$_2$O, CuSO$_4$·5H$_2$O, and (NH$_4$)$_6$MoO$_4$·4H$_2$O, respectively. The pH of all the nutrient solutions was adjusted to 6.0 with dilute NaOH. Nutrient solutions were prepared with purified water when used. The above nutrient salts used were of analytical-reagent grade (Sinopharm Chemical Reagent Shanghai Co., Ltd., China).

Plant material and experimental design

Celery (Apium graveolens L.) variety Shanghai Huangxin Qin was used as plant material. The experiment was performed in a greenhouse at the Chongming Modern Agricultural Zone, Shanghai (China). Treatments were distributed using a randomized complete block design. There were three replications of five pots each. On October 29, 2008, uniform 25-day-old celery seedlings were transplanted into each plastic pot. Three seedlings per pot were grown. Plants were irrigated with 100 ml of the treatment nutrient solutions per pot for the respective treatments every two days from November 1 to December 20, 2008. Additional irrigation water (purified water) was applied as needed using a weighing method every week to maintain field moisture capacity.

Sample collection and measurement

In situ EC values of growing media were measured every week from the onset of treatments by using an EC meter (model 2265FS; Beijing Aozuo Ecology Instrumentation Ltd., China). On Decem-
ber 21, 2008, one plant per pot was randomly selected and harvested. The plants were washed with tap water, rinsed in distilled water, and divided into roots, stalks and leaves. The above-ground fresh weight and leaf area per plant were investigated. Dry weights of the plant organs were measured after drying at 60°C for one week. Dry plant materials were ground to pass through a 917 μm mesh screen. The mineral compositions of plant were determined using inductively coupled plasma spectrometry (Iris Advantage 1000; Thermo Electron Corp., USA) following digestion in a nitric-perchloric acid mixture (Bao 2005). Plant N concentration was measured using an elemental analyzer (Vario EL III; Elementar Analysensyst GmbH, Germany). In addition, 0–15 cm depth substrate cores were collected and analyzed for inorganic N (NH$_4$-N + NO$_3$-N), P, K, Ca, Mg, Na, Fe, and Mn under natural moisture condition (Bao 2005). NH$_4$-N and NO$_3$-N in the substrate were extracted with 1:5 ratio of substrate to water (w/v) and measured by spectrophotometry with salicylic acid and ultraviolet light (HeλIOSγ Ultraviolet-Vis light spectrophotometer; Thermo electronic Co., USA) (Wei 1990). Available P in the substrate was analyzed by molybdenum-antimony colorimetry after NaHCO$_3$ extraction (Bao 2005). K, Na, Ca, and Mg were extracted with 1 mol/l acetic ammonium (Bao 2005). K and Na were measured using the atomic emission spectrometry, and Ca and Mg using atomic absorption spectrometry (AA6800; Shimadzu Corp., Kyoto, Japan). Fe and Mn were analyzed by atomic absorption spectrometry (AA6800; Shimadzu Corp., Kyoto, Japan) following DTPA-CaCl$_2$-TEA extraction (Bao 2005).

On December 22, 2008, remainder plants was collected and analyzed for soluble protein content in celery stalks by the Coomassie Brilliant Blue staining method, soluble sugar by the anthrone method, and ascorbic acid (vitamin C) by the colorimetry of xylene extraction (Li et al. 2000). In addition, leaf chlorophyll and carotenoids in celery leaves were measured as described by Li et al. (2000). The above measurements were replicated three times.

**Data analysis**

Analysis of variance of the data was carried out using the SAS 6.12 software (SAS Institute, Cary, NC, USA). Regression analysis was performed to identify relationships between possible parameters using the SigmaPlot 9.0 software.

**RESULTS AND ANALYSIS**

**Substrate EC**

The measurements of in situ EC every week indicated that EC values were relatively stable over time (Fig. 1). EC values were significantly affected by Ca and Mg level (A), P supply (B), and A × B interaction. Irrespective of P supply, EC value significantly increased with increasing Ca and Mg level, which could be partly attributed to increased Cl and SO$_4$ concentrations. In general, P supply could decrease substrate EC. Especially under the high level of Ca and Mg, P supply significantly (P < 0.05) decreased...
Fig. 2. Effect of P on growing media nutrient availability under low (●), medium (○) and high (▲) levels of Ca and Mg. The vertical bars represented the standard errors. A – Ca and Mg level; B – P supply; A × B – Ca and Mg level × P supply interaction; NS – not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
substrate EC compared to 0 mg/l P treatment. Fig. 1 indicates that substrate EC did not change significantly at 124–496 mg/l P treatments.

**Growing media nutrient availability**

Fig. 2 shows that Ca and Mg level, P supply, and A × B interaction significantly affected available P and Mg concentrations in the substrate (P < 0.05). Relationship between available P concentration in the substrate and P supply could be fitted using a power function (\( y = 1.37 + 8.44x^{0.67}, R^2 = 0.90, P < 0.001, n = 15 \)), indicating that available P in the substrate increased more rapidly per unit of applied P than it did at the higher levels of P supply. Moreover, the level of Ca and Mg did not result in a significant change of P concentration in the substrate at low P treatment (0–124 mg/l P), while P concentration in the substrate significantly increased with increasing the level of Ca and Mg at high P treatment (248–496 mg/l P). Phosphorus supply significantly increased Mg concentration in substrate compared to zero-P treatment at each of Ca and Mg levels. In addition, irrespective of P application, Ca and Mg level could increase Mg concentration in substrate. Inorganic N, Fe and Mn concentrations in the substrate were significantly affected by P application and the level of Ca and Mg, while not by A × B interaction (Fig. 2). Regression analysis revealed that inorganic N concentration quadratically decreased with increasing P application (\( R^2 = 0.72, P < 0.001, n = 15 \)). Fe concentration significantly decreased as a power function of P application (\( R^2 = 0.80, P < 0.001, n = 15 \)). Mn concentration decreased and then increased with increasing P supply. In general, increased Ca and Mg level increased Fe and Mn concentrations in the substrate. Fig. 2 shows that Ca, K and Na concentrations in substrate were significantly influenced by P supply, but not by Ca and Mg level, and A × B interaction. Regression analysis indicated that Ca concentration in the substrate linearly decreased with increasing P supply (\( R^2 = 0.76, P < 0.001, n = 15 \)). Potassium (\( R^2 = 0.51, P < 0.01, n = 15 \)) and sodium (\( R^2 = 0.98, P < 0.001, n = 15 \)) concentrations decreased and increased as power functions of P supply, respectively.

**Fig. 3. Effect of P on celery above-ground fresh weight, total dry biomass, leaf area and root/shoot ratio under low (●), medium (○) and high (▲) levels of Ca and Mg. The vertical bars represented the standard errors. A – Ca and Mg level; B – P supply; A × B – Ca and Mg level × P supply interaction; NS – not significant; *P < 0.05; **P < 0.01; ***P < 0.001**
During experiment period, it was observed that plant growth was poor and plants gradually perished with the three treatments without P. The symptoms were chlorosis of leaves and then stalk rotted at the base. Therefore, celery quality and mineral composition were not measured with the three treatments without P. No symptoms of P toxicity were observed even at the highest P level. Generally, growth inhibition could be observed at the high level of Ca and Mg, which could be partly attributed to high Cl and SO\textsubscript{4} concentrations in supplied nutrient solution.

Fig. 3 shows that celery above-ground fresh weight and total dry biomass were highly influenced by Ca and Mg level ($P < 0.001$), P supply ($P < 0.001$), and A × B interaction ($P < 0.05$). Celery leaf area and root/shoot (R/S) ratio were significantly affected by P supply and the level of Ca and Mg ($P < 0.001$), whereas no significant differences were observed due to A × B interaction. In general, celery above-ground fresh weight, total dry biomass and leaf area significantly increased from 0 to 124 mg/l P treatment, whereas they did not significantly change from 124 to 496 mg/l P treatment. The opposite trend was observed for R/S ratio (Fig. 3). Thus, 124 mg/l P application was proposed to be appropriate for celery growth in this study. Fig. 3 indicates that the medium level of Ca and Mg was suitable for celery growth compared to low and high levels of Ca and Mg.
Chlorophyll, carotenoids, soluble protein, soluble sugar and vitamin C

Leaf chlorophyll and carotenoids in celery leaves were significantly affected by P supply, and Ca and Mg level, but not by A × B interaction (Table 1). Total chlorophyll and carotenoids quadratically decreased with increasing P supply, respectively. Moreover, irrespective of P supply, leaf chlorophyll decreased with increasing Ca and Mg level.

Table 1 shows that P supply, Ca and Mg level, and A × B interaction significantly \( P < 0.001 \) affected soluble protein and soluble sugar concentration in celery stalks. Vitamin C was significantly influenced by P supply and A × B interaction, but not by Ca and Mg level. In general, soluble protein, soluble sugar and vitamin C had decreasing trends from 0 to 124 mg/l P, while they did not significantly change or had increasing trends from 124 to 496 mg/l P treatment. Table 1 indicates that soluble protein decreased significantly at the high Ca and Mg level compared to the medium and low levels. Mean value of soluble sugar at the medium level of Ca and Mg was low compared to the low and high levels.

Mineral composition

Phosphorus supply significantly affected N and P concentrations in celery leaves, whereas no significant differences were observed due to Ca and Mg level, and A × B interaction (Table 2). Regression analysis revealed that N concentration in celery leaves decreased logarithmically with increasing P application, whereas P concentration in the leaves increased logarithmically with increasing P supply.

Table 2 shows that Ca, Mg, K and Na concentrations in celery leaves were significantly affected by P supply and the level of Ca and Mg, but not by A × B interaction except Na. Calcium and Mg concentration increased and then decreased with increasing P supply. Irrespective of P supply, Mg concentration in celery leaves increased with increasing Ca and Mg level, while the opposite trend was observed for Ca concentration. Regression analysis indicated that K concentration decreased linearly in response to the increase in P supply. The concentration of Na as P concomitant increased logarithmically with increasing P supply. In general, increased Ca and Mg level decreased K and Na concentrations in celery leaves. Ca and Mg level significantly affected Fe and Mn concentration. In general, Fe and Mn concentrations were low at the low Ca and Mg level compared to the medium and high levels.

DISCUSSION

In intensive horticultural production, the matching of nutrient supply with plant demand is an important challenge for high income, low cost and reduced environmental pollution. Many studies (Hoagland, Arnon 1950; Sonneveld, Straver 1994; Adams 2002; Sonneveld 2002; Jones 2005) have indicated that the commonly recommended P concentration for growing plants in hydroponic systems are 31–62 mg/l, and the recommended ranges of Ca and Mg concentrations are 80–200 mg/l and 24–96 mg/l, respectively. The demand for P by celery was found to be similar to other vegetable crops (Dufault 1985; Espinoza et al. 1993; Alt et al. 1999). However, in greenhouse production, soil and substrate cultures are essentially different from hydroponic culture. Available nutrients in soil and organic substrate are affected by many factors such as precipitation/dissolution and adsorption/desorption reactions, microorganism activity, organic matter and moisture content, which differs from hydroponic culture. Fig. 1 shows that increased P supply decreased substrate EC values compared to the treatment without P, which is probably due to the formation of Ca-P compounds (Grattan, Greve 1999) and the buffering capacity of substrate. In addition, Valentine et al. (2001) found that the variations in the availability of P were often associated with the concomitant changes in the availability of other essential nutrients.

In this study, available P concentration in the substrate increased less per unit of applied P at the high levels of P supply because the relationship between available P concentration in the substrate and P supply could be fitted using a power function \( y = 1.37 + 8.44x^{0.67}, R^2 = 0.90, P < 0.001, n = 15 \), which indicates that there is some buffering effect of growing medium and could explain why celery plants did not show P toxicity symptoms at high levels of P supply (Gikaara et al. 2004). Figs 2 and 3 show that, irrespective of Ca and Mg levels, celery variety Shanghai Huangxin Qin exhibited a critical NaHCO\(_3\)-extractable P level for deficiency of about 23.5 mg/kg (124 mg/lP treatment). Meanwhile, about 0.25 mS/cm (medium Ca and Mg level) of substrate EC was found to be appropriate for celery growth.
Fig. 2 shows that different elements in the substrate responded differently as affected by P supply under different Ca and Mg levels, indicating that microbial activity, precipitation/dissolution and adsorption/desorption reactions in the substrate were very complex. In addition, the growth stage of plants can have an effect on elemental contents and their ratios in the substrate due to nutrient uptake, especially N and K. Some studies have shown that there is a competition between cations in the growing medium including monovalent and divalent (Li, Li 1998; Ao et al. 2008). In this study, Na concentration in the substrate was negatively correlated with K ($R^2 = 0.51$, $P < 0.001$, $n = 15$) and Ca ($R^2 = 0.50$, $P < 0.01$, $n = 15$) contents, respectively, but not Mg. In addition, Ca concentration in the substrate did not significantly increase with increasing Ca and Mg level, indicating that Ca was easily replaced by other ions in the growing media and leaked. Fig. 2 shows that P supply decreased DTPA-extractable Fe concentration in the substrate, which was probably the result of covalent bonding of phosphate ions to Fe (Zhang et al. 2001). Therefore, excessive P supply could result in Fe deficiency. On the contrary, Shuman (1988) believed that increased P can increase DTPA-extractable Fe concentration, which was probably due to the change of pH. In this study, DTPA-extractable Mn concentration in the substrate increased at high P treatments. Similarly, Shuman (1988) and Zhang et al. (2001) found that P supply increased DTPA-exchangeable and amorphous iron oxide-bound soil Mn.

### Table 2. Effect of P on mineral composition in celery leaves under different Ca and Mg levels (mg/g DM)

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Significance level $P$

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NS – not significant; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. 

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Interactions of nutrient elements in substrate significantly affected nutrient uptake of celery. High concentrations of available nutrients in the substrate did not always result in high nutrient uptake by plant due to the imbalance of nutrients (Medina et al. 2009). Regression analysis revealed that available P concentration in substrate had a logarithmic relation with the P contents in the leaves of celery ($R^2 = 0.88$, $P < 0.001$, $n = 15$), which was in agreement with the results of Marschner (1995). Meanwhile, celery grew well at a P concentration of approximately 0.1% in the leaves, which was similar to other crops (Marschner 1995). The K content in celery leaves linearly decreased with increasing Na in the leaves at each of Ca and Mg levels ($R^2 > 0.85$, $P < 0.05$, $n = 5$), indicating that there was a competition between K and Na in the leaves (Gratan, Grieve 1999). Increased Ca and Mg level increased Mg concentration in celery leaves, but not Ca, which was in agreement with the change of Ca and Mg in the substrate. In general, K concentration in celery stalks was 1.5–2.2 times higher than in the leaves, while Mg and Ca in stalks were 0.5–0.7 and 0.4–0.7 times higher than in the leaves, respectively. Fe in stalks was mainly the same as in leaves (data not shown). Meanwhile, appropriate Ca and Mg supply can enhance Fe concentration in celery shoots. Some information about K and Mg contents in different celery tissues was interesting from a nutritional point of view, because fruits and vegetables usually contribute about 35% and 24%, respectively, to the total K and Mg dietary intake of humans (Levander 1990). In addition, vegetable is one of important sources for Fe dietary intake of humans.

It is well known that crop growth and yield are negatively affected by imbalance of nutrients in the growing media (Savvas, Adamidis 1999). In the present experiment, celery above-ground fresh weight, dry shoot biomass and leaf area were significantly reduced at low P treatments (0–62 mg/l) as a result of P deficiencies. Especially, at the 0 mg/l P treatments (1.2, 1.3 and 1.5 mg/kg NaHCO$_3$-extractable P in the substrate under three Ca and Mg levels, respectively), celery stopped growing and gradually perished. Meanwhile, the high level of Ca and Mg salts (Ca 640 and Mg 384 mg/l) had negative effects on celery above-ground fresh weight, dry shoot biomass and leaf area, which was probably due to osmotic stress. The reduction in yield as a result of P deficiencies was reported previously in many plants (Silber et al. 2000; Gikaara et al. 2004; Johnston et al. 2006). The main effect of P deficiency is the reduction in celery growth rate leading to darker green and smaller leaves, shorter stature, fewer leaves and imbalance between shoot and root growth (Dufault 1985). The imbalance between shoot and root growth indicated that partitioning in the plants was significantly affected by the P concentration and the level of Ca and Mg. Westgate et al. (1954) found that increased soluble salt concentration can cause celery black-heart, which decreases celery quality. In this study, about 0.25 mS/cm of substrate EC was believed to be appropriate for celery yield. Table 1 shows that soluble protein, soluble sugar and vitamin C concentrations in celery stalks were high at the low P treatments (31 and 62 mg/l) compared to the high P treatments (124, 248, and 496 mg/l), indicating that low P supply had stress effects. In general, the results of this study suggest that 124–248 mg/l P supply under the conditions of medium level of Ca and Mg (Ca 320 and Mg 192 mg/l) were appropriate for the improvement of celery horticultural traits.

References


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