Calcium Effects on Yield, Mineral Uptake and Terpene Components of Hydroponic Chrysanthemum coronarium L.

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Abstract: An experiment was conducted to study the relationship between calcium level in the hydroponic solution on Chrysanthemum coronarium L. yield and essential oil contents. Regression analysis showed that the maximum yield was achieved in 30.9 mM of calcium in nutrient solution for leaves ($r=0.970^{**}$) and 38.0 mM for flowers ($r=0.950^{***}$). A highly positive correlation ($r=0.961^{***}$) was found between sesquiterpene lactone and calcium in the flower part. Maximum yield of essential oil in leaf part was achieved in 39.7 mM Ca treatment ($r=0.933^{***}$), and the correlation between essential oil and calcium contents of leaves was high ($r=0.967^{***}$). Therefore, the increase of calcium concentration in nutrient solution had positive effects on the contents and yields of effective component, terpine, from C. coronarium L. The content of sesquiterpene lactone increased with increase of calcium concentration in nutrient solution.

Key words: Chrysanthemum coronarium L., terpene, essential oil, calcium, mineral content

INTRODUCTION

Bioactive terpenes, such as dihydrochrysanolide and cumambrin A isolated from Chrysanthemum coronarium L., were proven to have the medicinal effects on cancer prevention[1,2] and blood-pressure reduction[9]. These terpenes (mainly sesquiterpenes and monoterpenes) are synthesized from acetyl CoA via the mevalonic acid pathway[8]. The elucidation of the bioactive substances from this plant is important to increase its vegetable value. The content of essential oil including terpenes and its composition in plant are affected by different factors, primarily by its genetics[5] and cultivation condition, such as harvesting time, climate and the use of fertilizer. Nitrogen fertilization was reported to reduce terpene content in Juniperus horizontalis[8], although it increase total oil yield in Thyme[11]. Nitrogen limiting conditions increase volatile terpenes production in annual herbal plants[8]. Soil application of P and N plus P fertilizer reduced the concentrations of terpenoid lactones in Ginkgo biloba seedling[9]. Mg is known as a cofactor in the biosynthesis of isoprenoid in tabacco[10]. Calcium is an essential plant nutrient which constitutes between 0.2 and 5.0% on a leaf dry weight basis[11]. In its ionic (Ca$^{2+}$) form, calcium plays important roles as a structural component of the cell wall and cell membranes, as a cytoplasmic second messenger, mediating various hormonal and environmental cues that provoke appropriate physiological responses, and as a counter cation for inorganic and organic anions in the vacuole[11]. Calcium deficiency disorder is usually alleviated by helping plants increase their Ca$^{2+}$ uptake from the surrounding media and transport the mineral to the Ca$^{2+}$-deficient organs[11]. Increasing Ca content in soil has shown to increase its content in the plant[12]. It was reported that essential oils terpene content in plant tissue of basil (Ocimum basilicum L.) showed a high coefficient correlation with calcium content, being higher than with nitrogen content[13]. However, study on agronomic factors concerning calcium fertilizations on terpene contents of C. coronarium L. was not investigated thoroughly till now. In this study we determined the relationship between calcium nutrient in hydroponics solution and the formation of effective components like sesquiterpene lactones and essential oils of flowerhead of C. coronarium L.
MATERIALS AND METHODS

Treatment and composition of nutrient solution: *Chrysanthemum coronarium* L. plants were grown in a hydroponic system with a nutrient solution based on the formulation suggested by the Applied Plant Research, Division Glasshouse Horticulture, the Netherlands[14]. Nutrient solution comprised of 2.0 mM KH₂PO₄, 19.0 mM Ca(NO₃)₂.4H₂O, 7.1 mM KNO₃, 1.3 mM NH₄NO₃, 4.0 mM MgSO₄.7H₂O, 2.0 mM Mg(NO₃)₂.6H₂O and micronutrient. Five grades of calcium ion concentrations were selected as calcium treatments; 10, 20, 30, 40, 50 and 70 mM, concentration curves of purified cumambrin A and dihydrochrysanolide was 6.59 and 13.57 µl: maximum absorption at 254 nm. The retention time of cumambrin A and dihydrochrysanolide as standards.

Five grades of calcium ion concentrations were selected as calcium treatments; 10, 20, 30, 40, 50 and 70 mM, adjusted by using CaCl₂. The electrical conductivities (EC) of each solution were 2.3±0.10, 2.2±0.08, 2.6±0.09, 2.8±0.09, 3.3±0.11 and 3.6±0.13 mS cm⁻¹, respectively, and pHs of each solution were 5.5±0.19, 5.5±0.20, 5.5±0.21, 5.5±0.21, 5.4±0.25 and 5.4±0.29, respectively.

Plant preparations and cultivation: The seeds of *C. coronarium* L. were purchased from Hungnong Seed Company in Seoul, Korea. The seeds were sown on the surface of sterilized sand saturated with deionized water. After 3 weeks, the plants with 10 cm height were transferred into treatment modified nutrient solution. On 12 February, the plant was transplanted in the container (60x 40x 15 cm, 18 L per pot) in the deep floating technique (DFT) system. Modified nutrient solutions was circulated continuously by air pump and were grown in a greenhouse under natural light conditions, a daytime temperature of 25°C and relative humidity of 65-70%. Containers were arranged in a completely randomized block design with three replications. The nutrient solution was replaced with a fresh solution every 7 days in the early growth stage (12 February - 12 March) and every 3 days in the later growth stage (13 March - 20 April). The leaves and flowers were harvested on March 12, 2001 and April 20, 2001, respectively. At harvesting time, the plant characteristics were investigated by the RDA methods[15].

Inorganic element and chlorophyll content: Flower-heads and leaf tissues were separated after harvesting and air-dried at 70°C for 6 days. Dried materials were ground and then digested in H₂SO₄ for total nitrogen or in a ternary solution (HNO₃:H₂SO₄:HClO₃ = 10:1:4 with volume) for the determination of P, K, Ca and Mg[16]. Chlorophyll was extracted by 80% (v/v) acetone and its contents were determined at 663 nm and 645 nm by a Hitachi U-2000 dual length spectrophotometer[17].

Essential oil and sesquiterpene content: Essential oil contents of *C. coronarium* L. were determined with simultaneous distillation extraction (SDE) apparatus, using the methods described by Schultz et al[18]. Cumanbrin A, a major active component of *C. coronarium* L. flowerheads, was analyzed by using HPLC (Waters 201, Waters, USA) after CHCl₃ extraction at room temperature for 2 days[19]. The operating conditions were as follows: Adsorbsphere silica 5 µm column and Lambda-max detector; eluent of a dichloromethane: isopropanol (49:1) mixture; column temperature at 25°C; sample size of 5 µl: maximum absorption at 254 nm. The retention time of cumambrin A and dihydrochrysanolide was 6.59 and 13.57 min. The individual peak area was calculated using concentration curves of purified cumambrin A and dihydrochrysanolide as standards.

Statistical analysis: All statistical analysis was conducted by using one-way ANOVA of the Statistical Analysis System (SAS) computer package[20]. The different between means of treatments were compared by using the Duncan's multiple range tests. The effects of treatments were considered as a significant when the probability for observed F-value was equal to or smaller than 5%. Multiple regression analysis was conducted to determine the effect of mineral element on productivity and effective components.

RESULTS AND DISCUSSIONS

Growth and yields: Growth and yield characteristics of *C. coronarium* L. are shown in Table 1. Plant height was significantly affected by calcium level, increased from 79.3 cm in 10 mM calcium to 86.3 cm in 40 mM calcium level. Stem diameter and leaf length showed similar pattern with those of plant height. Yield response showed similar tendency with growth characteristics. Dry weights of leaves and flowers of *C. coronarium* L. increased significantly with increasing calcium concentration in nutrient solution. Correlation coefficients were observed between leaf yields and calcium concentration in nutrient solution (γ=-0.0043X+0.266X+8.242, r=0.970, p<0.001), and flower yields and calcium concentration in nutrient solution(γ=-0.0041X²+0.312X+5.488, r=0.950, p<0.001). The maximum yields were achieved in 30.9 mM of calcium in nutrient solution for leaves and 38.0 mM for flowers. Chlorophyll content increased from 36 µg ml⁻¹ in 10 mM calcium to 48 µg ml⁻¹ in 40 mM calcium concentration in nutrient solution. Similarly, Takano and Yamamoto[21] reported that the yield of leaf was increased significantly by increasing calcium level in basil (*Ocimum basilicum* L.).

Mineral uptake: The nutrient content in leaves and flowers of *C. coronarium* L. was significantly affected by
calcium concentration levels in nutrient solution (Table 2). The calcium contents of leaves and flowers were increased significantly by increasing calcium concentration. However, P and Mg mineral contents of leaves and flowers in Ca treatment of hydroponics decreased significantly with increasing calcium concentration in nutrient solution. Many other studies reported similar results that the growth of leaf, stem and root part of crops increased with increasing calcium concentration in nutrient solution, and the calcium uptake of plants also showed the same tendency\textsuperscript{[22,23]}. Antagonism relationships of calcium and magnesium absorption of plants in the nutrient solution have also been reported in several studies\textsuperscript{[22,23]}.  

\textbf{Sesquiterpene lactone:} Calcium in the hydrophonic medium affected sesquiterpene lactones content in the flower of \textit{C. coronarium} L. and their yield (Table 3). Both cumambrin A and dihydrochrysanolide, as representatives of sesquiterpene lactone, showed a similar response to increasing calcium concentration in the nutrient solution, and a 71% increase in sesquiterpene lactones was achieved when calcium in the hydrophonic medium was increased from 10 mM to 40 mM. Regression analysis showed that maximum yields of cumambrin A and dihydrochrysanolide were achieved in 40.9 mM Ca treatment \textit{(Y=\textasciitilde0.0115X+0.941X+0.439, r=0.935, p<0.001)}. Total yields of the two compounds from the flower part increased to about 2.4 times in 40 mM Ca treatment, from

\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Ca\textsuperscript{2+} (mM)} & \textbf{Growth characteristics (cm)} & \textbf{Dry weight (g plant\textsuperscript{-1})} & \textbf{Chlorophyll content (ug ml\textsuperscript{-1})} \\
\hline
\textbf{Ca uptake (mg plant\textsuperscript{-1})} & \textbf{T-N} & \textbf{P} & \textbf{K} & \textbf{Ca} & \textbf{Mg} \\
\hline
\hline
10 & 79.3 & 8.9c & 12.2c & 10.6c & 8.4d & 32b & 14a & 46b \\
\hline
20 & 80.2b & 9.1bc & 12.7bc & 11.7c & 9.4d & 34a & 13a & 47b \\
\hline
30 & 85.2ab & 9.6b & 13.9ab & 12.1bc & 11.5c & 35a & 13a & 48a \\
\hline
40 & 86.3a & 9.9a & 14.5a & 12.7a & 11.8b & 35a & 13a & 48a \\
\hline
50 & 72.3bc & 9.2bc & 13.7a & 10.4a & 10.2a & 34a & 12a & 46b \\
\hline
70 & 64.5cd & 8.7c & 12.3c & 5.9b & 7.1b & 31b & 10b & 44c \\
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\hline
\end{tabular}
\caption{Yield and growth characteristics of \textit{C. coronarium} L. cultivated in the different calcium treatment}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Ca\textsuperscript{2+} (mM)} & \textbf{Mineral content (g kg\textsuperscript{-1})} & \textbf{Ca uptake (mg plant\textsuperscript{-1})} \\
\hline
\textbf{Plant height} & \textbf{Stem diameter} & \textbf{Leaf length} & \textbf{Leaf} & \textbf{Flower} & \textbf{Chl.a} & \textbf{Chl.b} & \textbf{Total} \\
\hline
10 & 79.3 & 8.9c & 12.2c & 10.6c & 8.4d & 32b & 14a & 46b \\
\hline
20 & 80.2b & 9.1bc & 12.7bc & 11.7c & 9.4d & 34a & 13a & 47b \\
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\hline
70 & 64.5cd & 8.7c & 12.3c & 5.9b & 7.1b & 31b & 10b & 44c \\
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\end{tabular}
\caption{Mineral composition and calcium uptake of \textit{C. coronarium} L. grown in the different levels of calcium treatment}
\end{table}
9.13 mg plant\(^{-1}\) in 10 mM Ca treatment. Calcium and sesquiterpene lactone contents in the flower part of \(C.\) coronarium \(L.\) have a highly positive correlation \((r=0.961, p<0.001)\) (Fig. 1). We found similar results in \(Chrysanthemum\) boreale \(M.\) [25]. In \(C.\) boreale, cumambrin A increased to 34 and 19% by lime and fly ash application in soil, and positive correlation between calcium content and cumambrin A in the flower part of \(C.\) boreale \(M.\) was also found [25]. This means that relative increases of sesquiterpene lactone cyclase and synthase by calcium were related directly in sesquiterpene lactone

\[
y = 0.051x + 0.713 \\
r = 0.897^{**}
\]

\(y = 0.119x + 7.223 \\
r = 0.853^{**}
\]
essential oil yield in leaves (Table 4). Regression analysis demonstrated that maximum yields of essential oil in leaf were achieved in 39.7 mM Ca treatment (Y\(=\)0.00001X\(^2\)+0.0069X-0.014, r=0.933, p<0.001), and a high correlation was observed between essential oil and calcium contents of leaves (r=0.967, p<0.001) (Fig. 2). Optimum fertilization could increase the yield of essential oils in the herbal plant like lemon-grass, bergamot mint and Japanese mint\(^{[1,3]}\). These results were contradictory to the results reported in basil (Ocimum basilicum L.) by Suh and Park\(^{[1]}\) that increasing calcium concentration in nutrient solution remarkably decreased the essential oil yield.

This study demonstrates the importance of calcium application to improve plant growth and essential oil yield in C. coronarium L.

REFERENCES