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Changes in Mineral and Terpene Concentration Following Calcium Fertilization of *Chrysanthemum boreale* M.

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Abstract: With increasing concerns for health-improving foods, the demand for the flowers of *Chrysanthemum boreale* M. has become higher than ever. To fulfill the increasing demand for a high quality flower we investigated the changes induced by calcium application on the terpene yield, mineral content and plant growth of *C. boreale* M. The results showed that the maximum flower yield was achieved at 2.1 ton $CaCO_3$ ha^{-1}. The dry weight of the flower and the total plant yield increased significantly up to 1.5 ton $CaCO_3$ ha^{-1} treatment. Further addition of calcium beyond this point was counterproductive. Photosynthetic responses were increased at 1.5 ton $CaCO_3$ ha^{-1} compared to the control. The calcium content and uptake by the flowers of *C. boreale* M. increased significantly with increasing calcium application rate. Increasing calcium application rate correlated positively with flower sesquiterpene lactone, essential contents and yield. At 1.5 ton $CaCO_3$ ha^{-1} terpene content was increased sesquiterpenes (30.4%) and monoterpenoids (9.5%), compared to the control. The most striking increase was in the concentrations of germacrene-D (39.4%) and epi-sesquiphellandrene (29.5%) compared to the control. This experiment suggests that calcium application could improve the yield and quality of the flower in *C. boreale* M.

Key words: *Chrysanthemum boreale* M., calcium, mineral uptake, photosysnthesis, terpene, essential oils

INTRODUCTION

Chrysanthemum boreale M. is an important medical plant that has been historically used in herbal medicine and in health foods throughout East Asia[1]. The edible flowerheads of *C. boreale* M. contain terpene components including monoterpenoid and sequiterpenoid, some of which have been reported to exhibit a wide range of biological activity[2,3]. Nam and Yang[4] found that cumambrin A, a sesquiterpene compound isolated from wild *C. boreale* M., has significant effects on blood-pressure reduction and is used as an industrial product[5]. Harvesting of *C. boreale* M. from natural resources has become more difficult, especially with the increasing concern for environmental protection. Future demand for *C. boreale* M. should be fulfilled primarily from the harvest of cultivated plants, and thus the management practice to optimize its sustainable production should be explored. The essential oil composition in a harvested herbal plant is affected primarily by its genetics[6,7] and other conditions such harvest time, climate and the use of fertilizer. Some researchers have reported that calcium fertilizers can increase essential oil and its composition in basil (*O. basilicum* L.)[8] and *C. coronarium* L.[9,10]. In addition, we have found that plant growth regulators (PGRs), such as abscisic acid (ABA) and salicylic acid (SA), can increase the yield and active components of *C. boreale* M. flowerheads[11]. However, studies on agronomic factors such as the calcium application on the terpene content of *C. boreale* M. has not been thoroughly investigated until now. In this paper, we report on the effect of calcium application on the mineral uptake, photosynthetic rate and terpene concentration (sesquiterpene lactones and essential oils) of flowers in *C. boreale* M. with the goal of developing a more efficient cultivation method.

MATERIALS AND METHODS

Plant cultivation: The effects of calcium application on *Chrysanthemum boreale* M. growth were examined in a greenhouse experiment using Aquepts Series, Typic

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Endoaquepts (USDA, Inceptisols) soil collected from Chinju, Kyungnam province, Korea. The chemical properties of the soil were as follows: pH 5.1, organic matter 6 g kg^{-1}, 4.5 g kg^{-1} available P_2O_5, and 2.2 cmol (+) kg^{-1} exchangeable calcium. About 18 kg of dried soil was packed in each 1/2000a Wagner pot, in which the following were applied: 0, 1.5, 13.0 and 4.5 ton $CaCO_3$ ha^{-1}, respectively. The application rate of chemical fertilizer during the present study was used as a basal fertilizer (105 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, 56 kg K_2O ha^{-1}) on May 23, 2000, and 30% of the required nitrogen and potassium were side-dressed at approximately 20 days before flowering. Treatments were replicated three times and assigned to a randomized complete block design. Rooted cuttings of *C. boreal* M. were transplanted on May 25, 2000 and harvested on October 20, 2000. Flowerhead yield was determined at the full bloom stage. The photosynthesis of plants was measured using a Li-Cor 6400 (Li-Cor Inc, Lincoln, Nebraska, USA) before plant harvest.

Analysis of inorganic chemicals: To analyze mineral elements, soil samples were sieved (2 mm screen) and analyzed for the following: pH (1:5 water extraction), organic matter content (Wakley and Black method[12]), available P content (Lancast[13]) and contents of exchangeable Ca^{2+}, Mg^{2+}, and K^+ (1 M NH_4-acetate pH 7, AA, Shimazu 660). Flower tissues were separated after harvesting and air-dried at 70°C for 5 days. Dried materials were grounded and then digested in H_2SO_4 for total nitrogen or in a ternary solution (HNO_3:H_2SO_4:$HClO_4$ = 10:1:4 with volume) for the determination of P, K, Ca and Mg.

Essential oil and cumambrin A: The essential oils of *C. boreale* M. were obtained from flowerheads with a simultaneous distillation extraction (SDE) apparatus, using the methods of Lee et al.[14]. Terpene compounds were identified by computer matching of the mass spectra and confirmed by GC retention times. Cumambrin A, a major active component of *C. boreale* M. flowerheads, was analysed using an HPLC machine (Waters 201, Waters, USA) after $CHCl_3$ extraction at room temperature for 2 days[15,16].

Statistical analysis: Yield and active component contents were analyzed statistically by an analysis of variance using the Statistical Analysis System (SAS) computer package[17]. When the analysis of variance showed significant treatment effects ($P < 0.05$), the least significant difference (LSD), at a 0.05 level of significance[17], was used to compare treatment means of plant growth, productivity and effective component variables.

RESULTS AND DISCUSSIONS

Plant growth: This study investigated the effect of calcium application on plant growth and terpene content in *Chrysanthemum boreale* M. The results of plant growth and yield are shown in Table 1. Total dry matter yield per plant increased with increasing application level up to 1.5 ton $CaCO_3$ ha^{-1}, after which any further increase decreased the yield. Total dry matter weight increased from 90.0 g $plant^{-1}$ in the control to 103.8 g $plant^{-1}$ at 1.5 ton $CaCO_3$ ha^{-1} treatment. The predicted maximum yield was achieved at 2.1 ton $CaCO_3$ ha^{-1} for flower weight ($Y = -0.6704X^2 + 2.7967X + 15.528$, r=0.776, $p<0.01$). Plant stem diameter and branch number per plant were also increased at 1.5 ton $CaCO_3$ ha^{-1} compared to the control. Photosynthetic responses of *C. boreale* M. to the treatments, demonstrated similar trends to that of total dry matter weight responses. Similar results was reported by Supanjani et al.[9] who reported that yields of the leaf and flower of *C. coronarium* L. increased with increasing lime levels up to 2 ton ha^{-1}. The calcium content of flowers of *C. boreale* M. increased with increasing calcium application rate, and so did the total amount of calcium per plant (Table 2). Similar results have been reported in many other studies. In tomato and radish plants it was reported that the growth of leaf, stem and root parts increased with increasing calcium concentration in the nutrient solution, and the calcium uptake of plants also showed the same tendency[19,20]. The increase in yields was attributed to the improvement of the soil nutrient status, since the soil was very low in nutrients. These results suggest that calcium fertilizer should be considered when cultivating *C. boreale* M. in acidic soil amendments or less fertile soils.

Essential oil concentration: The addition of calcium fertilizer increased essential oil yields in the flower of *C. boreale* M. up to a maximum of 0.249 ml $plant^{-1}$ at 1.5 ton $CaCO_3$ ha^{-1}, which was 33.8% higher compared to the control. However, any further increase over 3.0 ton $CaCO_3$ ha^{-1} reduced the essential oil yield in flowers (Table 3). Regression analysis demonstrated that maximum yields of essential oil in the flower were achieved at 2.1 ton $CaCO_3$ ha^{-1} ($Y=2.3419X^2 + 9.6396X + 36.179$, r=0.868, $p<0.001$). This increased the total essential oil concentration and yield of *C. boreale* M. Increases in essential oil concentration following NPK additions at optimum levels have been reported in other herbs, such as in basil, bergamot mint and Japanese mint[21,22]. Changes in essential oil concentrations, along with their relative percentage as affected by calcium fertilizer, are given in Table 4. Most of the essential oil components for plants

Table 1: Yield, photosynthetic rate and growth of *C. boreale* M. cultivated at different levels of calcium fertilizer

$CaCO_3$ (ton ha^{-1})	Yield (g $plant^{-1}$)				Chlorophyll content (ug ml^{-1})	Branch no. (No. $plant^{-1}$)	Stem diameter (cm)
	Leaf	Stem	Flower	Total			
0	16.8	58.0	15.2	90.0	18.6	33.3	1.05
1.5	18.5	66.2	19.1	103.8	18.0	38.0	1.16
3.0	17.1	63.7	17.0	97.8	18.9	37.8	1.10
4.5	14.4	55.1	14.8	84.3	19.0	20.5	0.96
$LSD_{0.05}$	1.1	2.9	0.9	3.5	0.7	2.0	0.05

Table 2: Mineral content and calcium uptake of the flower of *C. boreale* M. cultivated ate different levels of calcium fertilizer

$CaCO_3$ (ton ha^{-1})	Mineral content (g kg^{-1})					Ca uptake (mg $plant^{-1}$)
	T-N	P	K	Ca	Mg	
0	15.1	2.5	22.2	4.9	3.1	74
1.5	15.3	2.8	22.7	5.9	3.5	113
3.0	15.4	2.6	22.6	7.0	3.4	119
4.5	15.2	2.7	21.2	8.4	3.2	125
$LSD_{0.05}$	1.0	0.2	2.1	0.8	0.6	5.5

Table 3: Changes in essential oil concentration in the flower of *C. boreale* M.

$CaCO_3$ (ton ha^{-1})	Essential oil concentration (mL kg^{-1})	Essential oil yield (mL $plant^{-1}$)
0	0.233	35.5
1.5	0.249	47.5
3.0	0.246	41.9
4.5	0.221	32.9
$LSD_{0.05}$	0.08	3.7

grown in soils with applied calcium fertilizer were higher compared to the control. Calcium fertilizer treatments under 1.5 ton $CaCO_3$ ha^{-1} increased sesquiterpenes (30.4%) more than monoterpenoids (9.5%). However, any further increase over 3.0 ton $CaCO_3$ ha^{-1} reduced the essential oil concentration in flowers. The most striking increase was germacrene-D (39.4%) and epi-sesquiphellandrene (29.5%) compared to the control treatment. We found plant growth regulators (PGRs) such as abscisic acid (ABA) and salicylic acid

Table 4: Changes in the major terpene compound concentrations (%) in the flower of *C. boreale* M.

Compounds	0		1.5		3.0		4.5	
	Mean	SD [a]	Mean	SD	Mean	SD	Mean	SD
Monoterpenoids								
1,8-cineol	2.02	0.055	2.09	0.053	2.11	0.048	2.06	0.044
"-thujone	1.35	0.020	1.42	0.024	1.38	0.021	1.35	0.019
Camphor	18.14	0.308	20.03	0.336	19.82	0.305	19.12	0.323
cis-chrysanthenol	2.77	0.063	3.04	0.075	2.85	0.067	2.99	0.070
Total monoterpenoids (%)	24.28		26.58		26.16		25.52	
Sesquiterpenoids								
$–caryophyllene	3.21	0.062	3.89	0.054	3.76	0.059	3.60	0.062
epi-sesquiphellandrene	1.83	0.019	2.37	0.043	2.29	0.050	2.07	0.044
Germacrene D	3.12	0.046	4.35	0.042	4.08	0.048	3.76	0.046
Caryophyllene epoxide	2.15	0.028	2.84	0.030	2.80	0.030	2.73	0.035
Total sesquiterpenoids (%)	10.31		13.45		12.93		12.16	

[a] SD: standard deviation (n=3)

Table 6: Chemical properties of soils with different levels of calcium fertilizer after the harvest of *C. boreale* M.

$CaCO_3$ (ton ha^{-1})	pH (1:5, H_2O)	OM (g kg^{-1})	T-N (g kg^{-1})	Available P_2O_5 (mg kg^{-1})	Ex. Cations (cmol(+) kg^{-1})		
					K	Ca	Mg
0	5.0	5.5	0.8	5.9	0.38	1.1	0.55
1.5	5.1	6.0	0.6	5.2	0.30	2.9	0.58
3.0	5.8	6.3	0.6	4.8	0.31	4.0	0.59
4.5	6.7	6.2	0.5	4.7	0.33	5.3	0.57
$LSD_{0.05}$	0.7	0.5	0.1	1.0	0.05	0.9	0.04

Table 5: Changes in the cumambrin A concentration in the flower of *C. boreale* M.

$CaCO_3$ (ton ha^{-1})	Sesquiterpene lactone concentration (g kg^{-1}, D.W.)	Sesquiterpene lactone yield (mg $plant^{-1}$, D.W.)
0	2.25	34.2
1.5	2.87	54.7
3.0	2.67	45.4
4.5	2.38	35.3
$LSD_{0.05}$	0.5	3.4

(SA) increased the essential oil yield of *C. boreale* M. flowerheads[11]. This was especially the case with germacrene-D in the ABA treatment where it was increased by 39.1% compared to control. Our results with *C. boreale* M. are in agreement with those reported for basil by Suh and Park[8].

Cumambrin A: Application of calcium fertilizer in soil changed the sesquiterpene lactone concentration in the flower of *C. boreale* M. (Table 5). Regression analysis showed that maximum yields of cumambrin A were achieved at 2.2 ton $CaCO_3$ ha^{-1} ($Y= -3.4003X^2 + 14.902X + 35.673$, $r=0.796$, $p<0.01$). We found similar results in *C. coronarium* L.[9]. The total content of the two sesquiterpene lactone compounds, cumambrin A and dihydrochrysanolide, in the flower of *C. coronarium* L. increased from 1.36 g kg^{-1} in the control to 1.69 g kg^{-1} at 2 ton lime ha^{-1}. A positive correlation existed between sesquiterpene lactone content and calcium concentration in the flower ($r=0.604$, $p<0.05$). Our results with *C. boreale* M. are in agreement with those reported for basil by Suh and Park[8]; these authors found that increased calcium concentrations in nutrient solutions increased the sesquiterpene content more than the monoterpene concentration. This means that calcium treatment increased essential oil levels more by activating sesquiterpene than monoterpene biosynthesis and cyclase. The physiological role between sesquiterpene lactone and calcium is still unclear and further studies are required.

Soil properties: The chemical properties of soil at different levels of calcium application after the harvest of the flower of *C. boreale* M. are shown in Table 6. Soil pH and organic matter was increased from 5.0 and 5.5 to 6.7 and 6.2 by the $CaCO_3$ treatment, respectively. Exchangeable calcium was increased to 5.3 cmol(+) kg^{-1} by amending with $CaCO_3$, but other exchangeable cations in the soil at harvest did not increase. Since the soil was collected from a relatively sterile mountainous area, the soil nutrient content before transplanting was very low and scarcely changed after harvest.

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