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EFFECT OF CALCIUM, BORON AND MOLYBDENUM ON PLANT GROWTH AND BRACT PIGMENTATION IN POINSETTIA

EFECTO DEL CALCIO, BORO Y MOLIBDENO EN EL CRECIMIENTO DE PLANTA Y PIGMENTACIÓN DE BRÁCTEAS EN NOCHEBUENA

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SUMMARY

Foliar sprays of Ca (300, 400, and 500 mg L^{-1}), B (0.2, 0.5, and 0.8 mg L^{-1}), Mo (0.3, 0.4, and 0.5 mg L^{-1}), Ca + B (400 + 0.5 mg L^{-1}), Ca + Mo (400 + 0.4 mg L^{-1}), B + Mo (0.5 + 0.4 mg L^{-1}) and Ca + B + Mo (400 + 0.5 + 0.4 mg L^{-1}), were applied to improve the quality of poinsettia plants (*Euphorbia pulcherrima*) cv. 'Supjibi Red'. Treatments were applied three times at: beginning, middle, and end of the short photoperiod. Calcium at 400 mg L^{-1} increased significantly plant height by 15.3 %. Leaf chlorophyll concentration decreased by 25% when bract pigmentation initiated. Treatments did not affect the leaf chlorophyll contents. Calcium (300 mg) and B (0.8 mg) increased the number of transitional bracts (5.7 and 5.6, respectively) compared to 0.4 mg L^{-1} Mo treatment; while B (0.5 mg) increased the total number of colored bracts per shoot (8.36) compared to the rest of the treatments. Total chlorophyll concentration decreased by 95 % in transitional bracts, carotenoids decreased 89 % and anthocyanins increased considerably (from 21.4 to 296.7 mg g^{-1}). Foliar applications of calcium improved poinsettia plant height and the Ca plus B combination accelerated bract pigmentation.

Index words: *Euphorbia pulcherrima*, plant height, anthocyanins, chlorophyll, carotenoids.

RESUMEN

Aspersiones foliares de Ca (300, 400 y 500 mg L^{-1}), B (0.2, 0.5 y 0.8 mg L^{-1}), Mo (0.3, 04 y 0.5 mg L^{-1}), Ca + B (400 + 0.5 mg L^{-1}), Ca + Mo (400 + 0.4 mg L^{-1}), B + Mo (0.5 + 0.4 mg L^{-1}) y Ca + B + Mo (400 + 0.5 + 0.4 mg L^{-1}), se aplicaron para mejorar la calidad de plantas de nochebuena *Euphorbia pulcherrima* cv. 'Supjibi Red'. Los tratamientos se aplicaron tres veces: al inicio, mitad y final del fotoperiodo corto. El Ca (400 mg L^{-1}) incrementó significativamente la altura de la planta en 15.3 %. La concentración de clorofila en hojas disminuyó 25 % cuando las brácteas iniciaron la pigmentación. Los tratamientos no afectaron el contenido de clorofilas en las hojas. El Ca (300 mg) y B (0.8 mg) incrementaron el número de brácteas de transición (5.7 y 5.6, respectivamente), comparado con el tratamiento con 0.4 mg L^{-1} de Mo; mientras que el B (0.5 mg) incrementó el número total de brácteas coloreadas por brote (8.36) en comparación con el resto de los tratamientos. La concentración de clorofila total disminuyó 95 % en las brácteas de transición, los carotenoides disminuyeron 89 % y las antocianinas se incrementaron considerablemente (de 21.4 a 296.7 mg g^{-1}). Las aplicaciones foliares de calcio mejoraron la altura de la planta y la combinación de Ca más B aceleró la pigmentación de las brácteas.

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Palabras clave: *Euphorbia pulcherrima*, altura de planta, antocianinas, clorofila, carotenoides.

INTRODUCTION

Poinsettia (*Euphorbia pulcherima* Willd. ex Klotzch) is one of the most popular potted plants during the Christmas season. There is not official information available in México regarding the production of poinsettia potted plants; however, "Viveros Plantec", the leading nursery in poinsettia production in México, reported 8.5 million potted plants produced in 2002. The value of poinsettia production increased from 20 million pesos in 2000 to 126 million pesos in 2004. Twenty years ago, poinsettia was cultivated only in two states, Distrito Federal and Morelos, but currently it is also grown in the states of México, Michoacán, Puebla, Jalisco and Nuevo León (SIACON, 2004).

Good plant quality of poinsettia is determinant for a successful marketing because Mexican consumers are becoming more demanding. Thus, it is important for growers to know and control the main factors affecting plant growth. Nutrition and fertilization practices have a prominent impact on the growth and quality of poinsettias. It has been reported that poinsettia requires high levels of nitrogen and potassium (Martínez, 1995), as well as an unusually high requirement of calcium, magnesium and molybdenum (Dole and Wilkins, 2005). According to McAvoy and Bible (2000), the main factor for poinsettia bract necrosis development is nutrition.

Calcium (Ca), molybdenum (Mo) and boron (B) nutrition are critical for producing good quality poinsettia plants. Calcium deficiency is reported to reduce leaf growth, cause internode shortening near the apical bud, induce weak and malformed stems, as well as bract

necrosis (Stromme *et al.*, 1994). In addition to supply adequate Ca levels to the growing medium, Ca [$Ca(NO_3)_2$] can also be sprayed weekly at concentrations from 200 to 400 mg L^{-1}, starting at bract pigmentation and continuing until anthesis (Dole and Wilkins, 2005). Poinsettias are sensitive to B deficiency and toxicity. Boron deficiency symptoms include cessation of terminal growth and leaf and stem distortions (Ecke *et al.,* 2004; Dole and Wilkins, 2005). Plants with low B content in bracts are more susceptible to bract necrosis (McAvoy and Bible, 2000). Poinsettias have a high requirement of Mo, which is often supplied through a constant liquid fertilization program at 0.1 mg L^{-1}. Mo deficiency symptoms include marginal chlorosis, upward rolling and edge burn of recently mature leaves (Junk *et al.*, 1970; Dole and Wilkins, 2005).

Calcium and B are nutrients with very slow mobility within the plant because their transport is mainly driven by the transpiration stream. Mo is required for nitrogen metabolism (Marschner, 1995). Poinsettia bracts have a low stomatal density (Nell and Barrett, 1986) and are low-transpiring organs (Gislerød, 1999), which difficult the mobility of Ca, B and Mo to the bracts. It is necessary then the study of other alternatives to supplement these elements to poinsettia plants. Foliar spray may be such an alternative, since it allows the application of nutrients directly to the demanding organs, bypassing the slow mobility in the xylem and phloem due to low bract transpiration rates. In addition, there is little research concerning the role of foliar nutrient supply on the flower pigmentation and growth of poinsettia in México. Thus, the objective of the present research was to evaluate the effect of foliar sprays of Ca, B and Mo on plant growth and bract pigment content in poinsettia 'Supjibi Red'.

MATERIALS AND METHODS

The experiment was conducted during the Summer and Fall of 2003 in Texcoco, México located at 19° 31' NL and 19° 53' WL and 2310 m altitude. Poinsettia plants of cv. 'Supjibi Red' were transplanted in a modified tunnel greenhouse of plastic glazing with a 30 % shade intensity. 'Supjibi Red' is a potted poinsettia cultivar with vigorous and rapid growth, brilliant red bracts and dark green leaves (Martínez, 1995). Minimum and maximum temperatures and relative humidity were daily recorded with a digital thermo-hygrometer (Control Company, USA), and the monthly averages are shown in Table 1.

Liners of 'Supjibi Red' were transplanted on July 29th, 2003 in 17.8 cm diameter pots filled with a mixture of composted pine leaves and forest soil (7:3 v/v). The plants were soft-pinched one day before transplanting, leaving 5 to 7 buds remaining in the shoot, and allowed for vegetative growth for 50 d after transplanting (DAT); afterwards, artificial short days were imposed (10 h light + 14 h darkness) for eight weeks.

Table 1. Monthly average of maximum and minimum temperatures (°C) and relative humidity (RH) (%) prevailing during the experiment.

Month	Maximum temperature	Minimum temperature	Maximum RH	Minimum RH
August	29.3	17.2	72.1	44.0
September	28.6	17.6	77.0	42.0
October	29.2	16.2	78.6	40.2
November	26.7	15.1	76.1	37.5

One a week irrigation schedule was applied during the establishing period with a nutrient solution (mg L^{-1}) of: 300 N (NH_4NO_3), 80 P ($NH_4H_2PO_4$), 120 K (KNO_3), 350 Ca [$Ca(NO_3)_2 \cdot 4H_2O$] and 75 Mg ($MgSO_4 \cdot 7H_2O$), at pH 7. The volume of the irrigation solution was 250 mL per pot. Water used for solution preparation had pH of 7.2 and electrical conductivity (EC) of 0.24 dS m^{-1}, and a mineral composition (meq L^{-1}) of: 0.50 CO_3^{2-}, 1.49 HCO_3^-, 2.75 Cl, 0.93 Ca, 0.10 Mg, 1.07 Na and 0.12 B. Micronutrients were not supplied because the growing medium had high concentrations of Fe (1.2 mg L^{-1}), Cu (27.3 mg L^{-1}), Zn (109.4 mg L^{-1}), Mn (2.97 mg L^{-1}) and B (2.94 mg L^{-1}). Macronutrients content in the growing medium was (mg L^{-1}): 169.2 N, 0.11 P, 112.7 K, 18.8 Ca and 28.3 Mg. The growing medium had a pH of 7.2, cation exchange capacity (CEC) of 49.9 Cmol kg^{-1} and EC of 0.9 dS m^{-1}. In addition, plants were irrigated twice a week with 500 mL per pot, a volume enough to bring the substrate to container capacity and to allow a leaching fraction of 25-30 %.

The applied treatments were the foliar sprays with three concentrations of Ca: 300, 400 and 500 mg L^{-1} [$Ca(NO_3)_2 \cdot 4\ H_2O$], three concentrations of B: 0.2, 0.5 and 0.8 mg L^{-1} (H_3BO_3), three concentrations of Mo: 0.3, 0.4 and 0.5 mg L^{-1} ($Na_2MoO_4 \cdot 2\ H_2O$), plus the following combinations (mg L^{-1}): 400 Ca + 0.5 B, 400 Ca + 0.4 Mo, 0.5 B + 0.4 Mo and 400 Ca + 0.5 B + 0.4 Mo. The pH of the sprayed solution varied between 5 and 6. The solutions were sprayed until dripping (approximately 35 to 40 mL per plant) to both leaf surfaces, at 50, 72 and 99 DAT (at the beginning, at the middle and the end of the inductive short day treatment, respectively). A set of control plants with no foliar sprays was included. The 13 treatments were distributed in a completely randomized experimental design with ten replicates, each replication consisting of one plant (one pot).

The variables measured were: plant height, stem diameter, leaf area, specific leaf weight, number of shoots per plant, and length and diameter of lateral shoots (measured at the end of the experiment, 121 DAT). A recently mature,

entire and photosynthetically active leaf from the mid section of the plant was sampled from five plants per treatment, in order to measure total chlorophyll and chlorophylls a and b concentrations, at 50, 70, 99 and 121 DAT. One bract was also sampled from each of the five plants at 45, 65 and 79 d after initiation of the inductive short photoperiod, to evaluate concentrations of chlorophyll, carotenoids and anthocyanins, determined according to Witham *et al*. (1971), Lichtenthaler (1987) and Kannangara and Hansson (1998), respectively. The number of transitional bracts was determined when bract pigmentation initiated (75 DAT), while the total number of pigmented bracts per shoot was determined at the end of the growing period. Data were statistically analyzed with SAS by analyses of variance and Tukey's multiple mean comparison test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Vegetative growth

Foliar sprays of Ca at 400 mg L^{-1} produced taller plants (29.4 cm) compared to the control plants (25.5 cm) (Table 2). Plants treated with 400 mg L^{-1} Ca + 0.4 mg L^{-1} Mo had a similar plant height (25.7 cm) as that of the control. No significant differences were detected in the rest of the reatments. There was no treatment able to produce thicker stems than the control plants, but the solution containing 0.5 mg L^{-1} Mo significantly reduced the stem diameter (0.99 cm) compared to plants sprayed with 400 mg L^{-1} of Ca. No significant treatment effects were detected on leaf area and specific leaf weight (data not shown).

Table 2. Effect of foliar sprays of Ca, B and Mo on stem height and diameter of poinsettia plants.

Treatments (mg L^{-1})		Plant height (cm)	Stem diameter (cm)
Control (no foliar applications)		25.55 b	1.01 ab
Ca:	300	27.18 ab	1.05 ab
	400	29.45 a	1.10 a
	500	28.29 ab	1.07 ab
B:	0.2	27.98 ab	1.05 ab
	0.5	27.20 ab	1.07 ab
	0.8	28.29 ab	1.09 ab
Mo:	0.3	25.83 b	1.02 ab
	0.4	26.30 ab	1.04 ab
	0.5	25.93 b	0.99 b
Ca+ B:	400 + 0.5	27.24 ab	1.07 ab
Ca + Mo:	400 + 0.4	25.71 b	1.02 ab
B + Mo:	0.5 + 0.4	26.46 ab	1.05 ab
Ca + B + Mo:	400 + 0.5 + 0.4	26.63 ab	1.03 ab
LSD		3.59	0.11

Means followed by the same letter in each column are not statistically different (Tukey, 0.05). LSD = Least significant difference.

Foliar sprays of 400 mg L^{-1} Ca + 0.5 mg L^{-1} B significantly decreased the length of lateral shoots compared to the control plants (Figure 1A); however, the addition of 0.4 mg L^{-1} Mo to the previous mixture restored the lateral shoots length. Treatments had a non-significant effect on lateral shoot diameter, compared to the control plants (Figure 1B), but plants sprayed with 300 mg L^{-1} Ca produced significantly thinner lateral shoots compared to plants sprayed with 0.2 mg L^{-1} B. The apparent toxic effects of Ca on stem diameter were confirmed when treatment 400 mg L^{-1} Ca + 0.5 mg L^{-1} B was sprayed, since it significantly reduced stem length compared to plants sprayed with 0.2 mg L^{-1} B.

Leaf chlorophyll concentration

Compared to the control, the combination of B + Mo, either at 0.5 or 0.4 mg L^{-1}, did not significantly affect the leaf chlorophyll contents (Table 3). It should be pointed out that the initial concentrations of chlorophyll a, b and total were higher than in treated plants during the inductive short day photoperiod. However, when the chlorophyll concentrations at 50, 70, 99 and 121 DAT were pooled, a marked effect was detected on transitional bracts during maturation (Figure 2). At the end of the experiment, leaf chlorophylls a, b and total became decreased by 25 % compared to the initial concentration; this decrease in chlorophyll was first observed at the initiation of bract pigmentation (72 DAT) (data not shown), and the lowest content was reached six and nine weeks after the inductive photoperiod started (99 and 121 DAT).

Table 3. Effect of foliar sprays of Ca, B and Mo on chlorophylls a, b and total, in poinsettia leaves.

Treatments (mg L^{-1})		Chlorophyll a	Chlorophyll b (mg g^{-1} f. wt.)	Total Chlorophyll
Initial value (before short photoperiod started)		1.64 a	0.87 a	2.51 a
Control		1.22 bc	0.58 b	1.80 bc
Ca:	300	1.37 abc	0.71 ab	2.08 abc
	400	1.26 bc	0.59 b	1.84 bc
	500	1.17 c	0.57 b	1.74 bc
B:	0.2	1.28 bc	0.65 b	1.93 bc
	0.5	1.24 bc	0.60 b	1.84 bc
	0.8	1.29 bc	0.62 b	1.91 bc
Mo:	0.3	1.26 bc	0.59 b	1.86 bc
	0.4	1.22 bc	0.60 b	1.81 bc
	0.5	1.27 bc	0.61 b	1.89 bc
Ca+B:	400 + 0.5	1.24 bc	0.57 b	1.81 bc
Ca+Mo:	400 + 0.4	1.11 c	0.52 b	1.64 c
B +Mo:	0.5 + 0.4	1.45 abc	0.72 b	2.17 ab
Ca+B+Mo:	400+0.5+0.4	1.33 abc	0.62 b	1.96 bc
LSD		0.33	0.21	0.52

Means followed by the same letter in each column are not statistically different (Tukey, 0.05). LSD = Least significant difference.

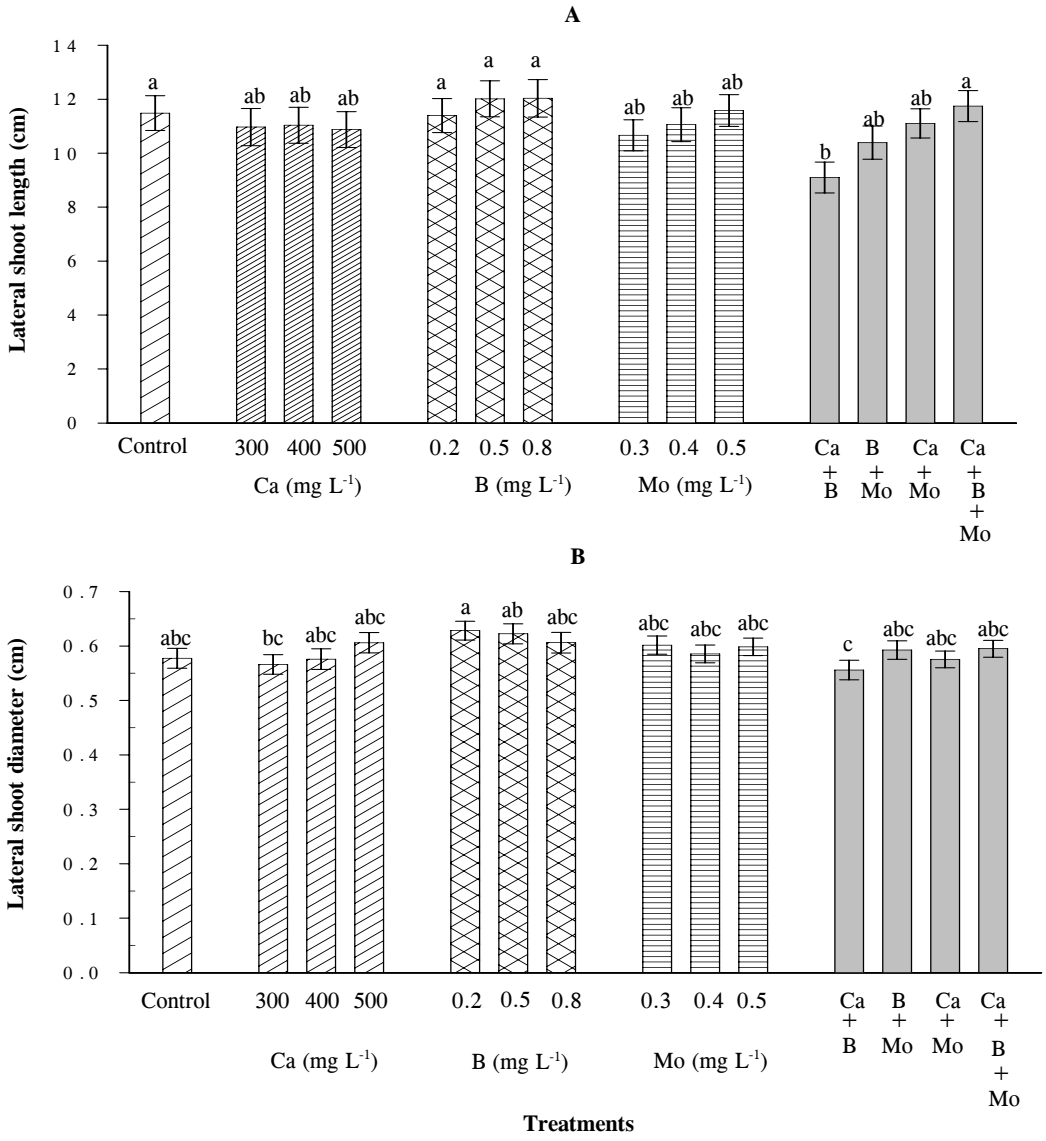

Figure 1. Effect of foliar sprays of Ca, B and Mo on lateral shoot length (A) and diameter (B) of poinsettia cv. 'Supjibi Red'.

Number of transitional bracts (initial pigmentation)

The initial pigmentation of leaves (transitional bracts) was registered 25 d after initiation of the inductive short days (75 DAT); by this time the effect of the two applications already sprayed became noticeable. Plants sprayed with 300 mg L^{-1} Ca and 0.8 mg L^{-1} B had effect on the number of transitional bracts compared to 0.4 mg L^{-1} Mo treatment (Figure 3A). The application of Ca and B did not promote initial pigmentation compared to the control, but an opposite effect was observed on both elements at high concentrations. Combinations of Ca, B, and Mo had not effect on this trait.

Total number of bracts per shoot

Foliar sprays of 400 mg L^{-1} Ca + 0.5 mg L^{-1} B and 400 mg L^{-1} Ca + 0.4 mg L^{-1} Mo caused a significant decrease in the total number of bracts, compared to 0.5 mg L^{-1} B treatment (Figure 3B).

Pigment concentration in transitional bracts

Pigment concentration in transitional bracts varied according to their maturity stage, whereas the foliar sprays had no effect. Chlorophyll and carotenoid concentrations had a significant decrease over time, while the

concentration of anthocyanins showed a significant increase (Figure 4). Total chlorophyll concentration decreased by 95 %, while anthocyanins increased from 21.4 to 296.7 mg g^{-1} f. wt. and carotenoids decreased by 89 %.

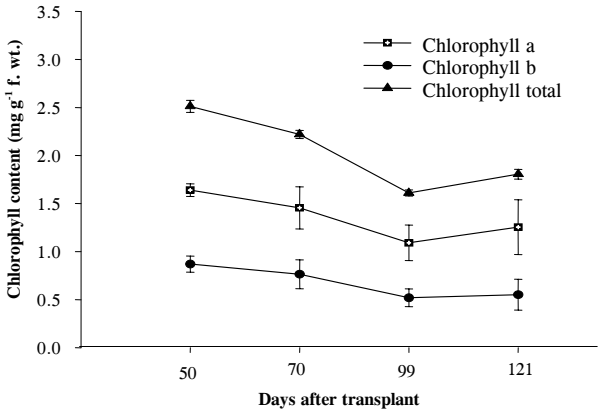

Figure 2. Fluctuation over time of chlorophylls a, b and total in poinsettia leaves cv. 'Supjibi Red'. Bars represent the standard error (n=70).

Calcium and B have been reported to affect plant growth due to their role in cell division and expansion (Marschner, 1995). In tulip (*Tulipa gesneriana*), for example, a Ca deficiency was associated to a decrease in plant height (Nelson and Niedziela, 1998), probably because Ca activates the enzymes involved in mitosis and cytokinesis, as demonstrated by Gislerød (1999). Boron participates in the synthesis of uracil, which is involved in RNA formation, and promotes cell division and differentiation, thus maintaining the meristematic activity (Marschner, 1995; Jones, 2003) and vegetative growth. Intracellular Ca at high level accelerates organ expansion rate due to the intracellular flux to the growing apex (Hepler and Wayne, 1985). The high growth rate in apical meristems, associated to a low transpiration rate, increases the risk of Ca deficiencies in expanding tissues, thus affecting plant cell wall stability and plasma membrane integrity (Marschner, 1995). Under these conditions, foliar sprays of Ca may be an option to supplement extra Ca to the sites of high demand and low supply of this nutrient via phloem (Marschner, 1995; Gislerød, 1999).

In the present study, poinsettia plants sprayed with Ca at 400 mg L^{-1} resulted with increased stem height, while B and Mo had a non significant effect on growth. The lack of promoting effect of Ca when combined with B suggests a possible antagonistic effect between Ca and B at the evaluated concentrations; it has been demonstrated that B affects Ca metabolism in plant species with low adaptation to high rates of B (Wang *et al.*, 2003). Therefore, it is possible that the total amount of B supplemented in the present experiment (B in foliar sprays + B in the water, 0.12 meq L^{-1} + B in the growing medium, 2.94 mg L^{-1}) could have affected the response of poinsettia to Ca sprays. The lack of effect of B and Mo may also be explained because these nutrients in the growing medium were enough to meet the demand of plants. Leaf area and specific weight were not affected, probably because foliar sprays were applied at the beginning of the inductive days, at 59 and 72 DAT, when all leaf primordia were already formed and most of the leaf expansion was completed.

There was a slight increase in chlorophyll concentration in leaves sprayed with B + Mo, whereas when sprayed separately neither B nor Mo affected the chlorophyll content, thus suggesting a possible synergistic effect between these two nutrients. The effect of Mo in chlorophyll is indirect since when N is supplied as nitrate, in the absence of Mo, plants have shown a poor growth and less chlorophyll (Marschner, 1995). Molybdenum is a component of nitrogenase and nitrate reductase (molibdoenzymes), the main enzymatic systems in plant N metabolism, particularly when it is supplied as nitrate (Fageria, 2001; Jones, 2003). The small increase in chlorophyll observed in poinsettia leaves sprayed with B + Mo could be due to a higher amount of N incorporated into the chlorophyll biosynthesis, since N is a constituent of this molecule (Jones, 1998; Raven *et al.*, 1999).

In soybean (*Glycine max* L.), Ca in the nutrient solution has produced a positive effect on the chlorophyll content, the a/b chlorophyll ratio and on photosynthetic carotenoids (Milivojevic and Stojanovic, 2003). There is no evidence of a direct participation of B in chlorophyll synthesis, but it influences Ca transport and metabolism (Yamauchi *et al.*, 1986; Fageria, 2001; Wang *et al.*, 2003). In tomato (*Lycopersicon esculentum* Mill.) plants it was observed that a B deficiency inhibited Ca translocation (Yamauchi *et al.*, 1986); in rose (*Rosa hybrida* L.) it was observed that a high Ca concentration in leaves was due to an increase of B in the nutrient solution (Ganmore-Neumann and Davidov, 1993).

There is limited information as to the roles of Ca and B on the pigmentation of poinsettia bracts. When sprayed separately, both Ca and B promoted an early pigmentation at 3.5 weeks after initiation of the short days, and 0.5 mg L^{-1} B also increased the number of colored bracts per stem. Calcium and B deficiencies in tulip were associated with anthocyanin loss in flowers (Nelson and Niedziela, 1998), suggesting the necessity to investigate the role of these nutrients regarding their participation in the anthocyanin biosynthetic pathway. Nonetheless, there is no

indication of B as an enzymatic component nor that it affects the activity of any enzyme (Marschner, 1995). Its role might be related to the metabolism and transport of carbohydrates, since anthocyanins are formed by an anthocyanidin and a carbohydrate (Saure, 1990), or by stimulating sugar synthesis during the anthocyanin synthesis (Vestrheim, 1970).

Although no significant differences were found among treatments regarding bract pigmentation, some changes in pigment concentration were observed over time.

It has been reported that accumulation of chlorophyll does not continue when young poinsettia leaves start to accumulate anthocyanins under short day conditions (Kannangara and Hansson, 1998). This response is similar to the response reported for red apples (*Malus domestica* Borkh) during the development of red color (Saure, 1990), since the anthocyanin synthesis coincides with a decrease in chlorophyll because all the enzymes involved in chlorophyll synthesis decrease their activity (Kannangara and Hansson, 1998).

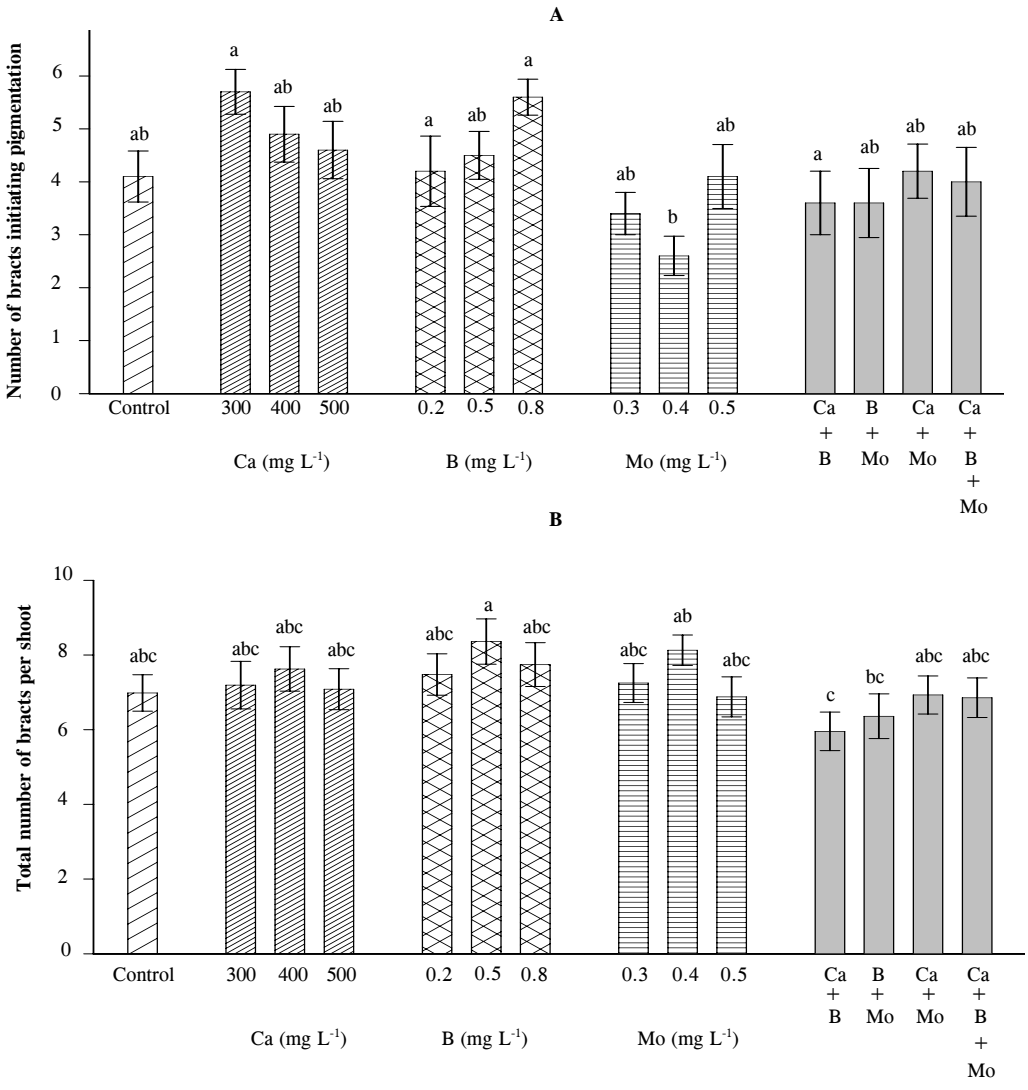

Figure 3. Effect of foliar sprays of Ca, B and Mo on the number of bracts initiating pigmentation (A) and the total number of colored bracts per shoot (B) in poinsettia cv. 'Supjibi Red'. Bars represent the standard error (n=10).

No reports were found about carotenoid synthesis in poinsettia bracts. However, during leaf senescence of some species, the chlorophyll loss is accompanied by a decrease in carotenoids (Dangl *et al.*, 2000); similarly, during ripening the tomato fruit shows an increase in total carotenoids and a simultaneous decrease in chlorophyll (Fraser *et al.*, 1994). Carotenoids are among the most widespread and important natural pigments, and are responsible for the orange-yellow colors observed in plant leaves. They play a minor role as accessory light-harvesting pigments, absorbing and transferring light energy to chlorophyll molecules (Malkin and Niyogi, 2000).

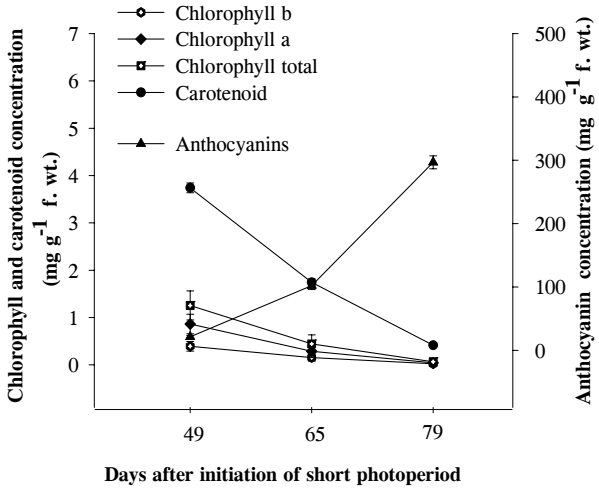

Figure 4. Fluctuation of chlorophyll, carotenoid and anthocyanin concentrations in transitional bracts of poinsettia cv. 'Supjibi Red'. Bars represent the standard error (n=70).

CONCLUSIONS

Foliar sprays of Ca at 400 mg L^{-1} increased plant height, and when Ca was combined with B at 0.5 mg L^{-1} it shortened the lateral shoots. The combined application of B (0.5 mg L^{-1}) and Mo (0.4 mg L^{-1}) produced a slight increase of the leaf chlorophyll content. Ca and B at 300 and 0.8 mg L^{-1} accelerated bract pigmentation, but they had no effect on the pigment concentration of bracts.

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