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Influence of calcium availability on deposition of calcium carbonate and calcium oxalate crystals in the idioblasts of *Morus australis* Poir. leaves

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ABSTRACT. In the leaves of *Morus australis*, calcium carbonate formed only in lithocysts of epidermal tissue while calcium oxalate crystals were found mostly in crystal idioblasts of bundle sheath. In order to identify a possible influence of calcium nutrition on the formations of these two kinds of calcium depositions, plants were grown with varying calcium supply. The results showed that the sizes of both lithocysts and calcium carbonate increased as the calcium concentration increased, but the distribution density of lithocysts was not affected. In addition, the average distribution density of calcium oxalate crystals was higher in the leaves grown in high calcium solution (3750 μmol Ca/l), and no calcium oxalate crystal was found in the leaves grown in low calcium solution (94 μmol Ca/l). Twenty-four days after plants were transferred from high to low calcium solutions, the size of lithocysts in the previously formed leaves remained the same, and that of calcium carbonate decreased slightly, but the density and size of calcium oxalate crystals decreased significantly. After transfer from low to high calcium, the size of existing lithocysts did not respond to the change of calcium concentration while the size of calcium carbonate and both the distribution density and size of calcium oxalate crystals changed.

Keywords: Calcium carbonate; Calcium nutrition; Calcium oxalate; *Morus australis.*

INTRODUCTION

Calcium is known to have influences on many biochemical and physiological processes in plant tissues and cells (Bush, 1995). In higher plants, calcium oxalate crystals are the most prominently deposited calcium salt and have been found in the cells of various tissues and organs (Arnott and Pautard, 1970; Franceschi and Horner, 1980; Borchert, 1985; Kuo-Huang and Zindler-Frank, 1998). The occurrence, the specific crystal shapes, and the locations of these crystals are useful taxonomic characters (Genua and Hillson, 1985; Wu and Kuo-Huang, 1997; Prychid and Rudall, 1999). The accumulation of calcium oxalate crystals in plant bodies has been studied for many years. However their function in normal plant growth and development is still unclear. They may represent a form of calcium and oxalate acid storage. They may act as depositories for regulation of cytosolic calcium concentration (Franceschi and Horner, 1980; Webb, 1999). The formation of calcium oxalate crystals in the crystal idioblasts is affected by the availability of calcium ions (Frank, 1972; Franceschi and Horner, 1979; Borchert, 1985; Kuo-Huang and Zindler-Frank, 1998). Calcium re-dissolved has been observed in times of calcium depletion (Franceschi and Horner, 1979; Borowitzka, 1984; Franceschi, 1989).

Amorphous calcium carbonate is found in several unrelated dicotyledonous families and is abundant in members of the order Urticales, such as Moraceae, Urticaceae, and Ulmaceae (Dickison, 2000). This calcium carbonate is in the form of cystolith and mostly occurs in the epidermal lithocysts of the leaves (Setoguchi et al., 1989; Okazaki et al., 1986, 1991; Taylor et al., 1993). The influence of the calcium nutrition on the precipitation of calcium carbonate has been discussed (Freisleben, 1933; Rabiger, 1951; Sugimura et al., 1999). However the bio-mineralization mechanisms giving rise to the production of amorphous calcium carbonate in plant cystoliths are not clear. Besides, the physiological function of these deposits is uncertain. There is no evidence of cystolith dissolution in either intact plants or detached leaves (Taylor et al., 1993). Nevertheless, amorphous deposits of calcium carbonate are found in gastropod mollusks, where they provide an ion source that can be mobilized for shell repair or acid-base balance (Mason and Nott, 1981).

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Deposits of calcium carbonate generally dissolve in acid while forming CO_2 bubbles (Nultsch and Grahle, 1988). Calcium oxalate crystals are insoluble in acetic acid, but they dissolve in hydrochloric and sulfuric acid without forming bubbles (Kuo-Huang, 1990). Most studies of calcium deposits in higher plants focus on the calcium oxalate crystals (Franceschi and Horner, 1980; Webb, 1999). Noticeably fewer reports concern the formation of calcium carbonate (Scott, 1946; Watt et al., 1987; Yu and Li, 1990; Taylor et al., 1993), and studies comparing both kinds of calcium deposits in a given species are rare (Wu and Kuo-Huang, 1997).

The calcium carbonate in the lithocyst idioblasts of mulberry (*Morus alba*) leaves was studied by Sugimura et al. (1999). However, their work made no mention of the occurrence of calcium oxalate crystals. In the leaves of *Morus australis* conspicuous calcium carbonate and calcium oxalate depositions were found (Wu and Kuo-Huang, 1997). The calcium carbonate depositions are formed mostly in the adaxial epidermal lithocysts while the prismatic or druse-shaped calcium oxalate crystals are frequently located in the bundle sheath cells. The purpose of this study was to examine the influence of calcium concentration on the development of plants and on the formations of calcium oxalate and carbonate depositions in the leaves of hydroponically grown plants of *Morus australis.*

MATERIALS AND METHODS

Mature fruits of mulberry plants (*Morus australis* Poir.) were collected from the mature healthy plants grown in the experimental farm of National Taiwan University. The seeds were washed out, dried in the oven at 40°C for 12 h, and then stored in plastic bags at 4°C. The prepared seeds were imbibed in distilled water for 2-4 h and then planted in vermiculite and watered with distilled water. After 12-14 days, as the hypocotyls hook was stretching, the seedlings were carefully transferred to modified Hoagland solutions in a growth chamber under conditions as described by Horner and Zindler-Frank (1982). The nutrient solutions used in this study contained one of the four calcium concentrations as $Ca(NO_3)_2$: 3750 μmol Ca/l (i.e. 5 Ca; high Ca); 750 μmol Ca/l (1 Ca; normal Ca), 94 μmol Ca/l (1/8 Ca; low Ca), and 47 μmol Ca/l (1/16 Ca; deficient). The nitrate contents of the low and deficient calcium solutions were adjusted to the high calcium solution by the addition of $NaNO_3$. During the hydroponical culture process, the growing length of the 5^{th} to 10^{th} leaves of the seedlings was measured every 2 days. After two months, five high Ca cultured plants were transferred to low Ca solutions, and another five low Ca cultured plants were transferred to high Ca solutions for another 24 days. Investigations were made with the 5^{th} to 7^{th} fully-expanded leaves of the plants grown in the four levels of calcium supply as well as with the latest and newly formed fully-expanded leaves of the transferred plants.

For light (LM) and scanning electron microscopy (SEM), the investigated leaves were fixed at 2 h after the beginning of the 12-h light period. Each experiment was done with leaves from at least two different plants from different sowing dates. Small squares were sliced out of the middle of the half leaf, placed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.2 at room temperature for 2-3 h, washed three times with buffer, and post-fixed in 1% osmium tetroxide in the same buffer for 4 h. The specimens were then rinsed three times with buffer, dehydrated through an acetone series (each step 20-30 min), and then either prepared for SEM observation or embedded in Spurr's resin (Spurr, 1969) for sectioning. Some one-μm thick sections were made and stained with 0.1% toluidine blue. Observations and photographs were made with a Leica Diaplan photomicroscope. The materials for SEM were then dried with a Hitachi Critical Point Dryer (HCP-1). A coating of about 30 nm was made with IB-2 ion coater and examined by a Hitachi S-2400 SEM. For clearing, the segments of leaves were hardened and decolorized in 95% ethanol. Then they were either cleared with 50% lactic acid solution or stored in 95% ethanol. Each cleared leaf segment was mounted on a slide in 50% aquatic glycerin (Sporne, 1948) and observed under a bright field or polarized light microscope. Counts and observations were made in the square areas (0.4 mm^2) distributed on the vein areas (for calcium oxalate crystals) and intercostals areas (for lithocysts) of the specimen (Figure 1). The results given are average values.

RESULTS

The shoot apexes of all the plants grown in the 47 μM Ca/l (1/16 Ca; deficient Ca) solutions withered after the formation of the first foliage leaf. The lengths of internodes and leaves of plants in 94 μM Ca/l (1/8 Ca; low Ca) solution were shorter than those grown in 750 μM Ca/l (1 Ca; normal Ca) or 3750 μM Ca/l (5 Ca; high Ca) solutions (Figure 2). The morphology of the plants grown in normal calcium and those grown in high calcium were similar.

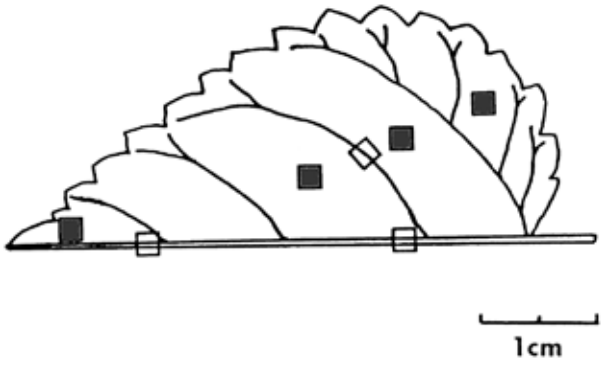

Figure 1. Localization of the test squares on the blade of *Morus australis*. ■, for lithocysts; □, for calcium oxalate crystals.

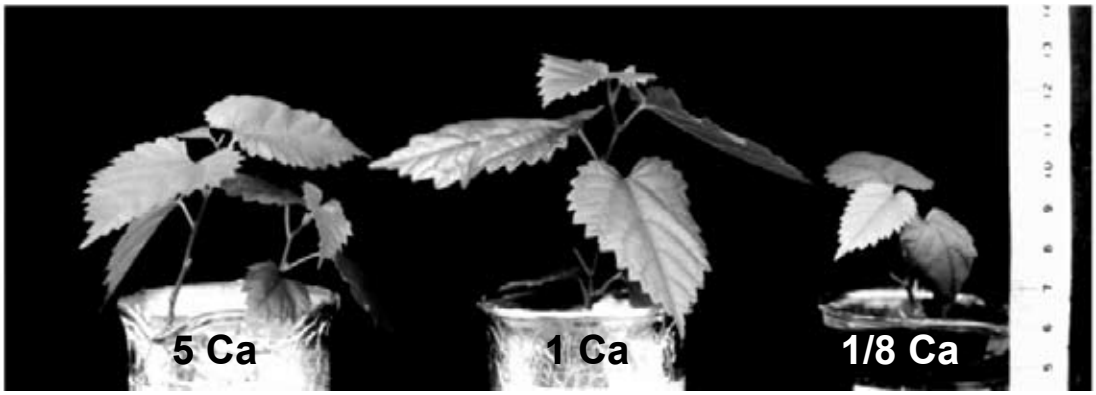

Figure 2. Young plants of *Morus* grown for 2 months with 5 Ca (high calcium), 1 Ca (normal calcium), and 1/8 Ca (low calcium) of calcium concentrations in the nutrient solution. Plant grown in low calcium solution showed signs of calcium deficiency. Bar=1 cm.

The average lengths of fully expanded leaves of the plants grown in low, normal, and high calcium solutions were 27.3 mm, 47.3 mm, and 45.3 mm, respectively (Figure 3). The thickness of leaf decreased as the calcium supply increased (Figures 4A, C, and E; Table 1). However the percentage of the areas of chloroplasts in palisade and spongy parenchyma were higher for the plants grown with high calcium supply than those grown in normal or low calcium supplies (Figures 4 A-F; Table 1).

The formation of calcium carbonate deposition was affected by the calcium concentrations. The size of both lithocyst and calcium carbonate deposition increased as the calcium concentration increased (Table 1). In the leaves of the plants grown in the low calcium solution, most lithocysts contained only the stalk (Figures 4A and B), but most plants grown in normal calcium solution had both the stalk and the cystolith body structures (Figures 4C and D). In the plants grown in the high calcium solu-

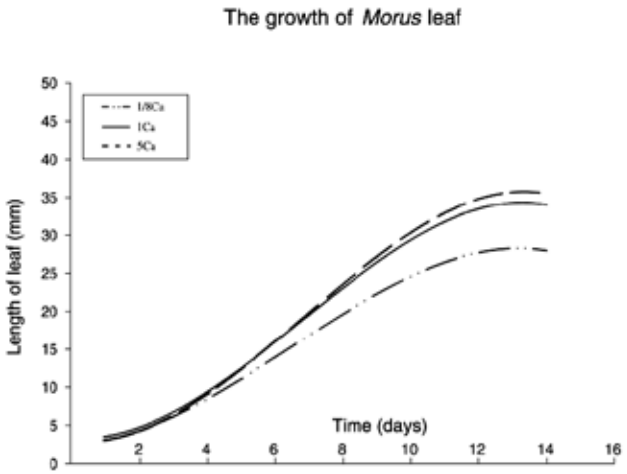

Figure 3. Growth curves of leaves of the plants grown with different calcium concentrations in the nutrient solutions.

Table 1. The anatomical characteristics in the cross sections (C.S.) of the leaves of *Morus australis* grown with different concentrations of calcium in the nutrient solution.

Characters	1/8 Ca	1 Ca	5 C a	P
Thickness of leaf (μm) (n=6)	[a] 104.7±5.2	[b] 87.9±4.7	[c] 68.9±5.0	0.01
Area % of palisade tissue in the C. S. of leaf (n=6)	[a] 29.46±3.73	[a] 33.10±3.00	[a] 33.35±2.30	0.05
Area % of chloroplasts in the C. S. of palisade cell (n=6)	[a] 80.39±3.24	[b] 88.03±2.51	[c] 92.22±3.35	0.05
Area % of chloroplasts in the C. S. of spongy cell (n=6)	[a] 61.05±3.55	[b] 78.36±5.25	[b] 77.63±6.66	0.05
Density of lithocyst (No./mm^2) (n=20)	[a] 768.5±175.24	[a] 680.4±189.28	[a] 816.9±178.63	0.20
Area of C.S. of lithocyst (μm^2) (n=25)	[a] 2851.1±918.78	[a] 2554.3±959.24	[b] 3569.9±1170.6	0.001
Area of C.S. of Ca carbonate deposition (μm^2) (n=25)	---	[a] 341.41±314.95	[b] 1403.1±766.20	0.005

The values with different letters in the same row are significantly different by LSD.

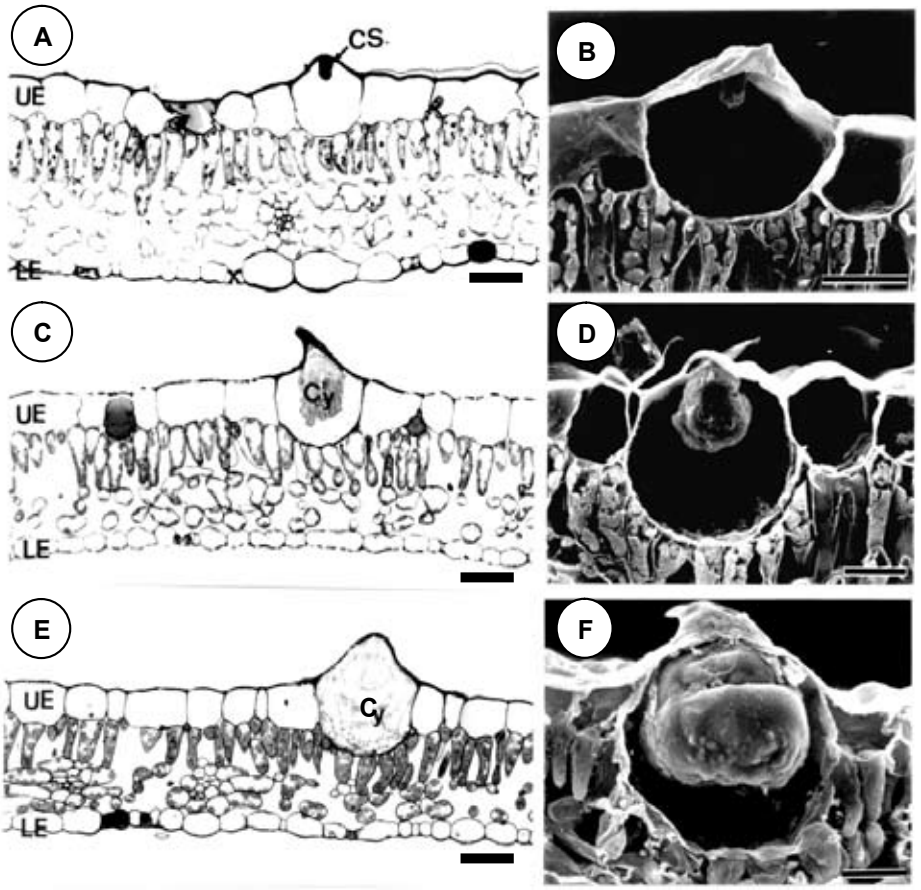

Figure 4. Light and scanning electron photographs of cross sections of leaves from the plants grown with different calcium concentrations in the nutrient solutions. The sizes of the chloroplasts in palisade parenchyma and the calcium carbonate depositions in the lithocysts were smaller in the plants grown with low calcium supply (A and B) than those with normal (C and D) or with high (E and F) calcium supplies. Lithocyst in the plants grown with low calcium contains only the structure of cystolith stalk (CS), but in the plants grown in normal or high calcium solutions, the calcium carbonate depositions formed both the structures of stalk and cystolith body (Cy). In the plants grown in the high calcium solution the lithocyst was almost filled with the cystolith body (Cy). (Bar=25 μm)

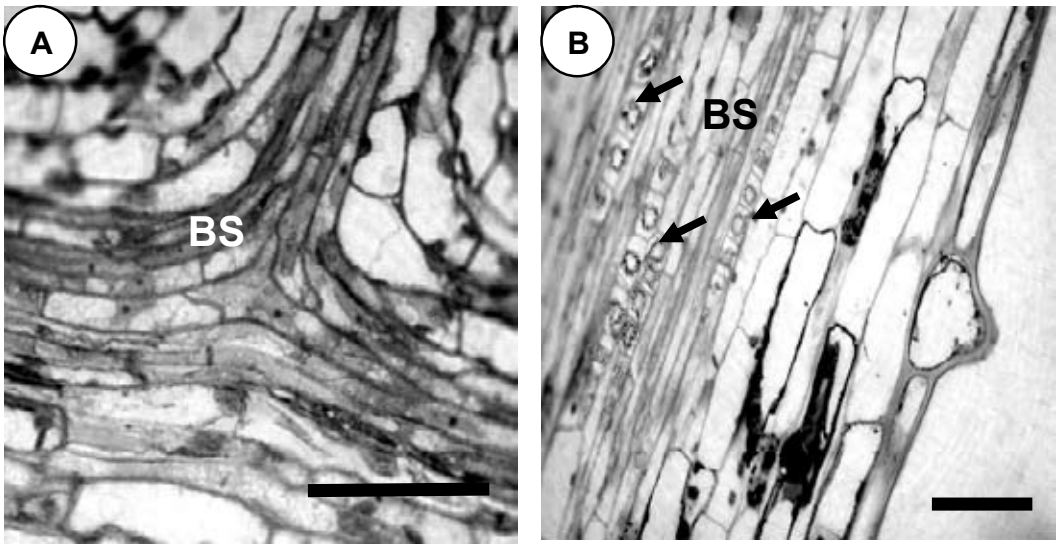

Figure 5. Paradermal sections of leaves from the plants grown with low (A) and normal (B) calcium concentrations in the nutrient solution. No calcium oxalate crystals were found in the low calcium supply, but many druses (arrows) occurred in the cells of bundle sheath. (Bar=50 μm)

tion, the lithocyst was always filled with calcium carbonate deposition (Figures 4E and F). In contrast, the calcium oxalate crystal idioblasts, the density of lithocysts was not affected by the different concentrations of calcium (Table 1). The formation of calcium oxalate crystals in the cells of fully-expanded leaves was also related to the calcium concentration of the solution. No oxalate crystal was found in the leaf of the plants grown in low calcium solution (Figure 5A). In the bundle sheath cells, many druses and prismatic crystals were observed in the leaves for the plants grown in high and normal calcium solutions (Figure 5B). The distribution density of calcium oxalate crystals was higher in the high calcium solution group. There were about 9,200 and 29,100 crystal idioblasts per mm^2 leaf area in the mature leaf of the plants grown in the normal calcium and high calcium solutions, respectively.

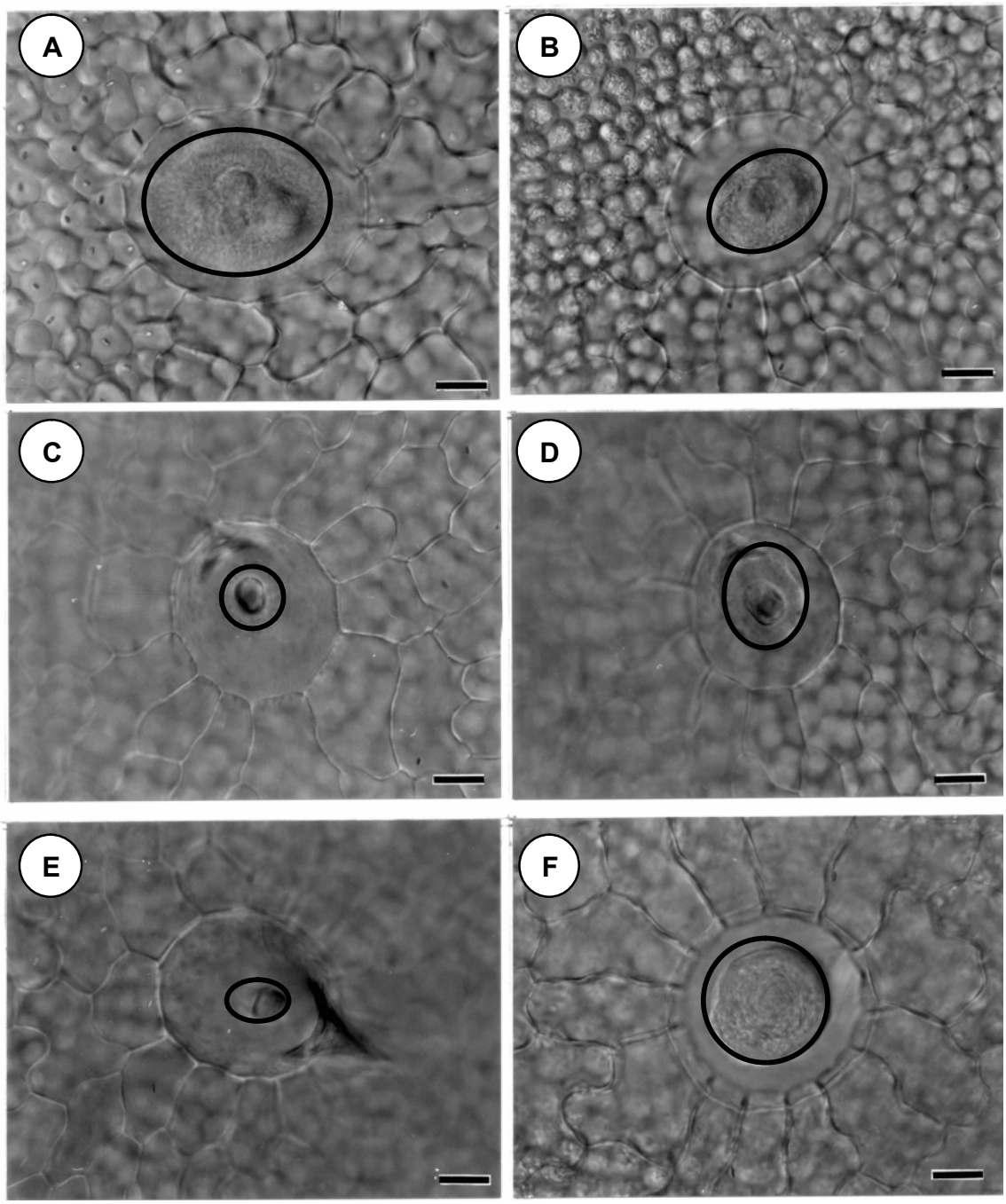

Figure 6. The morphology of lithocyst and calcium carbonate depositions before and after the transfer experiments from high to low (A, B, and E) and from low to high (C, D, and F) calcium concentration. Mature leaves before transferring experiments (A and C). Previously formed mature leaves after transferring experiments (B and D). Newly formed mature leaves after transferring experiments (E and F). (Bar=20 μm)

Twenty-four days after transfer from the high to low calcium concentrations, the plants grew equally well. In the previously formed mature leaves, the density of lithocysts remained the same, and the size of calcium carbonate depositions decreased slightly (Figures 6 A and C), but the density of calcium oxalate crystals decreased (Figures 7A and C) from 29,000 to 23,300 crystal idioblasts per mm^2 leaf area. After transfer from low calcium to high calcium, the size of existing calcium carbonate depositions in the previously formed mature leaves increased (Figures 6B and D). In addition, the distribution density of calcium oxalate crystals increased (Figures 7B and D) from 0 to 2,400 crystal idioblasts per mm^2 leaf area. As expected, the newly formed calcium carbonate and calcium oxalate in the mature leaves that followed the lowering of the calcium concentrations of the growth solutions was similar to those in the leaves of plants grown in unaltered calcium concentrations throughout the experiment (Figures 6E and F; 7E and F).

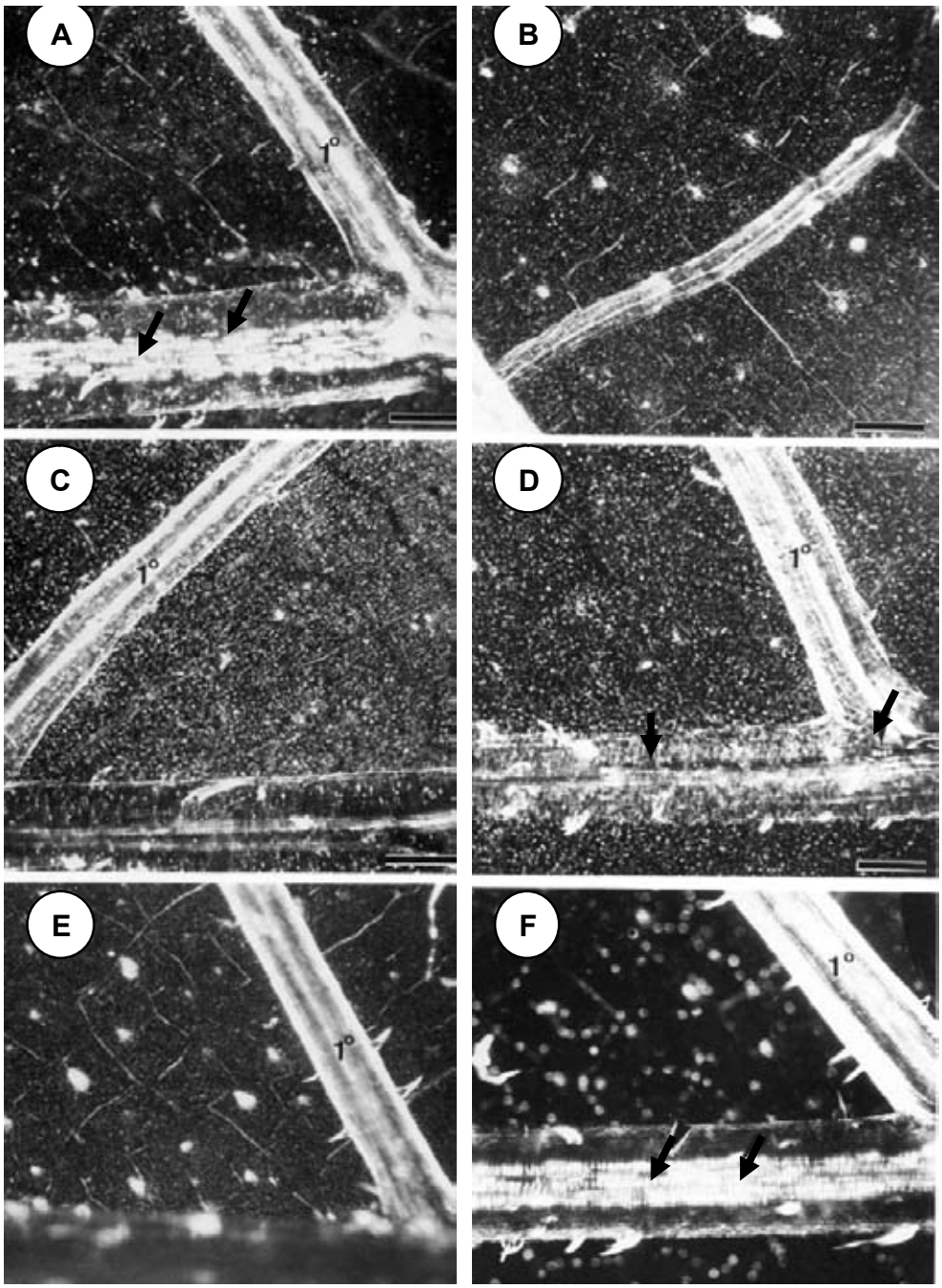

Figure 7. The distribution of calcium oxalate crystals (arrows) in the cleared leaves before and after the transferring experiments from high to low (A, C, and E) and from low to high (C, D, and F) calcium concentration. Mature leaves before transferring experiments (A and C). Previously formed mature leaves after transferring experiments (B and D). Newly formed mature leaves after transferring experiments (E and F). (Bar= 200 μm).

DISCUSSION

Calcium deficiency generally has a rapid and strongly deleterious effect on the growth of higher plants. *Morus australis* plants grown with a low calcium supply (94 µmol Ca/l) showed retardation. The average length of fully expanded leaves of the plants grown in low calcium solution was only about 60% of the length of leaves grown in normal and high calcium solutions. The thickness of leaf also decreases at the calcium supply increased. It is interesting to find that the percentage of areas containing chloroplasts in palisade and spongy parenchyma was higher for plants grown with a high calcium supply than for those grown in normal or low calcium supplies.

All the seedlings grown in the deficient solution (47 µmol Ca/l) died out before the expansion of the first foliage. For *Morus australis*, a calcium supply below 94 µmol Ca/l will reduce the growth, and plants will survive only when the calcium concentration in the growth solution is higher than 47 µmol Ca/l. The minimum required calcium concentration differs between plant species. Plants of *Phaseolus vulgaris* grow well in the solutions with 47 µmol Ca/l (Zindler-Frank et al., 1988), but for *Justicia procumbens* the minimal calcium requirement is 188 µmol Ca/l (Kuo-Huang and Lin, 1997).

In plant cells and tissues, the formation of calcium depositions is not a random event but occurs in a series of biologically organized steps (Webb, 1999). The results of this study demonstrated that the depositaries of calcium oxalate and calcium carbonate in cells may act as Ca-sinks. These cells enable the plant tissues to accumulate surplus Ca absorbed under experimental conditions and, implicitly, natural conditions either as Ca-oxalate or as Ca-carbonate, and to maintain relatively low concentrations of soluble calcium in the vacuoles and apoplast of the tissues. If the capacity of existing Ca-sinks is insufficient to precipitate most unnecessary Ca, the content of soluble Ca in the tissues will rise and induce the formation of new calcium oxalate crystal cells or of larger calcium carbonate depositions in the lithocysts.

The formation of calcium oxalate crystals may involve the induction of mechanisms for the transport of calcium into the vacuole as well as for the crystal initiation. The literature cited has shown that the number of crystal idioblasts formed is related to the amount of calcium available to the plant (Frank, 1972; Zindler-Frank, 1975; Franceschi and Horner, 1979; van Balen et al., 1980). Depending upon the species and the test conditions, both the number and size of the calcium oxalate crystals will be influenced by calcium nutrition to a certain degree. In this study, the distribution density of calcium oxalate crystals in the mature leaves of plants grown in high calcium solution was three times higher than that of the plants grown in normal calcium solution. No oxalate crystal was found in the leaves of the plants grown in low calcium solution, but following transfer from low to high calcium solutions, the formation of calcium oxalate crystal in the mature bundle sheath cells occurred. However, the distribution density of these crystal idioblasts was much lower than that of plants grown either in high or normal calcium solutions in this study. In the mature leaves of *Alibizia julibrissin*, a higher concentration of calcium acetate was required to induce the mesophyllous cells to form calcium crystals (Borchert, 1985).

The density of lithocysts in the mature leaves of *Morus australis* was not significantly affected by the different concentrations of calcium. The results suggested that extra lithocysts were not induced under the high calcium supply, nor were fewer lithocysts formed under a low calcium supply. Only the sizes of lithocyst and calcium carbonate depositions increased from the low calcium to the high calcium solutions. The initial number of lithocysts in the epidermal tissue was genetically determined, but their differentiation was affected by the amount of Ca available. During leaf development, a continuous surplus of Ca^{+2} influx from the high calcium solution enables the lithocyst initially to form a larger cell and a larger calcium carbonate deposition inside. Otherwise, when under calcium deficiency the lithocysts are smaller, and most calcium carbonate depositions contain only the stalk with no obvious body structure. In *Morus alba*, the increase in the Ca content of leaves was proportional to the increase in leaf age, and it was found to be closely related to the Ca-sink capacity of the developing lithocysts (Sugimura et al., 1999). The seasonal dynamics of intracellular Ca^{2+} concentration in the apical bud cells of *Morus bomciz* have a close relationship to growth cessation and the development of dormancy and cold hardiness (Jian et al., 2000).

Since crystals appear to be reabsorbed during calcium deficient conditions, it was suggested that calcium oxalate serves as a form of calcium storage in plants (Franceschi and Horner, 1979; Borowitzka, 1984; Franceschi, 1989). The results of the transferring experiments of this study showed that the number and size of lithocysts in the previously formed mature leaves did not change though the size of calcium carbonate depositions changed slightly. However, the density and size of the calcium oxalate crystals changed significantly. After transfer from high calcium to low calcium, the size of the first 2-3 newly formed leaves was larger than those grown only in the lower calcium throughout the experiment. This suggests that in the mature leaves of *Morus australis*, both calcium oxalate and calcium carbonate could serve as forms of calcium storage, and after transferring to the low calcium conditions, the calcium depositions were dissolved as the calcium ions could be utilized for the growth of new leaves. This phenomenon was revealed during the development of new leaves because the formation of lithocysts with calcium carbonate depositions was found to occur earlier than the bundle sheath cells with calcium oxalate crystals. However, the calcium oxalate crystals were dissolved and transported to the other tissues and organs earlier than the calcium

carbonate depositions. It would be interesting to test the hypothesis that the timing of dissolution is related to the distances between the vascular tissue and the calcium deposition cells. Further experiments are needed to understand the regulation mechanisms associated with the formations and redistributions of calcium oxalate and calcium carbonate.

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LITERATURE CITED

Arnott, H.J. and F.G.E. Pautard. 1970. Calcification in plants. *In* H. Schraer (ed.), Biological Calcification. New York: Appleton-Centry Crofts, pp. 375-446.

Borchert, R. 1985. Calcium-induced patterns of calcium-oxalate crystals in isolated leaflets of *Gleditsia triacanthos* L. and *Albizia julibrissin* Durazz. Planta **165:** 301-310.

Borowitzka, M.A. 1984. Calcification in aquatic plants. Plant Cell & Eviron. **7:** 457-466.

Bush, D.S. 1995. Calcium regulation in plant cells and its role in signaling. Ann. Rev. Plant Physiol. Plant Mol. Bio. **46:** 95-122.

Dickison, W.C. 2000. Integrative Plant Anatomy. Academic Press, A Harcourt Science and Technology Company, USA, pp. 40-44.

Franceschi, V.R. 1989. Calcium oxalate formation is a rapid and reversible process in *Lemna minor* L. Protoplasma **148:** 130-137.

Franceschi, V.R. and H.T. Horner. 1979. Use of *Psychotria punctata* callus in study of calcium oxalate crystal idioblast formation. Z. Pflanzenphysiol. **92:** 61-75.

Franceschi, V.R. and H.T. Horner. 1980. Calcium oxalate crystals in plants. Bot. Rev. **46:** 361-427.

Frank, E. 1972. The formation of crystal idioblasts in *Canavalia ensiformis* DC. at different levels of calcium supply. Z. Pflanzenphysiol. **67:** 350-358.

Freisleben, R. 1933. Untersuchungen über Bildung und Auflösung von Cystolithen bei den Urticales. Flora **27:** 1-45.

Guena, J.M. and C. J. Hillson. 1985. The occurrence, type and location of calcium oxalate crystals in the leaves of fourteen species of Araceae. Ann. Bot. **56:** 351-361.

Horner, H.T. and E. Zindler-Frank. 1982. Calcium oxalate crystals and crystal cells in the leaves of *Rhynchosia caribaea* (Leguminosae: Papilionoideae). Protoplasma **111:** 10-18.

Jian, L.C., J.H. Li, and P.H. Li. 2000. Seasonal alteration in amount of Ca^{2+} in apical bud cells of mulberry (*Morus bombciz* Koidz): an electron microscopy-cytochemical study. Tree Physiol. **20:** 623-628.

Kuo-Huang, L.L. 1990. Calcium oxalate crystals in the leaves of *Nelumbo nucifera* and *Nymphaea tetragona*. Taiwania **35:** 178-190.

Kuo-Huang, L.L. and E. Zindler-Frank. 1998. Structure of crystal cells and influences of leaf development on crystal cell development and vice versa in *Phaseolus vulgaris* (Leguminosae). Bot. Acta **111(5):** 337-345.

Kuo-Huang, L.L. and M.L. Lin. 1997. Morphological and X-ray microanalytical study on the calcium carbonate crystals in the leaves of *Justicia procumbens* L. Sup Electron Microscop*y* (Japan), 32, 22-IV-1030, pp. 157.

Kuo-Huang, L.L., S.H. Chen, and S.J. Chen. 2000. Ultrastructural study on the formation of sclereids in the floating leaves of *Nymphoides coreana* and *Nuphar schimadai*. Bot. Bull. Acad. Sin. **41:** 283-291.

Mason, A.Z. and J.A. Nott. 1981. The role of intracellular biomineralized granules in the regulation and detoxification of metals in gastropods with special reference to the marine prosobranch *Littorina littorea*. Aquat. Toxicol. **1:** 239-256.

Nultsch, W. and A. Grahle. 1988. Mikroskopisch-Botanisches Praktikum. 8th Ed. Georg Thieme Verlag Stuttgart, pp. 53-56.

Okazaki, M., H. Setoguchi, and E. Hisanaga. 1991. Inorganic composition of cystoliths isolated from leaves of higher plants. *In* S. Suga and H. Nakahara (eds.), In Mechanisms and Phylogeny of Mineralization in Biological Systems. Tokyo, Springer-Verlag, pp. 173-177.

Okazaki, M., H. Setoguchi, H. Aoki, and S. Suga. 1986. Application of soft X-ray microradiography to observation of cystoliths in the leaves of various higher plants. Bot. Mag. Tokyo **99:** 281-287.

Prychid, C.J. and P.J. Rudall. 1999. Calcium oxalate crystals in monocotyledons: A review of their structure and systematics. Ann Bot. **84:** 725-739.

Rabiger, F.H. 1951. Untersuchungen an einigen Acanthaceen und Urticaceen zur Function der Cystolithen. Planta **40:** 121-144.

Scott, F.M. 1946. Cystoliths and plasmodesmata in *Beloperone*, *Ficus* and *Boehmeria*. Bot. Gaz. **107:** 372-378.

Setoguchi, H., M. Okazaki, and S. Suga. 1989. Calcification in higher plants with special reference to cystoliths. *In* R. E. Crick (ed.), Origin, Evolution, and Modern Aspects of Biomineralization in Plants and Animals, New York, Plenum Press, pp. 409-418.

Sheue, C.R., Y.P. Yang, and L.L. Kuo-Huang. 2003. Altitudinal variation of resin ducts in *Pinus taiwanensis* Hayata (Pinaceae) needles. Bot. Bull. Acad. Sin. **44:** 305-313.

Sporne, K.R. 1948. A note ona rapid clearing technique of wide application. New Phyto. **47:** 290-291.

Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26:** 31-34.

Sugimura, Y., T. Mori, I. Nitta, E. Kotani, T. Furusawa, M. Tatsumi, S. Kusakari, M. Wada, and Y. Morita. 1999. Calcium deposition in idioblasts of mulberry leaves. Ann. Bot. **83:** 543-550.

Taylor, M.G., K. Simkiss, G.N. Greaves, M. Okazaki, and S.

Mann. 1993. An X-ray absorption spectroscopy study of the structure and transformation of amorphous calcium carbonate from plant cystoliths. Proc. Royal Soc. Lond. **252:** 75-80.

Van Balen, E., S.C. van de Gejn, and G.M. Desmet. 1980. Autoradiographic evidence for the incorporation of cadmium into calcium oxalate crystals. Z. Pflanzenphysiol. **97:** 123-133.

Watt, W.M., C.K. Morrell, D.L. Smith, and M.W. Steer. 1987. Cystolith development and structure in *Pilea cadierei* (Urticaceae). Ann. Bot. **60:** 71-84.

Webb, M.A. 1999. Cell-mediated crystallization of calcium oxalate in plants. Plant Cell **11:** 751-761.

Wu, C.C. and L.L. Kuo-Huang. 1997. Calcium crystals in the leaves of some species of Moraceae. Bot. Bull. Acad. Sin. **38:** 97-104.

Yu, F.G. and Z.I. Li. 1990. Anatomy of the lithocyst in the epidermis of leaf in *Broussonetia papyrifera*. Acta. Bot. Sinica **33:** 249-255.

Zindler-Frank, E. 1975. On the formation of the pattern of crystal idioblasts in *Canavalia ensiformis* D.C. VII. Calcium and oxalate content of the leaves in dependence of calcium nutrition. Z. Pflanzenphysiol. **77:** 80-85.

Zindler-Frank, E., E. Wichmann, and M. Korneli. 1988. Cells with crystals of calcium-oxalate in the leaves of *Phaseolus vulgaris* - a comparison with those in *Canavalia ensiformis*. Bot. Acta **101:** 246-253.

鈣對小葉桑葉部異型細胞內碳酸鈣與草酸鈣堆積的影響

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小葉桑（*Morus australis*）葉部之鐘乳體僅形成於位於表皮之石胞中，而草酸鈣晶體則大都位於葉脈鞘細胞之結晶異型細胞中。本研究為探討鈣對小葉桑葉部異型細胞內此兩種鈣堆積可能的影響，而以不同濃度之鈣營養液供植株生長。結果顯示石胞與碳酸鈣的大小隨著鈣離子濃度的增加而增大，但石胞的密度並未隨著鈣離子濃度不同而有所差異。此外，草酸鈣結晶分布之平均密度於較高濃度之鈣營養液中 (3750 μmol Ca/l) 最高，而在較低濃度之鈣營養液中 (94 μmol Ca/l) 則未觀察到草酸鈣結晶。於植株移植試驗中，由高鈣營養液移植至低鈣營養液 24 天後，在移植前已達成熟階段的葉片內，其石胞的大小並沒有明顯變化，而其內之鐘乳體之體積稍變小，但草酸鈣晶體的密度顯著的減少而體積亦明顯變小；而由低鈣營養液移植至高鈣營養液的試驗，其石胞的大小亦沒有反應鈣濃度的變化，然而其內之鐘乳體之體積與草酸鈣晶體的密度與體積均明顯變大。

關鍵詞：碳酸鈣；鈣養分；草酸鈣；小葉桑。