

# Size of In Vitro Plantlets Affects Subsequent Tuber Production of Acclimated Calla Lily

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**Abstract.** Tuber production of calla lily (*Zantedeschia elliottiana* Spreng cv. Super Gold) was investigated using three size ranges (7–10, 4–7, and <4 mm shoot diameter) of in vitro plantlets acclimated in either pots or soil beds in a protected house. The shoots and tubers of large plantlets exhibited higher rates of dry-matter accumulation than did those of small plantlets. The diameter of tubers harvested from pots ranged from 0.67 to 4.1 cm with median values of 2.7, 2.1, and 1.9 cm for the plants derived from large, medium, and small plantlets, respectively. Plants grown in soil beds, regardless of size, produced larger tubers than did those grown in pots. Tubers >3 cm in diameter developed on 25% and 52% of plants grown in pots and soil beds, respectively. Our results suggest that improved calla lily production could be realized by using larger in vitro plantlets as the source material and growing them in soil beds in a protected house.

Calla lilies and their hybrids are commercially available as cut flowers and potted plants (Corr, 1993; Letty, 1973). Growth habits of calla lily have been studied extensively (Corr and Widmer, 1991; Plummer et al., 1990), with major efforts focused on flowering control and tuber storage (Corr and Widmer, 1987, 1988). Growth regulator treatments have been evaluated with particular emphasis on increasing the number of flowers rather than on increasing the proportion of flowering-sized tubers (Funnell et al., 1988; Tjia, 1987).

Tuber size is one of the major flowering determinants in calla lily. Tubers >3 cm in diameter are graded as flowering-sized, although small tubers can also flower after treatment with gibberellic acid (Corr, 1993; Dennis et al., 1994). From the growers' viewpoint, an ideal system for tuber production of calla lily should stress large tuber size and minimal loss due to infection by *Erwinia carotovora* ssp. *carotovora* or *Erwinia chrysanthemi* (Welsh and Clemens, 1992). Flowering-sized tubers of calla lily can be produced from either seeds or small tubers. Commercially, annual multiplication of calla lily through offsets and tuber division is practiced to increase the number of tubers of flowering size (Welsh and Clemens, 1992). However, this system is subject to the spread of cucumber mosaic virus (CMV) and dasheen mosaic virus (DsMV) (Zettler and Hartman, 1987). In addition, division of field-grown

tubers are subject to infection by *Erwinia* soft rot, which causes serious losses.

Tissue culture is now used to increase new cultivars and provide clean materials free of known viruses (Cohen, 1981; Yao et al., 1995). Tissue culture-derived plantlets have been proposed as an alternative planting material for tuber production in calla lily (Clemens and Welsh, 1993; Cohen, 1981). Although in vitro micropropagation methods for calla lily have been described (Cohen, 1981), little information is available on tuber production using tissue culture-derived plantlets. This study was designed to examine whether the quality of such plantlets could influence subsequent tuber growth and size distribution.

## Materials and Methods

The experiment was conducted at the Taiwan Seed Improvement and Propagation Station from Oct. 1997 to Apr. 1998. Tissue culture-derived plantlets of calla lily, cv. Super Gold, were used as planting materials. In vitro plantlets were produced from stock tubers free of CMV and DsMV. Tubers were surface-disinfected with 0.5% sodium hypochlorite solution for 15 min followed by four rinses in sterile distilled water. Buds excised from the tubers were cultured on multiplication medium containing MS basic salts (Murashige and Skoog, 1962) supplemented with 3% sucrose, 0.054  $\mu\text{M}$   $\alpha$ -naphthaleneacetic acid (NAA), 9.29  $\mu\text{M}$  kinetin, and 2.22  $\mu\text{M}$  benzyladenine (BA), and solidified with 0.8% agar (Sigma, St. Louis). The pH was adjusted to 5.7 before autoclaving. Cultures were incubated under a 16-h photoperiod (50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) with a temperature of  $25 \pm 2^\circ\text{C}$ . Adventitious buds were taken through 8–10 subcultures at 4-week intervals. When sufficient shoots was available, single shoots were transferred to rooting medium containing MS

basic salts supplemented with 3% sucrose and 0.46  $\mu\text{M}$  kinetin, and solidified with 0.8% agar. These cultures were first incubated at  $25 \pm 2^\circ\text{C}$  with a 16-h photoperiod (100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) for 2 weeks, and then placed on adjacent benches in a plastic-covered greenhouse (mean maximum light intensity 1050  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; mean temperature  $22^\circ\text{C}$ ) for another 2 weeks of preacclimation. Preacclimated plantlets were rinsed in water to remove any medium, and misted with water to prevent wilting. Rooted plantlets were graded into three size groups (large, medium, and small) by shoot diameter and then transplanted in pots or in soil beds. The ranges of diameters of the basal part of the shoot for large, medium, and small plantlets were 7–10, 4–7, and <4 mm, respectively. For growth analysis, 600 plantlets of each size were planted in 11-cm (0.52-L) pots with a growing medium of 5 sphagnum peat : 1 perlite (by volume). A randomized complete-block design was used with three replicates of each plantlet size. For comparing the difference in size distribution of tubers grown in pots and in soil beds, 120 plantlets of each size were first planted in pots for 4 weeks, then transferred to sandy soil beds at a density of 10 plants/ $\text{m}^2$ . A randomized complete-block design was used with three replicates of 40 plants each per plot. All plants were grown in a nonshaded greenhouse under natural sunlight (mean maximum light intensity during the experiment was 1950  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , relative humidity 70% to 80%, and mean maximum and minimum temperatures  $\approx 25^\circ\text{C}/16^\circ\text{C}$ ). The plants were watered by drip irrigation system once a day at 0900 HR. Liquid fertilizer containing 200  $\text{mg}\cdot\text{L}^{-1}$  N (Peter's 20N–20P–20K; Scotts-Sierra Horticultural Products, Maryville, Ohio) was applied through drip irrigation with an average 100 mL/plant every 10 d. No additional fertilizers were applied during the experiment.

Twenty-four plants grown in pots were sampled randomly from each size treatment at 7-d intervals for 24 weeks. Leaf numbers and areas were determined immediately after sampling. For shoot and tuber dry-weight determination, samples were oven-dried at  $80^\circ\text{C}$  for 2 d. Tubers were harvested 24 weeks after planting. The freshly harvested tubers were placed on benches in a shaded net house for 1 week to air-dry. The air-dried and cured tubers were graded according to the smallest diameter across the top of the tuber.

Data were analyzed by analysis of variance, and means were separated by the least significant differences (LSD) test. Linear regression was also used to characterize growth response over sampling stage for plants and tubers.

## Results

**Growth in pots.** In the pot culture system, leaf area (LA) of all plants increased slowly during the first 6 weeks after planting (Fig. 1A). Thereafter, the LA increased linearly to a maximum  $\approx 11$ –14 weeks after planting. The rate of increase in LA was considerably higher in plants derived from large and medium plantlets, whereas the time of reaching maximum

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LA appeared to be independent of size. The mean maximum LAs attained by large, medium, and small plantlets were 81, 73, and 55 cm<sup>2</sup> per plant, respectively (Fig. 1A). The LA started to decrease after 14 weeks of cultivation when leaves began to senesce. The decrease in LA was presumably due to the onset of leaf abscission, as indicated by the decrease in leaf number per plant (Fig. 1B). The pattern of change in leaf number during the culture period resembled that of change in leaf area (Fig. 1B). Leaf number increased rapidly during the first 11 weeks and reached a plateau  $\approx$ 11–14 weeks after planting. The maximum leaf numbers attained by plants derived from large, medium, and small plantlets were 8.1, 7.2, and 7.6 per plant, respectively, at 14 weeks after planting. The change in leaf number after week 11 was similar for all treatments.

Dry-matter accumulation of shoots, regardless of plantlet size, changed little during the first 3 weeks of culture (Fig. 2A). Thereafter, it increased to a maximum at 14 weeks after planting, with a growth rate ranging from 27 to 40 mg·week<sup>-1</sup> per shoot (Table 1).

Tuber dry weight began to increase when LAs approached the maximum and continued to increase linearly until harvest (Fig. 2B). Tuber growth occurred only after leaf area had reached its maximum when small tubers were used for propagation (Clemens and Welsh, 1993). This was also the case in the present study with *in vitro* plantlets. Clemens and Welsh (1993) reported that the initiation and subsequent rate of tuber growth was primarily related to the availability of assimilates in excess of the demands of shoot development. This tendency is probably due to limitation of assimilate supply during the early stage of growth (Koble and Stephan-Beckmann, 1997). Our data showed little change in tuber dry weight until the shoots attained 75% to 80% of their maximum weight (Fig. 2A). Tuber growth rate (TGR), estimated by linear regression between 11 and 20 weeks after planting, ranged from 81 to 168 mg/tuber per week (Table 1). The rate and duration of tuber growth was positively related to the original size of mother plantlets (Fig. 2B). About 95% of the final dry weight was accumulated by week 24, 22, and 20 for plants derived from large, medium, and small plantlets, respectively.

The difference in rate of tuber dry-matter accumulation was reflected in the size of tubers (Fig. 3A). For plants grown in pots, tuber size ranged from 0.7 to 4.1 cm in diameter, with median values of 2.7, 2.1, and 1.9 cm in diameter for the plants derived from large, medium, and small plantlets, respectively (Fig. 3A). For the small plantlets, 79% of the tubers were <2.5 cm and only 0.4% were >3 cm in diameter, whereas all tubers produced from large plantlets were >2 cm, and  $\approx$ 25% were >3 cm in diameter (Fig. 3A).

**Growth in soil beds.** Tubers grown in soil beds showed a size trend similar to that of grown in pots. However, the pattern of tuber size distribution differed significantly from that of tubers grown in pots (Fig. 3B). Tuber size in soil beds ranged from 0.9 to 4.3 cm in diameter, with median values of 3.3, 2.8, and

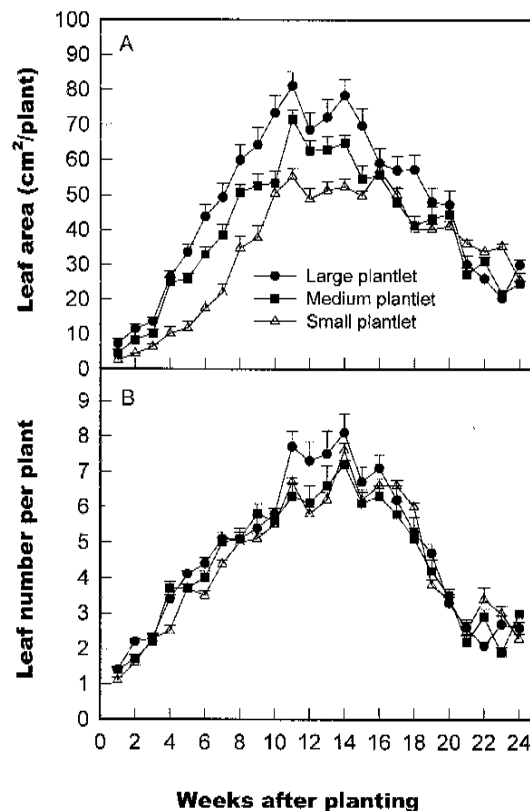


Fig. 1. (A) Leaf area and (B) leaf number of calla lily during plant development. The plants originated from large (●), medium (■), or small (△) tissue-culture-derived plantlets. Each point is the mean for 24 plants. Vertical bars denote  $\pm$ SE.

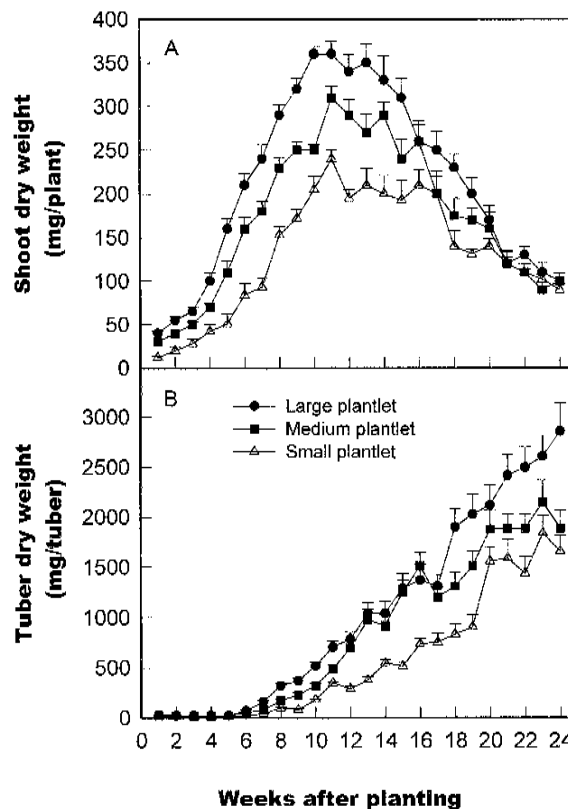


Fig. 2. Dry-matter accumulation in (A) shoot and (B) tuber of calla lily during plant development. The plants originated from large (●), medium (■), or small (△) tissue-culture-derived plantlets. Each point is the mean for 24 plants. Vertical bars denote  $\pm$ SE.

Table 1. Shoot and tuber growth rate of *Zantedeschia* produced from in vitro plantlets of three sizes.

Size of plantlets <sup>z</sup>	Shoot growth rate <sup>y</sup> (mg week <sup>-1</sup> )	Tuber growth rate <sup>x</sup> (mg week <sup>-1</sup> )
Large	40	168
Medium	33	132
Small	27	81
LSD <sub>0.05</sub> <sup>w</sup>	4	19

<sup>z</sup>Size of plantlets was graded by measuring diameter at basal part of shoot. The shoot diameter for the large, medium, and small plantlets was 7–10 mm, 4–7 mm, and <4 mm, respectively.

<sup>y</sup>Shoot growth rate was estimated by fitting a linear regression to the data for dry weight between 3 and 11 weeks after planting.

<sup>x</sup>Tuber growth rate was estimated by fitting a linear regression to the data for dry weight between 11 and 20 weeks after planting.

<sup>w</sup>Mean separation within columns by LSD,  $P \leq 0.05$ .

2.5 cm for the plants derived from large, medium, and small plantlets, respectively (Fig. 3B). Plants grown in soil beds, regardless of plantlet size, tended to produce larger tubers than did those grown in pots. For the large plantlets, especially, the proportion of tubers that produced flowers was 25% in pots vs. 52% in soil.

### Discussion

For calla lily growers, production requires a high proportion of flowering tubers (Corr and Widmer, 1988; Welsh and Clemens, 1992), and production of plantlets in vitro has become an alternative method (Cohen, 1981). Generally, tissue culture plantlets require two growing cycles ex vitro for tubers to reach sufficient diameters (3–6 cm) for flowering. Welsh and Clemens (1992) reported that micropropagated plants produced small tubers in their first growing season and reached flowering size in their second season. In the present study, however, we found that the variation of tuber size distribution depended on both the size of mother plantlets and the culture method. About half of the tubers produced by the larger in vitro plantlets grown in soil beds reached flowering size in only one growth cycle, whereas only 0.4% of tubers produced from small plantlets grown in pots reached flowering size within one season (Fig. 3).

The variation in size of in vitro plantlets is typical, and growers do not select for size for tuber production. Many researchers have suggested that the multiplication rates in vitro can be controlled by adjusting the form and/or concentration of plant growth regulators in the medium (Pierik, 1991). However, the conditions that favor adventitious bud formation (i.e., high multiplication rate) would usually limit plantlet development (i.e., plantlet size) during in vitro culture. Therefore, balancing the rate of multiplication and the size of plantlets in a tissue culture system is important for establishing a highly efficient system of tuber production that uses in vitro plantlets as mother stock.

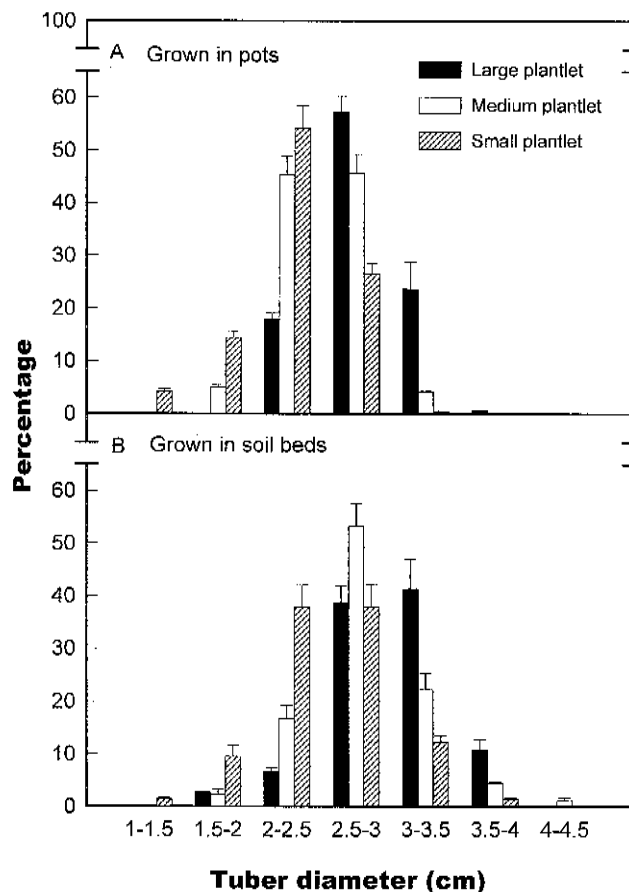


Fig. 3. Size distribution of calla lily tubers grown in (A) pots and in (B) soil beds under a protected house. Data were taken 24 weeks after planting. Vertical bars denote  $\pm$ SE.

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