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Growth Optimization and Organogenesis of *Gerbera jamesonii* Bolus ex. Hook f. *in vitro*

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Abstract: Regeneration potentials in *Gerbera jamesonii* Bolus ex. Hook f. from tissues culture system was studied using leaf, petiole and root explants. *In vitro* regeneration, callus induction and root formation were optimized by manipulation of growth regulators during organogenesis. Various kinds of plant growth regulators such as 6-Benzylaminopurine (BAP), α-Naphthalene acetic acid (NAA), 2, 4-Dichlorophenoxyacetic acid (2,4-D), Indole-3-acetic acid (IAA), Indole-3-Butyric acid (IBA), N^6-[2-Isopentenyl]adenine (2iP), Kinetin and Zeatin were used to initiate cultures. These plant growth regulators were added to Murashige and Skoog medium in different combinations and concentrations. Adventitious shoots were obtained from petiole explants cultured on Murashige and Skoog (MS) medium supplemented with 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA. Effectiveness of shoot regeneration medium, type of growth regulator used and duration of induction period were investigated. Leaf explants cultured on MS medium supplemented with 1.0 mg L^{-1} BAP and 2.0 mg L^{-1} 2, 4-D showed the best results for callus induction. Root explants were found to be non-regenerative in all experiments conducted. Petiole segment was identified as the best explant for regeneration of this species. Regenerated plants were rooted on Murashige and Skoog basal medium. Plantlets were then transferred to field with 75% survival rate.

Key words: *Gerbera jamesonii*, organogenesis, *in vitro*, callus induction, Murashige and Skoog

INTRODUCTION

Tissue culture technique has long been used since the cell theory was established. Many scientists have tried to prove the totipotency concept, which is the ability of a single cell to form complete individual. Today, tissue culture technique is being used widely realizing its potentials in mass propagation and preservation of elite plants. The most important aspect in plant tissue culture is the capability of cultured cells and tissues to regenerate into complete plants. This technology is being utilized commercially in the ornamentals industry and in other plant production organizations worldwide (Chu, 1992; Huetteman and Preece, 1993; Mantell *et al*., 1985; Pierik, 1987). Many temperate and tropical plants have been successfully propagated via tissue culture. *In vitro* response of plant tissues depends on genotype, the physiological status of the donor plant, the type of explant, the culture medium and their interactions (Tosca *et al*., 1999). The physiological status of the donor is determined by environmental conditions such as temperature, light intensity, day-length and light wavelength. Plant regeneration via direct organogenesis is much preferred over regeneration through somatic embryogenesis and callus culture (Arockiasamy *et al*., 2002). Adventitious organogenesis or shoot formation is a preferred system as it enables to retain the clonal fidelity since many ornamental species are cultivars that are propagated for one or more unique characteristics (Kantia and Kothari, 2002). The propagation rates via organogenesis can be much higher than axillary shoot proliferation (Chun, 1993).

In the present study, propagation of *Gerbera jamesonii* through tissue culture techniques was done and the factors influencing the growth of this plant were studied. *Gerbera jamesonii* Bolus ex. Hook f., commonly known as *Gerbera* daisy or Barberton daisy is a temperate perennial flowering plant which belongs to the Asteraceae family. They are planted outdoors in full sun and useful as cut flowers, pot plant and also bedding plant. This species consists of many cultivars with variety of colors and shapes and they are popular commercial plants. *Gerbera jamesonii* is also used in the preparation of traditional Chinese medicine, tu-er-feng, for curing cold and also for treating rheumatism (Ye *et al*., 1990). Plant propagation by tissue culture technique is mainly aimed to produce plants with very high multiplication rates. Through indirect organogenesis, multiple shoots can be produced *in vitro* from callus. Because genetic variability within the *Gerbera* genus is relatively limited, breeding

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potential for new flower colours and patterns as well as resistance to biotic or abiotic stresses is also limited (Orlikowska *et al.*, 1999).

In the present study, experiments were conducted to investigate organogenesis from various sources of *Gerbera* explants. The effects of various concentrations of plant growth regulators on the multiplication of shoots were examined.

MATERIALS AND METHODS

The study was conducted at laboratory B2.5 and the green house at the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. Explants were obtained from 8-week-old aseptic seedlings. *Gerbera* seeds were first soaked in distilled water for 30 min with addition of 1-2 drops of Tween-20 followed by 40% (v/v) sodium chloride solution and gently agitated. The seeds were then rinsed 3 times with distilled water and then soaked in 70% (v/v) alcohol for 1 min. Finally the seeds were rinsed 3 times with sterile distilled water. Sterilized seeds were cultured on basal MS (Murashige and Skoog, 1962) medium. pH of the medium was adjusted at 5.8 before being autoclaved at 121°C for 21 min. Petioles and leaves obtained from aseptic young plantlets formed from the seedlings were used as source of explants. Leaves and petioles were cultured for shoot induction on MS medium supplemented with 3% sucrose and 0.8% technical agar containing 6-Benzylaminopurine, BAP (0.5-2.0 mg L^{-1}) and α-Naphthalene acetic acid, NAA (0.5-1.0 mg L^{-1}). Induction of rooting *in vitro* was also observed when petiole and leaf explants were cultured on MS medium fortified with BAP, 2, 4-D, NAA, Zeatin, IAA and 2iP (Fig. 1). Callus induction was initiated on MS medium with BAP, Kinetin, Zeatin and 2iP (0.5-2.0 mg L^{-1}) and NAA, IAA, IBA and 2, 4-D (0.5-2.0 mg L^{-1}).

Plantlets were transferred to soil and maintained in culture room for 2-3 weeks for adaptation process before being transferred to field environment. *In vitro* propagated *Gerbera* plants were compared morphologically with the intact plant. Survival rate of micro propagated plants that were transferred to soil were investigated (Fig. 2).

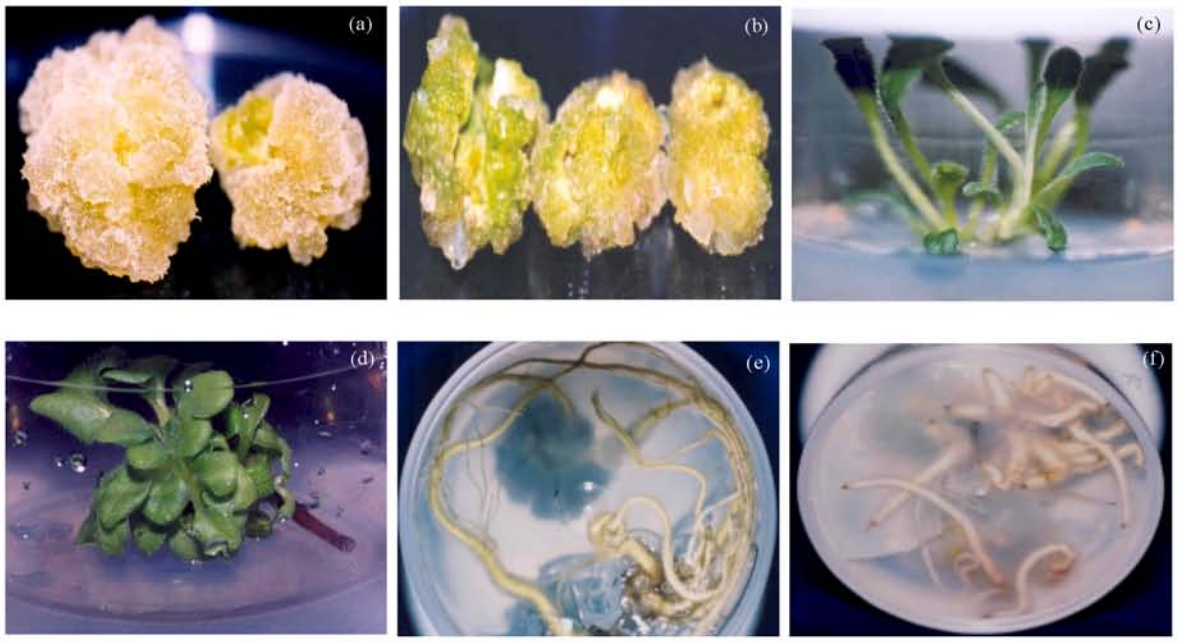

Fig. 1: (a) Callus formed on MS medium supplemented with 1.0 mg L^{-1} BAP and 2.0 mg L^{-1} 2,4-D, (b) Callus formed on MS medium added with 1.0 mg L^{-1} BAP and 2.0 mg L^{-1} NAA, (c) Regeneration of shoots from petiole explants cultured on MS medium supplemented with 2.0 mg L^{-1} Zeatin and 0.5 mg L^{-1} IBA, (d) Regeneration of shoots from petiole explants cultured on MS medium supplemented with 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA, (e) Development of roots from leaf explant cultured on MS medium supplemented with 0.1 mg L^{-1} BAP and 2.0 mg L^{-1} NAA and (f) Roots formed from leaf explants cultured on MS medium fortified with 2.0 mg L^{-1} NAA

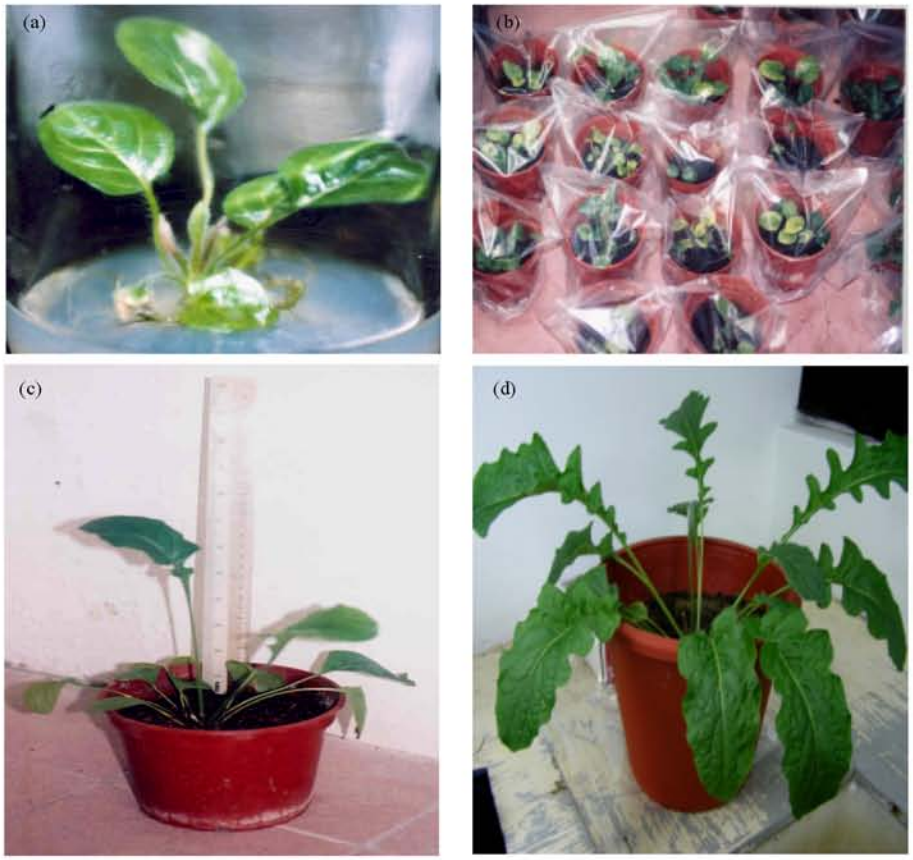

Fig. 2: (a) Plantlet ready to be transferred to soil, (b) Young *Gerbera* plantlets were transferred to soil, (c) *Gerbera* plantlet after 8 weeks being transferred to soil and (d) Six- months-old *Gerbera* plant successfully acclimatized

RESULTS AND DISCUSSION

Plant hormones and type of explants play very important roles in determining regeneration of *Gerbera jamesonii in vitro*. Many commercial ornamental plants are being propagated by *in vitro* culture on the culture medium containing auxins and cytokinins (Preil, 2003; Rout and Jain, 2004). Ornamental plants and woody plant species are also propagated commercially by axillary bud proliferation (Mantell *et al*., 1985; Pierik, 1987; Chu, 1992). Various explants have been used for direct shoot formation. Different types of auxin and cytokinin combinations were used in order to obtain complete regeneration of *Gerbera in vitro*. BAP strongly enhanced regeneration of shoots in petiole explants of *Gerbera jamesonii*. The right combination of auxin and cytokinin in the culture medium determined the effectiveness of micro propagation of *Gerbera* shoots. In the present study, regeneration of shoots increased when BAP was added with NAA. Highest numbers of shoots from petiole explants (9.3±0.6 per explant) were obtained when explants were cultured on MS medium supplemented with 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA (Table 1). Shoots were obtained after 28 days of culture and shoot growth was normal. The lowest shoot formation was observed when explants were cultured on MS medium supplemented with 0.1 mg L^{-1} BAP and 2.0 mg L^{-1} NAA with 1.6±0.7 shoots per explant (Table 1).

Table 1: Percentage of shoot formation and number of shoots per explants produced on MS medium supplemented with different hormones and concentrations after 8 weeks in culture

MS+hormone (mg L^{-1})	Explants	Shoot regeneration (%)	No. of shoots per explants
2.0 mg L^{-1} BAP+0.5 mg L^{-1} NAA	Petiole	94.3±2.5	9.3±0.6
2.0 mg L^{-1} BAP+1.5 NAA	Petiole	79.2±2.0	5.2±1.2
2.0 mg L^{-1} Zeatin+0.5 mg L^{-1} IBA	Petiole	83.7±1.5	7.4±0.9
0.1 mg L^{-1} BAP+2.0 mg L^{-1} NAA	Petiole	4.6±0.8	1.6±0.7
1.0 mg L^{-1} BAP+2.0 mg L^{-1} NAA	Petiole	15.4±2.7	3.5±1.8
1.5 mg L^{-1} BAP+1.0 mg L^{-1} NAA	Petiole	83.1±0.5	8.3±1.1
0.5 mg L^{-1} BAP+0.5 mg L^{-1} NAA	Petiole	33.4±1.2	4.7±0.5
2.0 mg L^{-1} BAP+0.5 mg L^{-1} IAA	Petiole	33.6±3.4	3.5±2.6
2.0 mg L^{-1} BAP+0.5 mg L^{-1} IBA	Petiole	39.7±2.1	5.1±1.3
2.0 mg L^{-1} Kinetin+0.5 mg L^{-1} IBA	Petiole	66.7±0.8	6.2±0.7
2.0 mg L^{-1} Zeatin+0.5 mg L^{-1} 2,4-D	Petiole	22.1±1.5	2.4±1.6
2.0 mg L^{-1} Zeatin+0.5 mg L^{-1} IAA	Petiole	73.2±1.4	5.6±0.8
2.0 mg L^{-1} 2ip+0.5 mg L^{-1} IBA	Petiole	11.9±2.3	2.9±1.7
2.0 mg L^{-1} 2ip+0.5 mg L^{-1} IAA	Petiole	26.3±2.0	2.4±1.0
3.0 mg L^{-1} BAP	Petiole	80.4±1.6	8.8±0.9

Addition of strong auxin (NAA) with BAP promoted better shoot formation compared to weak auxin (IAA) (Pierik *et al*., 1973). However, higher addition of auxin compared to cytokinin in the culture medium resulted in the inhibition of shoot formation. Shoot formation was observed when capitulum explants were cultured on MS modified medium supplemented with 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} IAA (Eduardo *et al*., 1991). Jones *et al*. (2007) reported that the supplementation of TDZ (thidiazuron) in culture medium resulted shoots regeneration in *Echinacea purpurea* L. Lower concentration of TDZ promoted direct shoot regeneraration and higher concentrations promoted callus induction in *Hagenia abyssinica* (Bruce) J.F. Gmel. (Feyissa *et al*., 2005). The present study showed that only petiole explant produced optimum results for shoot formation. Capitulum explant promoted callus development. Vardja and Vardja (2001) reported that, the addition of 10 mg L^{-1} BAP in culture medium increased the formation of adventitious buds of *Gerbera* at initiation and multiplication stage. However, in this study, at a very high concentration of BAP and other growth regulators, development and growth of shoots were inhibited.

It is reported that a good combination of cytokinin and auxin in the culture medium enhanced good shoot formation and plantlet regeneration from explants, for example, adventitious *Gerbera* shoots were regenerated from flower buds of greenhouse grown plants (Pierik *et al*., 1973, 1975; Laliberte *et al*., 1985). Martin *et al*. (2003) obtained indirect shoot bud regeneration from lamina explants of *Anthurium andraeanum* on MS medium supplemented with 0.25 mg L^{-1} BAP, 0.2 mg L^{-1} IAA and 0.1 mg L^{-1} Kinetin. Takayama and Misawa (1982) reported that medium containing 0.3 mg L^{-1} BAP or 0.1 mg L^{-1} Kinetin along with 1.0 mg L^{-1} NAA showed rapid regeneration of shoot buds from leaf and petiole segments of *Begonia* sp. The combination of low concentration of cytokinin and auxin initiated propagation of *Begonia* species (Reuter and Bhandari, 1981). Cytokinin alone in the culture medium induces shoot formation in many plants. However, in *Gerbera jamesonii*, cytokinin alone in the medium induces the formation of microshoots. Combination of auxin and cytokinin induces the formation of adventitious shoots and roots. Addition of auxins together with cytokinins becomes essential for shoot induction and multiplication depending on the plant type. Micropropagation of *Saintpaulia ionantha* was optimized when petiole explants were cultured on MS medium supplemented with 2.0 mg L^{-1} Zeatin and 1.0 mg L^{-1} IAA (Daud, 2005). Rancillac and Nourrisseau (1989) improved the performance of micropropagated strawberry plants by decreasing the cytokinin concentration and limiting the number of subcultures. High concentrations of cytokinin in culture medium were found to be unsuitable for shoot formation from leaf or petiole explants in some ornamentals. *Gerbera* plantlets formed were transferred to soil with 75.03% survival rate. The regenerated plants were observed to be normal.

The first step towards de novo regeneration is to establish callus or cell suspension cultures. Tissues of explants generally show distinct planes of cell division, various specializations of cells and organization into specialized structures such as the vascular system. Formation of callus from explants tissues involves the development of progressively more random planes of cell division, less frequent specialization of cells and loss of organized structures (Thorpe, 1980; Wagley *et al*., 1987). In *Gerbera jamesonii*, BAP was also required for the formation of callus. Based on the present study, all combinations of hormones used were able to induce callus. Callus with good and compact structure was obtained when explant was cultured on MS medium supplemented with the combination of BAP and 2, 4-D. Relatively, in this experiment, callus formation was optimum when leaf explant was cultured on MS medium supplemented with 1.0 mg L^{-1} BAP+2.0 mg L^{-1} 2, 4-D (Table 2). This callus gave the highest dry weight. Callus

Table 2: Percentage of callus and root formation per explants produced on MS medium supplemented with different hormones and concentration after 8 weeks in culture

MS+hormone (mg L^{-1})	Explants	Callus (%)	Root (%)	No. of roots per explant
2.0 mg L^{-1} BAP+	Leaf	89.4± 2.3	0	0
0.5 mg L^{-1} NAA	Root	67.5± 4.7	0	0
2.0 mg L^{-1} Zeatin+	Leaf	65.8±3.3	2.5±0.7	0.8±0.7
0.5 mg L^{-1} IBA	Root	31.7±5.2	0	0
0.1 mg L^{-1} BAP+	Leaf	58.9±3.1	73.7±2.3	22.1±2.3
2.0 mg L^{-1} NAA	Root	49.7±3.9	63.5±0.5	19.1±0.5
0.5 mg L^{-1} BAP+	Leaf	86.3±0.6	71.3±1.5	21.4±1.5
2.0 mg L^{-1} NAA	Root	58.3±1.6	65.0±0.9	19.5±0.9
1.0 mg L^{-1} BAP+	Leaf	90.7±0.9	21.4±2.1	6.4±2.1
2.0 mg L^{-1} NAA	Root	79.3±3.4	26.3±1.8	7.9±1.8
1.5 mg L^{-1} BAP+	Leaf	87.3±0.6	1.2±0.8	0.4±0.8
1.0 mg L^{-1} NAA	Root	55.6±1.2	7.4±0.5	2.2±0.5
2.0 mg L^{-1} BAP+	Leaf	66.3±2.4	0	0
0.5 mg L^{-1} IAA	Root	42.8±3.6	0	0
2.0 mg L^{-1} BAP+	Leaf	78.4±2.7	0	0
0.5 mg L^{-1} IBA	Root	29.1±3.2	0	0
1.0 mg L^{-1} BAP+	Leaf	98.4±0.6	0	0
2.0 mg L^{-1} 2,4-D	Root	83.2±1.5	0	0
2.0 mg L^{-1} BAP+	Leaf	87.1±2.2	0	0
2.0 mg L^{-1} 2,4-D	Root	60.4±1.8	0	0
1.0 mg L^{-1} BAP+	Leaf	57.0±2.0	0	0
0.1 mg L^{-1} 2,4-D	Root	30.5±1.0	0	0
2.0 mg L^{-1} IAA+	Leaf	63.6±2.3	54.7±1.7	16.4±1.7
0.5 mg L^{-1} 2iP	Root	43.5±1.9	23.1±2.1	7.0±2.1
2.0 mg L^{-1} IBA+	Leaf	86.5±1.2	16.7±1.6	5.0±1.6
2.0 mg L^{-1} 2iP	Root	50.3±2.4	9.6±1.0	2.9±1.0
2.0 mg L^{-1} 2,4-D+	Leaf	91.3±1.6	68.4±0.5	20.5±0.5
0.5 mg L^{-1} Zeatin	Root	66.4±2.3	0	0
3.0 mg L^{-1} BAP	Leaf	5.3±0.5	0	0
	Root	2.6±1.7	0	0
2.0 mg L^{-1} NAA	Leaf	10.4±2.4	70.7±2.4	21.2±2.4
	Root	5.5±0.3	60.5±1.0	18.1±1.0

formed in these experiments were mainly green. However, white, creamy friable callus was obtained when explants were cultured on MS medium supplemented with 2, 4-D alone. The lowest callus formation was obtained when root explant was cultured on MS medium supplemented with 3.0 mg L^{-1} BAP (Table 2). In *Begonia*, the addition of kinetin and zeatin in the culture medium (Murashige and Skoog, 1962) induced multiple shoots (Jain, 1997). Callus is capable of forming adventitious roots. Root formation occurred when explants were cultured on medium with higher auxin concentration and lower cytokinin concentration. Highest root formation was obtained when leaf explants were cultured on MS medium supplemented with 0.1 mg L^{-1} BAP and 2.0 mg L^{-1} NAA with an average of 22.1±2.3 roots per explants (Table 2). However, regeneration of root was not observed when explants were cultured on MS medium supplemented with BAP and 2, 4-D. Bigot (1981) reported that the addition of 1-2 g L^{-1} activated charcoal in the culture medium showed vigorous rooting from excised shoots of *Begonia*× *hiemalis*. However, in *Gerbera*, addition of charcoal is not necessary for rooting.

Roest *et al*. (1981) successfully induced roots in the adventitious shoots of chrysanthemum in the liquid MS medium containing 1.0 mg L^{-1} IAA. Shoots and roots of chrysanthemum were developed on a single medium containing 1.0 mg L^{-1} BAP and 1.2 mg L^{-1} kinetin. In this study, roots were induced from adventitious shoots of *Gerbera jamesonii* on solid MS medium fortified with various concentration and combination of BAP, NAA, IBA, 2iP and Zeatin. Root explants itself also induced the formation of root *in vitro*.

Rooted plantlets of *Gerbera* were successfully established and all plantlets were transferred to soil and maintained in the green house. Plantlets survival rate achieved was 75.03%.

CONCLUSIONS

The main purpose for propagation of ornamental plants is for its aesthetic value. Thus, improvements of plant quality need to be studied and more research needs to be done. One of the most important techniques in plant improvements is via micro propagation. Successful *in vitro* propagation of ornamental plants is now being widely used in commercialization purposes. In conclusion, the research done has proven that micro propagation of *Gerbera jamesonii* Bolus ex. Hook f. *in vitro* could be successfully obtained. Petiole explants have been identified as the most regenerative explants for multiple shoot formation. Studies of *Gerbera* clonal propagation could also be efficiently adapted for other ornamental plants.

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