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In vitro Flowering of Shoots Regenerated from Cultured Nodal Explants of *Gypsophila paniculata* L.

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Abstract

A protocol for the regeneration of *Gypsophila paniculata* L. using nodal explants from 2-month-old field grown plants was established. The induction of multiple shoots was best obtained on Murashige and Skoog (MS) medium supplemented with 13.3 µM BA. Callus growth was observed on MS medium containing 44.3 µM BA. Calluses were transferred to MS medium supplemented with 2, 4-D (4.5, 13.5, 22.6 µM), NAA (5.3, 16.1, 26.8 µM) or BA (4.4, 13.3, 22.1 µM) for 2 months to induce shoot formation. After 6 weeks of initial culture, multiple shoots were regenerated from calluses cultured on MS medium supplemented with 13.3 µM BA. All regenerated shoots produced roots on 16.1 µM NAA containing MS medium within 4 weeks. Rooted plantlets were hardened and established in pots at 100% survival. For induction of *in vitro* flowering, regenerated shoots could be induced to flower efficiently when cultured on MS medium containing 13.3 µM BA and 50 g/l sucrose.

Keywords: *Gypsophila paniculata*, *in vitro* flowering, micropropagation, nodal culture, sucrose

Introduction

Gypsophila (*Gypsophila paniculata* L.), commonly known as Baby's breath, is a very popular ornamental plant in the florist trade. It belongs to the family Caryophyllaceae and is a native of Eastern Europe. The flowers of *G. paniculata* are sterile and do not produce seeds hence breeding programs are limited (Rady, 2005). Thus, micropropagation seems to be the most promising method for the large-scale production of plantlets for use in horticultural markets. The other advantages of clonal propagation over conventional propagation techniques include: progeny are true to type, multiplication is rapid, and production costs are lower. Tissue culture systems for *G. paniculata* have been reported that utilize callus and cell culture (Salman, 2002); leaf explants (Zuker *et al*., 1997); stem segments (Ahroni *et al*., 1997) and shoot tips (Rady, 2005). The switch from the vegetative stage to the reproductive stage of growth is one of the most critical events in the life of a plant. An *in vitro* flowering system is considered to be a convenient tool to study this process. Recent advances in flowering research, for instance control of the flowering mechanisms, can be studied with an *in vitro* experimental system. An *in vitro* flowering system has been described for several plants such as bamboo (Nadgauda *et al*., 1997); orchid (Kostenyuk *et al*., 1999) and rose (Vu *et al*., 2006). Recently, the applications of cytokinins and optimum sucrose concentrations that promote *in vitro* flowering have been well documented for many plant species.

In this context we have devised and described an effective tissue culture technique that yields large numbers of shoots from nodal explants and can be induced to produce *in vitro* flowering of *G. paniculata*. This study is part of a larger program designed to investigate the *in vitro* flowering mechanisms of *G. paniculata*.

Materials and methods

Plant materials and in vitro conditions

Nodal explants containing lateral buds of 2-month-old field grown *G. paniculata* plants were used for the multiplication experiments. They were cut into 2-3 cm length segments and washed in running water to remove any dirt. These segments were then surface disinfested using 70% ethanol for 10-20 sec followed by immersion in 20% (v/v) Clorox™ solution containing 2 drops of Tween-20 emulsifier for 20 min to aid wetting. After surface decontamination the sterilized explants were washed 2-3 times with sterile distilled water to remove any disinfectant solution. They were trimmed down to 1 cm long pieces prior to transfer to the culture medium.

Medium preparation and culture conditions

MS (Murashige and Skoog, 1962) salts and vitamins supplemented with 30 g/l sucrose were used as the basal medium. Mermaid™ agar (8.2 g/l) was used as a gelling agent. The pH of all media was adjusted to 5.8 with 1 N NaOH or 1 N HCl prior to autoclaving at 1.05 kg/cm^2,

121°C for 20 min. Cultures were maintained at 25±1°C air temperature in a culture room with a 16/8h light/dark photoperiod under an illumination of 20 μmol $m^{-2}s^{-1}$ photosynthetic photon flux intensity provided by cool-white fluorescent lights. Plant materials were stored in glass-capped culture jars (115 ml capacity) each containing 20 ml of medium.

Influences of BA on shoot multiplication and callus formation

To test the effect of BA (6-benzyladenine) on the formation of multiple shoots, nodal explants were excised and cultured on MS medium containing 0, 4.4, 13.3, 22.1 or 44.3 μM BA for 6 weeks. After this period callus formation was observed and the effect of 4.5, 13.5 or 22.6 μM 2, 4-D (2, 4-dichlorophenoxyacetic acid), 5.3, 16.1 or 26.8 μM NAA (α-naphthaleneacetic acid) and 4.4, 13.3 or 22.1 μM BA on the regeneration of shoots from the callus culture was recorded. To establish root proliferation, green and normal adventitious shoots from shoot multiplication cultures were excised and placed on MS containing 16.1 μM NAA. When adequate rooted shoots were obtained, the plantlets were transferred to 330 ml screw-topped jars containing sterile vermiculite for 2 weeks for hardening.

Effect of sucrose and BA on in vitro flowering

Three concentrations of sucrose (0, 30 and 50 g/l) were added to the MS medium supplemented either with 0, 13.3 or 22.1 μM BA. Explants were incubated as previously described for 8 weeks.

Statistical analysis

One explant (1 cm long) was planted per jar and all experiments were carried out with 20 replicates and the experiments were repeated on three consecutive days. Data were submitted to ANOVA and the difference between the mean values was compared using Duncan's Multiple Range Test at $p \leq 0.05$.

Results and discussion

After 6 weeks of initial culture, nodal explants cultured on MS medium with several concentrations of BA developed multiple shoots. Results obtained revealed that 13.3 μM BA gave the highest number (6.0±1.05) shoots with green expanded leaves (Fig. 1a). There were significant differences ($p \leq 0.05$) in shoot number per nodal (Tab. 1). This may indicate that bud formation in this cultivar required BA. Ahroni *et al.* (1997) obtained different results with different cytokinins. They reported that thidiazuron was the most efficient cytokinins they tested for adventitious shoot regeneration from stem-segment explants. Therefore MS medium containing 13.3 μM BA was considered to be currently optimal for shoot proliferation and we routinely use this protocol for multiplication of shoots used in further experiments.

No callus was formed in the presence of 0, 4.4, 13.3, or 22.1 μM BA; however, callus formation was observed only with medium containing 44.3 μM BA (Fig. 1b). The initial callus was pale yellow to green in color and friable in appearance and callus growth was rather slow. After a 4-week culture period the speed of callus formation became faster. In order to optimize the shoot regeneration from callus, a second experiment was conducted by transferring these callus forms to MS medium supplemented with 2, 4-D, NAA and BA at different concentrations. No shoot bud induction was formed when 2, 4-D or NAA was added to the medium. With 13.3 μM BA incorporated into the medium, a yellow friable callus was formed simultaneously with shoot development within 8 weeks of culture at a frequency of 3-4 shoots and callus-derived shoots did not produce any roots on this medium (Fig. 1c).

In vitro flowering was seen on MS medium containing BA and sucrose after 8 weeks of culture (Tab. 2). The flowers were small, had normal petals and sepals and proceeded to open (Fig. 1d). Cytokinins are believed to induce molecular changes associated with floral transition (Bernier *et al.,* 2002). BA has been used for most experiments on *in vitro* flowering of many plant species such as *Cymbidium niveomarginatum* (Kostenyuk *et al*., 1999), *Bambusa*

Tab. 1. Effect of BA on multiple shoot formation in *Gypsophila paniculata* L.

MS medium + BA (μM)	Number of shoots per explant (Mean ± SE)
0	1.3±0.48^{c}
4.4	0.5±0.7^{d}
13.3	6.0±1.2^{a}
22.1	2.8±0.7^{b}
44.3	0.1±0.3^{d}

The different letters within a column identify significant differences using ANOVA and Duncan's Multiple Range Test at p≤0.05

Tab. 2. Effect of different combinations of BA and sucrose on *in vitro* flowering of *Gypsophila paniculata* L. cultured on MS medium

BA (μM)	Sucrose (g/l)	Number of flowers per explant (Mean ± SE)
0	0	0^{d}
0	30	0^{d}
0	50	0^{d}
13.3	0	0^{d}
13.3	30	3.3±0.48^{c}
13.3	50	6.8±0.63^{a}
22.1	0	0^{d}
22.1	30	2.9±0.73^{c}
22.1	50	5.0±0.66^{b}

The different letters within a column identify significant differences using ANOVA and Duncan's Multiple Range Test at p≤0.05

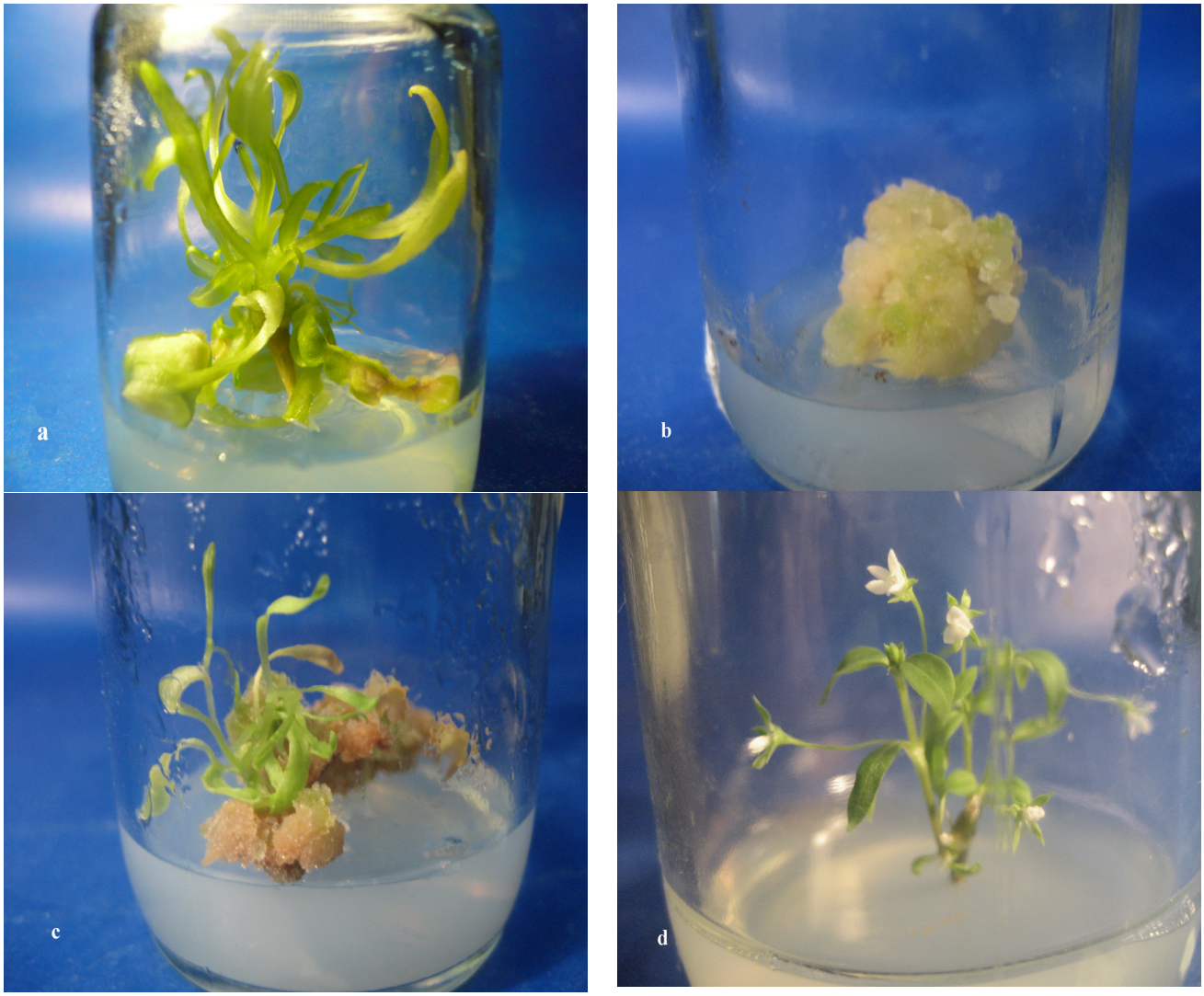

Fig. 1. Plant regeneration and *in vitro* flowering in *Gypsophila paniculata* L. (a) Multiple shoot formation. (b) Nodal derived callus. (c) Shoots regenerated from callus culture. (d) Regenerated shoots that flowered *in vitro*

edulis (Lin *et al*., 2003), *Kniphofia leucocephala* (Taylor *et al*., 2005) and *Rosa hybrida* (hybrid tea) (Vu *et al*., 2006). Sucrose is generally known as carbon sources for the vegetative growth and development of flowers. The effect of sucrose on shoots bearing floral buds has been reported in a number of species such as *Fortunella hindsii* (Jumin and Nito, 1996), *Fagopyrum esculentum* (Kachonpadungkitti *et al*., 2001), *Rosa hybrida* (hybrid tea) cv. 'First Prize' (Vu *et al*., 2006). BA and sucrose together could induce flowering in our system and indicates that Gypsophila is not cultivar-dependent.

To establish complete *Gypsophila* plants, regenerated shoots were excised and transferred to MS medium containing 16.1 μM NAA to induce roots. Roots that developed on this medium were thick, long and fibrous. Two weeks of rooting was adequate before transplanting to polystyrene pots containing soil mixture (1 sand: 1 manure: 1 decayed leaves v/v). *In vitro*-derived plants did not display any phenotypic variation during subsequent vegetative development.

Conclusions

A micropropagation system was developed for *G. paniculata* utilizing nodal explants. Callus culture with its regenerative capacity reported here could be useful for a genetic transformation system in *G. paniculata*. Regenerated shoots could flower in the presence of certain BA and sucrose concentrations. Although *in vitro* flowering was observed, a more consistent culture regime may need to be developed for commercial exploitation.

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References

Ahroni A, Zuker A, Rozen Y, Shejtman H, Vainstein A (1997). An efficient method for adventitious shoot regeneration from stem-segment explants of *Gypsophila*. Plant Cell Tissue and Organ Culture 49:101-106.

Bernier G, Corbesier L, Perilleux C (2002). The flowering process: on the track of controlling factors in *Sinapsis alba*. Russian Journal of Plant Physiology 49:445-450.

Jumin HB, Nito N (1996). *In vitro* flowering of *Fortunella hindsii* (Champ.). Plant Cell Reports 15:484-488.

Kachonpadungkitti Y, Romchatngoen S, Hasegawa K, Hisajima S (2001). Efficient flower induction from cultured buckwheat (*Fagopyrum esculentum* L.) node segments *in vitro*. Plant Growth Regulation 35:37-45.

Kostenyuk I, Oh BJ, So IS (1999). Induction of early flowering in *Cymbidium niveo-marginatum* Mak *in vitro*. Plant Cell Reports 19:1-5.

Lin CC, Lin CS, Chang WC (2003). *In vitro* flowering of *Bambusa edulis* and subsequent plantlet survival. Plant Cell Tissue and Organ Culture 72:71-78.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15:473-497.

Nadgauda RS, John CK, Parasharami VA, Joshi MS, Mascarenhas AF (1997). A comparison of *in vitro* with *in vivo* flowering in bamboo: *Bambusa arundinacea*. Plant Cell Tissue and Organ Culture 48:181-188.

Rady MR (2005). *In vitro* culture of *Gypsophila paniculata* L. and random amplified polymorphic DNA analysis of the propagated plants. Arab Journal of Biotechnology 8:155-168.

Salman MN (2002). Establishment of callus and cell suspension culture from *Gypsophila paniculata* leaf segments and study of the attachment of host cells by *Erwinia herbicola* pv. *Gypsophilae*. Plant Cell Tissue and Organ Culture 69:189-196.

Taylor NJ, Light ME, Van Staden J (2005). *In vitro* flowering of *Kniphofia leucocephala*: influences of cytokinins. Plant Cell Tissue and Organ Culture 83:327-333.

Vu NH, Anh PH, Nhut DT (2006). The role of sucrose and different cytokinins in the *in vitro* floral morphogenesis of rose (hybrid Tea) cv. 'First Prize'. Plant Cell Tissue and Organ Culture 87:315-320.

Zuker A, Ahroni A, Shejtman H, Vainstein A (1997). Adventitious shoot regeneration from leaf explants of *Gypsophila paniculata* L. Plant Cell Reports 16:775-778.