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Induction, growth and direct rooting of adventitious shoots of *Begonia* × *hiemalis*

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Abstract. The efficiency of commercial micropropagation programs for *Begonia* × *hiemalis* depends on the production of large adventitious shoots for easy handling and on effective rooting and acclimatization procedures. Maximum induction of adventitious buds on petiole segments occurred in response to NAA (0.1 mg,l^{-1}) and BA (0.5 mg l^{-1}), but continued shoot growth was limited. With a lower concentration of BA (0.1 mg l^{-1}) fewer shoots were produced but shoot growth was enhanced. With a combined agar/liquid culture program the low BA (0.1 mg l^{-1}) medium produced 50 percent more shoots larger than 1 cm than did the high BA (0.5 mg l^{-1}) medium. In vitro rooted explants developed weak root systems and acclimatization losses occurred during adaptation to greenhouse conditions. Adventitious shoots treated with commércial rooting powder and placed directly in mist frames produced much stronger root systems and could be adapted to greenhouse conditions without loss. The elimination of the in vitro rooting stage also simplifies the micropropagation program.

Introduction

The high degree of organogenic potential exhibited by *Begonia* × *hiemalis* explants has been used to develop micropropagation procedures. Such schemes take advantage of the induction of very large numbers of adventitious buds in response to combinations of auxins and cytokinins [1, 9, 10]. Although several studies have indicated the potential for producing vast numbers of plants by these means [2, 3, 7], in practice this potential is rarely realized. Many of the adventitious buds remain too small to be rooted, and considerable plant loss also occurs at the rooting and acclimatization stages [4].

In preliminary studies it was observed that plantlets growth was limited on auxin/cytokinin combinations which resulted in the induction of the greatest numbers of adventitious buds. The purpose of this investigation was to determine culture conditions which provide the highest production of shoots large enough for successful rooting and to modify rooting procedures so that acclimatization loss could be reduced.

Materials and methods

Plant material

Stock plants of *Begonia* × *hiemalis* (Fotsch.) cv. Schwabenland Pink were maintained in controlled environment cabinets at 25 °C ± 0.5 °C in a 16 h photoperiod provided by a mixture of fluorescent light (Sylvania CW/VHO/135) 180 uE m^{-2} s^{-1} and incandescent light 20 uE m^{-2} s^{-1} at pot height. Light measurements were made with a Li-Cor Model Li-185A radiometer (400–700 nm bandwidth with cosine correction). The plants were grown in six-inch pots in a mixture of peat : soil : sand (4 : 2 : 1) and fed once per week with $N : P_2O_5 : K_2O$ (20 : 20 : 20) fertilizer (1.25 g l^{-1}).

Micropropagation

Petioles of leaves 3–6 cm diameter were excised, surface sterilized by immersion in 70% ethyl alcohol for 30 s, followed by 15 min in 7% (W/V) calcium hypochlorite solution and three rinses in sterile distilled water. Petiole segments (5 mm long) were obtained aseptically and each one transferred onto a nutrient medium in a 115 ml glass jar. The shoot induction medium consisted of Murashige & Skoog [5] major and minor elements (MS medium) sucrose at 30 g/l and various concentrations of naphthalene acetic acid (NAA) and 6-benzylaminopurine (BA). The pH was adjusted to 5.7 with 0.2 N KOH and the medium solidifed with 0.8% agar. Shoot growth medium was the same as this except that agar was omitted. For in vitro rooting, BA was eliminated from the agar cultures. All media were autoclaved at 125 °C and 20 p.s.i. for 15 min. The cultures were grown in a 16 h photoperiod provided by fluorescent light (Sylvania CW/20) 40 uE m^{-2} s^{-1} at shelf level. Liquid shake cultures (15 ml/250 ml erlenmeyer flask containing 4 explants) were maintained for 3 weeks on a rotary shaker operated at 100 r.p.m. Further growth of adventitious shoots was obtained in static liquid culture. Flasks were transferred directly to shelves in the culture room, without replenishing the medium, and maintained for another 3–6 weeks before shoots were harvested. Shoot initiation and growth on agar medium in response to NAA and BA were measured after 6 weeks in culture when shoots were sufficiently large for quantitative analysis. The following micropropagation sequence was used to obtain shoots for rooting studies. Petiole explants were cultured on agar for 3 weeks and then transferred to liquid medium and maintained for 3 weeks on a shaker followed by 3 weeks static culture.

Direct rooting in the mist frame

Shoot explants (1 cm) were excised from shoot multiplication cultures, the basal region dipped in commercial rooting powder (Stim-Root, Plant Products Co. Ltd., Bramalea, Ontario, Canada) containing either 0.1% or 0.4% indolebutyric acid (IBA), and planted in cell-packs containing peat or

peat:sand (1:1). These were transferred to mist frames with bottom heat at 21 °C. Timed misting was controlled by an electronic leaf moisture sensor. For the first week the plants were misted 24 h day^{-1} and then for 16 h day^{-1} for another two weeks. The plants were then transplanted into three-inch pots containing soil mix (4:1:2:1), fed immediately with $N:P_2O_5:K_2O$ (10:52:10) fertilizer (0.9 g l^{-1}) and grown in a shaded greenhouse. The shade was removed after seven days and the plants fed once per week with $N:P_2O_5:K_2O$ (20:20:20) fertilizer (1.25 g l^{-1}).

All treatments included at least 20 explants and experiments were repeated at least twice.

Results

Adventitious shoots were induced on petiole explants in response to various combinations of NAA and BA. Shoot initiation was earliest on medium with NAA:BA (0.1:0.5 mg l^{-1}) (Figure 1B). This treatment also produced most shoots per explants (Figure 7). Shoot initiation was delayed on medium with lower BA (NAA:BA, 0.1:0.1 mg l^{-1}) (Figure 1A) and fewer shoots were formed (Figure 7) but subsequent shoot growth was better on this low BA (0.1 mg l^{-1}) medium than on the higher BA (0.5 mg l^{-1}) medium (Figures 2 and 7). After six weeks on medium with BA 0.1 mg l^{-1} each explant produced ten shoots longer than 20 mm while with BA 0.5 mg l^{-1} only five such shoots developed and BA 1.0 mg l^{-1} medium produced only two per explant (Figure 7). Similarly, on a micropropagation schedule, which comprised three weeks agar culture followed by three weeks liquid shake and three weeks liquid static culture, explants on BA 0.1 mg l^{-1} yielded 9 shoots longer than 1 cm while those on BA 0.5 mg l^{-1} produced only 6 such shoots.

Maximum in vitro rooting of these shoot explants was obtained after three weeks on medium with NAA 0.05 or 0.1 mg l^{-1} (Table 1). Each explant initiated approximately four roots but these were relatively short and poorly developed (Figure 3). These plants did not readily establish in soil; plant loss (10–30%) occurred during acclimatization and transfer to greenhouse conditions. Shoot explants smaller than 1 cm were considered unsatisfactory for commercial micropropagation procedures because they were difficult to manipulate during rooting and acclimatization procedures and more transplantation losses occurred.

Root initiation and development was improved on the larger shoot explants by rooting them directly in mist frames. After a three week rooting period the directly rooted explants produced much more vigorous root systems than did in vitro rooted explants (cf Figures 3 and 4). Peat was superior to peat:sand (1:1) substrate and IBA (0.4%) rooting powder was better than IBA (0.1%) for root initiation (Table 2). Direct rooting increased root initiation two-fold over in vitro rooting levels. These plants acclimatized to lower levels of relative humidity during the rooting period and could be

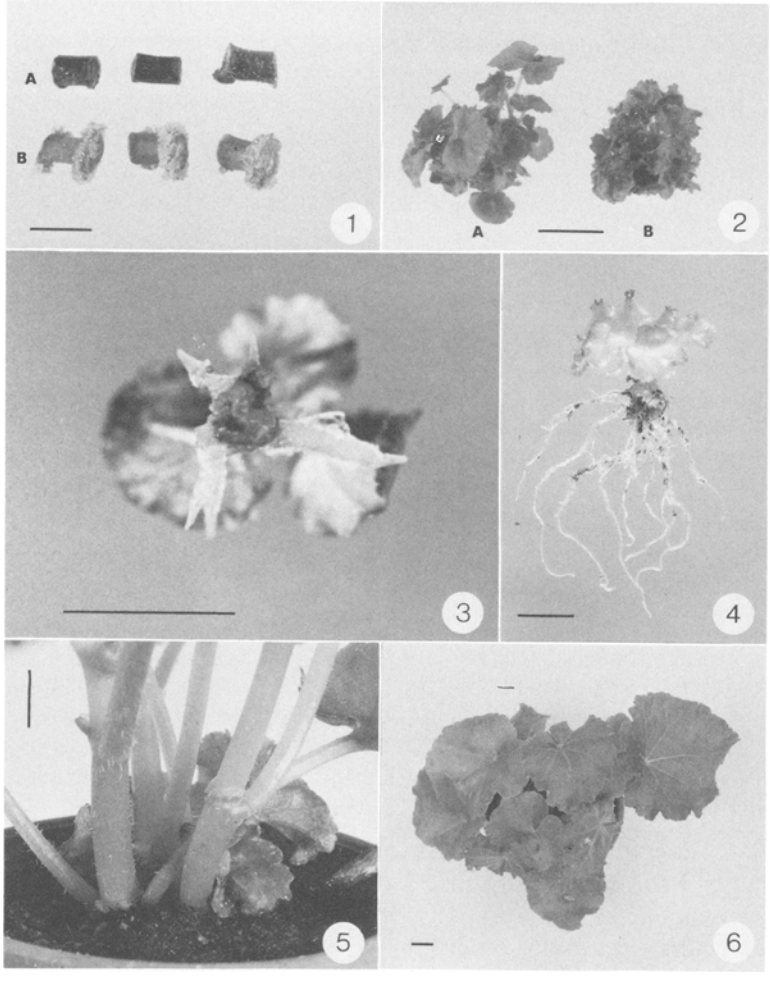

Figure 1. Adventitious shoot induction on petiole explants of *B.* × *hiemalis*. Incubation three weeks on agar medium,
a. NAA (0.1 $mg\,l^{-1}$), BA (0.1 $mg\,l^{-1}$);
b. NAA (0.1 $mg\,l^{-1}$), BA (0.5 $mg\,l^{-1}$).

Figure 2. Adventitious shoot induction on petiole explants of *B.* × *hiemalis*. Incubation, three weeks on agar medium, and six weeks liquid culture.
a. NAA (0.1 $mg\,l^{-1}$), BA (0.1 $mg\,l^{-1}$);
b. NAA (0.1 $mg\,l^{-1}$), BA (0.5 $mg\,l^{-1}$).

Figure 3. In vitro root production on adventitious shoots of *B.* × *hiemalis*. Incubation, three weeks on agar medium with NAA (0.1 $mg\,l^{-1}$).

Figure 4. Direct rooting of adventitious shoots of *B.* × *hiemalis* in mist frame in response to rooting powder (IBA, 0.4%). Incubation, three weeks in peat substrate.

Figures 5, 6. Multi-stemmed plants in soil mix and maintained in a greenhouse.

Bar scale on each figure represents 1 cm.

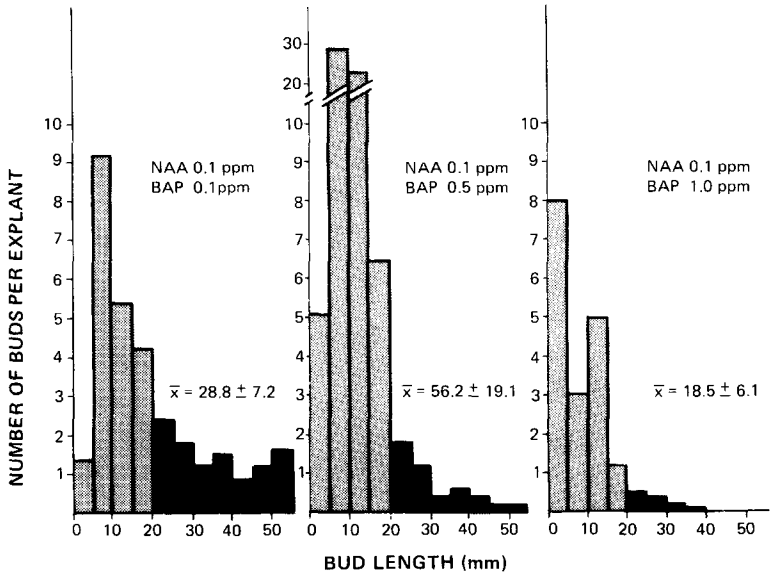

Figure 7. Influence of BA on induction and growth of adventitious buds of *B.* × *hiemalis*. Incubation six weeks on agar medium.

transferred directly to the greenhouse without losss where they developed into robust multi-stemmed plants (Figures 5 and 6).

Discussion

Maximum adventitious shoot formation on petiole explants occurred in response to NAA ($0.1\,mg\,l^{-1}$) with BA ($0.5\,mg\,l^{-1}$) which was in agreement with previous observations [4]. However, efficient micropropagation procedures require not only the production of large numbers of shoots but also on their continued development until they can be manipulated for rooting and acclimatization. It has been reported that only larger shoots can be used for micropropagation; when a broader spectrum of developing shoots was used only 49% survived to become established greenhouse plants [4]. In another proposed micropropagation system, which used NAA ($1.0\,mg\,l^{-1}$) in the shoot proliferation medium, many buds were produced on agar medium but growth ceased and they remained too small for rooting. Shoot growth was improved by a further period in liquid shake culture but affects on plant establishment were not reported [8]. In this study adventitious shoot growth was increased when shoot production was limited on low BA ($0.1\,mg\,l^{-1}$) medium. These shoots could be readily rooted in vitro but transplantation losses still occurred. Poor root development and callus production at the root/shoot union are factors which would decrease root efficiency during

Table 1. In vitro root development on micropropagated shoot explants of *Begonia* x *hiemalis* cv. Schwabenland Pink

Response[1]	NAA ($mg\,l^{-1}$)					
	0.01	0.05	0.1	0.5	1.0	5.0
Percent explants with roots	68	96	91	67	20	0
No. roots per explant[2]	5.4 ± 3.2	3.6 ± 2.6	4.1 ± 3.0	4.8 ± 2.8	5.4 ± 3.6	0

[1] Incubation, three weeks on agar medium.
[2] Mean ± S.D. of 20 replicates.

Table 2. Direct rooting of adventitious shoots of *Begonia* x *hiemalis* cv. Schwabenland Pink in substrate under mist.

Response[1]	Peat : sand (1 : 1) IBA		Peat IBA	
	0.1%	0.4%	0.1%	0.4%
Percent explants with roots	100	100	100	100
No. roots per explant	6.3a[2]	6.1a	7.4b	9.0c
Root length (cm)	3.3a	3.6a	3.2a	4.1a

[1] Incubation, three weeks in mist frame.
[2] Values followed by the same letter, within rows, are no significantly different ($p = 0.05$) by Duncan's multiple range test.

subsequent acclimatization procedures. Transplantation loss of micropropagated apple rootstocks was decreased by rooting the shoots directly in substrate in mist frames [6]. Similarly, during a three-week rooting period, adventitious shoots of *B.* x *hiemalis* developed larger, more vigorous root systems in an aerobic substrate than in agar and these plants could be established, without loss, in the greenhouse.

In conclusion, it was demonstrated that the induction of very large numbers of adventitious buds can be inhibitory to the subsequent growth of the shoots, which is not desirbale for micropropagation programs. Secondly, directly rooted adventitious shoots produced more efficient root systems than did in vitro rooted shoots. This is of particular economic importance for micropropagation of this variety because elimination of the in vitro rooting stage reduces labour and material costs, acclimatization to lower levels of relative humidity during the rooting period saves time and transplantation losses can be decreased.

Acknowledgement

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References

1. Appelgren M (1976) Regeneration of *Begonia hiemalis* in vitrol Acta Hortic 64: 31–38.
2. Hilding AS, Welander T (1976) Effect of some factors on propagation of *Begonia* × *hiemalis* in vitro. Swed J Agric Res 6:191–199
3. Khoder M, Villemur P, Jonard R (1981) La multiplication végétative de l'éspèce florale *Begonia elatior* (cultivar Rieger) à partir de différents organes cultivés in vitro CR Acad Sc Paris Ser III 293:403–408
4. Mikkelsen EP, Sink KC (1978) In vitro propagation of Rieger Elatior Begonias. Hort Science 13:242–244
5. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473–497
6. Simmonds J (1983) Direct rooting of micropropagated M26 apple rootstocks. Scientia Hortic 21:233–241
7. Takayama S, Misawa M (1981) Mass propagation of *Begonia* × *hiemalis* plantlets by shake culture. Plant Cell Physiol 22:461–467
8. Takayama S, Misawa M (1982) Factors affecting differentiation and growth in vitro, and a mass-propagation scheme for *Begonia* × *hiemalis* Scientia Hortic 16:65–75
9. Welander T (1977) In vitro organogenesis in explants from different cultivars of *Begonia hiemalis* Physiol Plant 41:142–145
10. Welander T (1979) Influence of medium composition on organ formation in explants of *Begonia* × *hiemalis* in vitro. Swed J Agric Res 9:163–168