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Bud Proliferation and Plant Regeneration in Liquid-Cultured Philodendron Treated with Ancymidol and Paclobutrazol

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Abstract: Philodendron plants propagated in liquid shake or bioreactor cultures proliferated profusely in the presence of paclobutrazol (PAC) and to a lesser extent in the presence of ancymidol (ANC). The growth retardants inhibited leaf development and induced the formation of bud clusters. Short transient treatments with low concentrations (1.7–3.4 μM) of the growth retardants limited leaf growth and proliferation to a lesser extent than higher concentrations (6.8–17 μM). The growth retardants had a carryover dwarfing effect in the semi-solid hardening medium, which was more pronounced at the higher concentrations or prolonged exposure periods. Regenerated plants resumed normal growth 3–6 weeks after transplanting. Treatment with growth retardants may become a useful method in the prevention of abnormal leaf growth in large-scale liquid cultures, as well as in enhancing bud proliferation.

In vitro propagation of several economic plant species is often handicapped by the lack of modern methods to overcome intensive labor manipulation (Levin and Vasil 1989). Scaling-up, using liquid cultures, along with a certain degree of automation, can provide an efficient economic micropropagation system (Levin et al. 1988). Bioreactors were used for somatic embryo development in alfalfa (Stuart et al. 1987), carrot (Ammirato and Styer 1986), celery (Nadel et al. 1990), and also for fern buds (Ziv and Hadar 1990) and gladiolus protocorms (Ziv 1990a). Plants propagated in liquid cultures exhibit malformed shoots, the leaves being the first organs to be affected, becoming vitreous (Ziv 1990c). Leaves formed in liquid culture do not survive transplanting *ex vitro* (Ziv 1986). The control of shoot morphogenesis *in vitro* requires culture conditions which will limit leaf development and at the same time enhance bud proliferation. Treatment with growth retardants, inhibitors of gibberellin (GA) biosynthesis (Graebe 1987), was reported to inhibit leaf expansion and enhance bud and/or protocorm proliferation in *Aechmea faciata* (Ziv et al. 1986) and gladiolus (Ziv 1989, 1990a). In liquid-cultured gladiolus, paclobutrazol and high sucrose in the liquid medium enhanced corm development (Steinitz and Lilien-Kipnis 1989). Philodendron is a popular house plant propagated quite extensively *in vitro* (Pierik 1990). Bud explants in the presence of benzylaminopurine (BA) and napthalene acetic acid (NAA) proliferated, forming shoot clusters which were separated for further subculture (Murashige 1977).

The objective of the present work was to examine the effects of growth retardants on leaf growth and bud proliferation, in philodendron plants cultured in liquid shake and bioreactor cultures.

Materials and Methods

Philodendron hastatum × *imble* × *wendlandii* cv. "Burgundy" shoot clusters were supplied in agar cultures by Biological Ind, Beit Haemek, Israel. The clusters were transferred to 150 ml medium in 500-ml Kimax flasks. Stocks were kept routinely by subculturing once every 30 days to Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 3% sucrose, 0.54 μM NAA, and 44 μM BA, inoculated with 4–5 g plant clusters. Proliferation experiments (stage 2; Murashige 1977) were conducted in 250-ml flasks containing 50 ml medium (same as above) inoculated with 2–3 g bud clusters. The flasks were rotated at 100 rpm on a gyratory shaker. Proliferation experiments in large-scale liquid cultures were carried out in 1 L bubble bioreactors containing 800 ml proliferation medium. The bioreactors were constructed as previously described (Ziv and Hadar 1990). Aeration and circulation were achieved by bubbling air at 0.6–0.7 vvm (volume air/volume medium/minute). The cultures were incubated under continuous photon flux of 3 $\mu E\ m^{-2}\ s^{-1}$ at 25 ± 1°C. Before every subculture, the clusters were separated and the leaves were removed with a scalpel. Each experiment

Table 1. The effect of a transient or a continuous treatment with ANC on growth of philodendron bud clusters in liquid shake cultures.

ANC (μM)		Leaves (%)[a]	Growth value[b]
0	T[c]	23.2 ± 3	1.3 ± 0.2
	C	15.8 ± 1.8	1.1 ± 0.18
19.5	T	11.8 ± 1.3	0.9 ± 0.04
	C	11.0 ± 1.0	0.8 ± 0.06
39.0	T	10.5 ± 0.9	0.7 ± 0.05
	C	6.9 ± 0.8	0.4 ± 0.05

[a] Leaves development was determined by % of 2 g FW bud cluster samples.
[b] Growth value = $(FW_{final} - FW_{initial})/FW_{initial}$.
[c] T, Transient treatment for 3 days was followed by transfer to ANC-free medium; C, continuous ANC for 32 days.

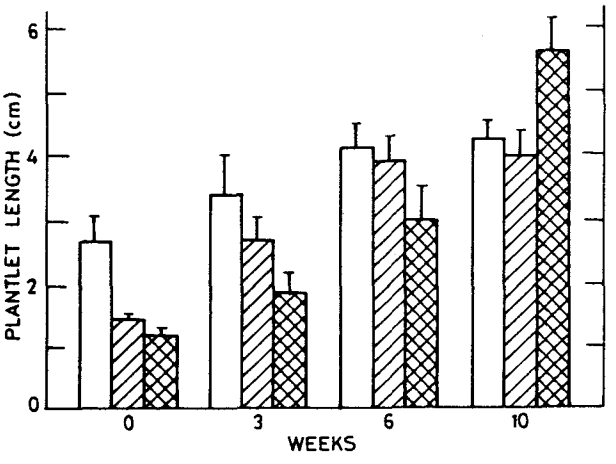

Fig. 1. The 3-day effect of transient treatment with ANC during the proliferation stage in liquid medium on plant growth (after transplanting to the greenhouse). ☐, 0 μM ANC; ▨, 19.5 μM ANC; ▩, 39 μM ANC. Bars indicate SE of the mean.

was repeated twice and consisted of six to eight flasks and two to three bioreactors. The hardening medium in stage 3 (Murashige 1977) included one-half strength MS minerals, full vitamins and other organic additions, 20.0 mg/L adenine sulfate, and 8% agar. Ancymidol [Elanco; α-cyclopropyl-α-(4-methoxy-phenyl)-5-pyrimidine methanol (ANC)] was added as an aqueous solution after the autoclaving by sterilization through a Millipore membrane to the cooled (35°C) medium. Paclobutrazol [ICI PP333; (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol (PAC)] was added as an aqueous solution prior to autoclaving. Stage 3 cultures in 10 × 10 × 6 cm boxes containing 80 ml medium were incubated under 16 h photoperiod of 50–70 $\mu E\ m^{-2}\ s^{-1}$. Plants were transferred to Milcap plugs in trays for acclimatization in the greenhouse. The plants in the plugs were watered with one-quarter strength Hoagland solution by flooding twice daily for 30 min. During the first 3 days the plants were mist sprayed for 10 s once every 2 h. The temperature was 25 ± 1°C under natural day length, and the radiation varied from 30–100 $\mu E\ m^{-2}\ s^{-1}$. Growth is expressed as a value calculated by dividing the difference between final and initial fresh weight (FW) with the initial FW: $(FW_{final} - FW_{initial})/FW_{initial}$. Leaf development was determined by calculating leaf FW in grams as the percent of leaves in 2 g FW plant cluster samples, five samples per treatment.

Results

Liquid-cultured philodendron buds in the presence of 0.54 μM NAA and 44 μM BA developed shoots with several vitreous leaves. The leaves contributed as much as 30% to the FW of clusters. Leaves developing during the proliferation stage in agitated liquid cultures became brown, degenerated, and released toxic substances into the medium, which caused tissue necrosis. Growth retardants were used to control leaf expansion. As can be seen in Table 1, both a short inductive and a continuous treatment with 19.5 or 39 μM ANC reduced leaf growth. The presence of ANC caused a significant inhibition of shoot growth which was the greatest in the 39 μM continuous ANC treatment. The transfer of the clusters to fresh medium in the absence of ANC enhanced leaf development, probably due to the renewal of the medium. Shoot clusters separated and subcultured to an agar-solidified hardening medium were dwarfed, and the developing leaves were oval instead of the spear-shape, typical of this variety. Treatment with ANC for 3 days inhibited plant development for a period of 6–10 weeks after transplanting *ex vitro*. Following recovery from the inhibitor, enhancement in growth was observed in plants treated with 39 μM ANC (Fig. 1). PAC, another inhibitor of GA biosynthesis (Grossmann 1990), was tested at lower concentrations, in the range of 1.7–17 μM, given as a continuous treatment for 32 days in shaken cultures. Increasing PAC concentration in the medium decreased leaf expansion, giving a smaller growth value (plant biomass). Treatments with 8.5 and 17 μM PAC reduced leaf percent (as part of total FW of the aggregates) to one fifth, and the growth value to about one half of the controls or the lower PAC levels (Table 2). PAC had an inhibiting effect on philodendron growth after the plants were transplanted to the greenhouse (Fig. 2). Even after 6 weeks *ex vitro*, 8.5 and 17 μM PAC had a carryover inhibiting effect. Treated plants were 2.0–2.5 cm high as compared to 6.0 cm in the control.

In order to reduce the inhibiting effect of PAC, short transient treatments of 1 or 3 days were compared to control or to the continuous presence of PAC for 26 days. Leaf expansion was reduced extensively even after 1 day of PAC treatment; from 22–13.5% of the total FW of the clusters. The pres-

Table 2. The effect of PAC on growth of philodendron bud clusters after 32 days in liquid shake cultures.

PAC (μM)	Leaves (%)[a]	Growth value[b]
0.0	25 ± 0.4	2.0 ± 0.4
1.7	10.6 ± 1.7	2.1 ± 0.1
3.4	10.4 ± 3.0	2.1 ± 0.1
8.5	8.4 ± 1.3	0.7 ± 0.5
17.0	5.2 ± 0.8	0.9 ± 0.04

[a,b] See legend to Table 1.

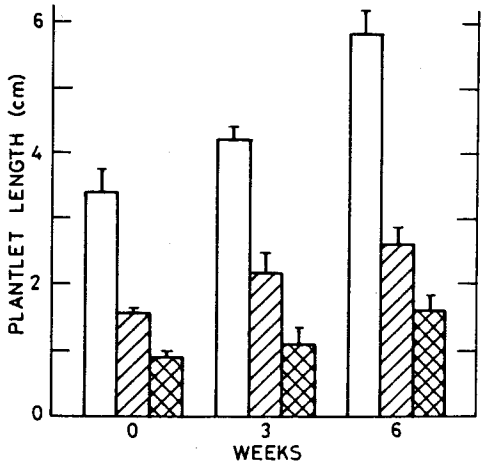

Fig. 2. The effect of PAC given continuously during the proliferation stage on subsequent growth and development of plantlet *ex vitro*. ☐, 0 μM PAC; ▨, 8.5 μM PAC; ▩, 17.0 μM PAC.

ence of PAC for 3 or 26 days in the medium reduced the leaves to 6 and 5% of the total FW, respectively. The growth value was inversely proportional to the length of time PAC was present in the medium. PAC had an enhancing effect on bud proliferation (Fig. 3a,b), which increased from 2.5 in controls to 18 and 21 buds per cluster in media where PAC was present for 3 or 26 days, respectively. However, PAC had a carryover effect on plant growth in the greenhouse, which was observed even after 8 weeks *ex vitro*. The presence of PAC for 26 days completely inhibited the growth of the plants *ex vitro*, whereas its presence for 1 or 3 days during the proliferation stage, reduced the plants FW to about 50% of the control (Fig. 4).

The effect of a transient PAC treatment was compared to the continuous presence of PAC in shake or bioreactor cultures (Table 3). The continuous presence of PAC in the medium reduced the leaves FW by about 30% and growth by 50% as compared to the transient treatment in both shake and bioreactor cultures. In contrast, bud proliferation was enhanced by the continuous presence of PAC, by 38 and 30% in shake or bioreactor cultures, respectively.

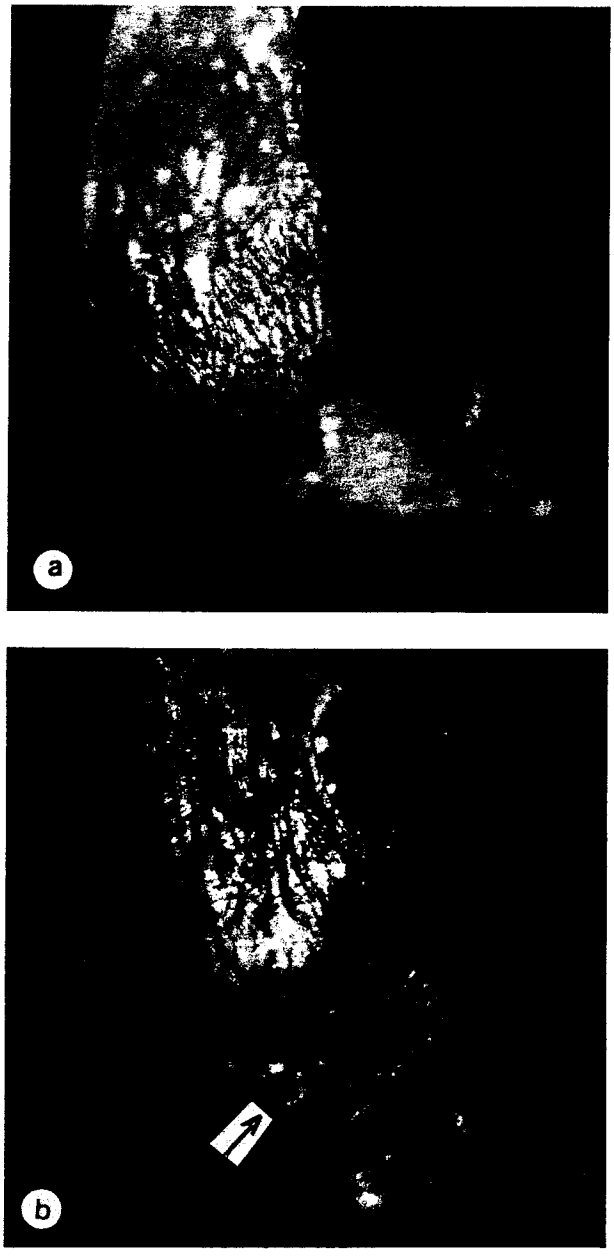

Fig. 3. Liquid-cultured shoots: (**a**) control, no buds; (**b**) paclobutrazol treated, showing several buds in the leaf axile. (a) original magnification, ×50; (b) original magnification, ×60.

Discussion

Liquid cultures, used to scale-up micropropagation, were reported to induce malformed (vitreous) shoots

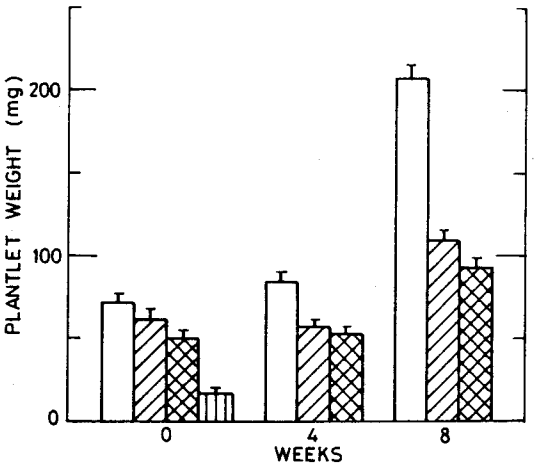

Fig. 4. The effect of continuous or short transient treatments with PAC during the proliferation stage on subsequent plant growth *ex vitro*. ☐, 0-day; ▨, 1-day; ▩, 3-day; ▥, 26-day treatments with PAC.

Table 3. The effect of 3.4 μM PAC on growth and proliferation of philodendron bud clusters in liquid shake or bioreactor cultures after 32 days.

Culture treatment[a]		Leaves (%)[b]	Growth value	Bud proliferation[d]
Shake	T[c]	16.4 ± 3.0	2.1 ± 0.1	8.2 ± 0.7
	C	11.2 ± 0.9	0.9 ± 0.06	21.3 ± 2.3
Bioreactor	T	9.6 ± 1.3	3.2 ± 0.3	18.7 ± 1.9
	C	6.0 ± 0.5	1.5 ± 0.14	26.4 ± 3.0

[a] 2.5 g/100 ml medium in flasks on a 100 rpm shaker or 15 g/750 ml medium in the bioreactor aerated at 0.6 vvm.
[b] See legend to Table 1.
[c] T, Transient treatment for 3 days was followed by transfer to PAC-free medium; C, continuous PAC treatment for 32 days.
[d] Number of buds per 2 g FW samples, five samples per treatment.

(Ziv 1990c). In order to solve the problem of abnormal leaf formation and to reduce foliage growth, ANC and PAC, inhibitors of GA synthesis (Graebe 1987), were added to liquid-cultured philodendron plants. Both of these inhibiting substances, given at various levels for different treatment periods, caused a significant reduction in leaf growth. The treatments induced compact bud cluster formation and enhanced bud proliferation, especially by PAC. Plant growth retardants employed to reduce abundant vegetative growth are known to act through inhibition of GA synthesis by blocking a particular step in the biosynthetic pathway of GA (Grossmann 1990, Rademacher 1989). Leaf growth was apparently inhibited by low GA, which caused a reduction in cell elongation (Jung et al. 1987). Enhanced bud proliferation in philodendron, reported also in *Aechmea faciata* (Ziv et al. 1986) and gladiolus (Ziv 1989, 1990a), could be the result of impaired apical dominance or changes in sink and source correlation. Growth retardants were found also to inhibit ethylene production (Grossmann et al. 1989) and to stimulate the translocation of assimilates to growing seeds (Luib et al. 1987). Some of the effects of growth retardants are assumed to be mediated through the elevated concentration of cytokinins (Fletcher and Arnold 1986, Grossmann et al. 1987, Izumi et al. 1988). Increased cytokinins and reduction in ethylene could be the factors involved in amplified bud development in philodendron. Changes in tissue polarity due to continuous agitation and circulation in shake or bioreactor cultures could have also contributed to adventitious buds in addition to axillary bud development. The continuous presence of ANC and PAC in the medium had a dwarfing effect on regenerated philodendron plants. A 3-day treatment with PAC was effective in reducing leaf growth and enhancing bud proliferation. However, it also had a dwarfing carryover effect on the regenerated plants, although not as pronounced as the continuous presence of PAC in the medium. Shortening of the treatment period resulted in a lower proliferation rate. The addition of GA to philodendron cultures at stage 3 was tested and was found to significantly improve the plants growth *ex vitro;* however, PAC- and ANC-treated plants were still 35–40% smaller than controls (Ariel 1987).

The production of compact bud clusters in philodendron, as well as in fern (Ziv and Hadar 1990), or protocorms in geophytes (Ziv 1989; Ziv 1990b), in shake and bioreactors cultures can provide an efficient and economical *in vitro* micropropagation system. However, the exact method and stage of application and the duration of the presence in culture of the growth retardants needs further study, before the method can be employed for other plant species.

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