BIOLOGIA PLANTARUM 39 (2): 179-186, 1997

Micropropagation of *Calathea ornata* Koern.

M. PODWYSZYŃSKA

Research Institute of Pomology and Floriculture, Waryńskiego St. 14, 96-100 Skierniewice, Poland

Abstract

In vitro cultures of *Calathea ornata* Koern. cv. Sanderiana shoots were maintained on modified medium of Murashige and Skoog. With the aim to find effective micropropagation method the effects of cytokinins, paclobutrazol, saccharose, mineral salts and shoot-tip damage on the shoot growth and quality were studied. The highest number of shoots was obtained on a medium containing 2.5 mg dm^{-3} benzylaminopurine, 2.5 mg dm^{-3} kinetin, 4.5 % saccharose and raised concentrations of Ca, Mg, Fe and Mn. Shoot branching was markedly stimulated by the shoot-tip damage.

Additional key words: cytokinins, mineral composition, paclobutrazol, saccharose.

Introduction

Calathea sp. and other species belonging to *Marantaceae* are highly appreciated ornamental plants characterized by very decorative foliage. The current study was performed in order to develop an effective micropropagation method of pathogen-free plants; microcuttings are especially in demand. There has been scant information on *Marantaceae* propagation *in vitro* (Duston and Sutter 1984, Van Mil and Van Telgen 1990). However, our observation showed that the main barrier for *Calathea* sp. mass production *in vitro* appeared to be the cytokinin/auxin ratio which makes it possible to achieve simultaneously buds, small shoots and microcuttings suitable for transplanting into a greenhouse (data not published). At a high concentration cytokinin strongly stimulates bud formation; however, buds develop into the shoots very slowly (3 - 5 months) and are often deformed. Hyperhydricity and the ensuing tissue necrosis of the oldest leaves represent another problem. These symptoms disqualify microcuttings for export.

Received 18 December 1995, *accepted* 27 August 1996.

Abbreviations: BAP - benzylaminopurine; kin - kinetin (6-furfurylamino-purine); NAA - 1-naphthylacetic acid; PP333 - paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol].

Acknowledgements: The author wishes to thank ICI Chemicals for kindly providing paclobutrazol and Mrs. Jadwiga Białek for excellent technical assistance. This work was supported by a Grant No. PB 0166/S3/93/04 from the State Committee for Scientific Research (Poland).

The aim of this study was to develop a micropropagation method and obtain: *1*) clumps of buds and small shoots suitable for further multiplication and *2*) high-quality microcuttings ready for direct transplanting to soil in a greenhouse.

Materials and methods

Adult plants of *Calathea ornata* Koern. cv. Sanderiana were used as donor plants supplying the initial explants - apical and lateral buds (2 - 4 mm). They were isolated from shoot cuttings which were previously disinfected in 0.1 % $HgCl_2$ for 10 min and rinsed 3 times in sterile distilled water. The explants were grown for 2 months on a modified BM containing a lower level of NH_4NO_3 and KNO_3 (1/2 MS), 1 mg dm^{-3} BAP and 0.1 mg dm^{-3} NAA. Then shoots were transferred to a basal medium (BM) containing Murashige and Skoog (1962) mineral salts, 170 mg dm^{-3} NaH_2PO_4, 100 mg dm^{-3} myo-inositol, 80 mg dm^{-3} adenine sulfate, 1 mg dm^{-3} thiamine, 1 mg dm^{-3} pyridoxine, 1 mg dm^{-3} nicotinic acid, 2 mg dm^{-3} glycine, 3 % saccharose, 0.2 % *Phytagel* (*Sigma*), 2.5 mg dm^{-3} BAP and 0.1 - 1 mg dm^{-3} NAA; pH was adjusted to 5.6 before autoclaving (20 min at 120 °C and 1 MPa). This BM was modified and used in all experiments. Shoots were cultured in food jars (330 cm^3) containing 40 cm^3 of medium, and subcultured at 6 weeks intervals. Plant cultures were kept at temperature 25 °C, 16-h photoperiod, photosynthetic photon flux density (PPFD) 40 μmol m^{-2} s^{-1} provided by cool-white fluorescent lamps.

The experiments were done with uniform shoots with a length over 3.5 cm having 2 - 3 buds, or with younger shoots (below 1.5 cm in length) originating from 2-year-old *in vitro* cultures. In order to establish shoot cultures on the experimental media, the plants were subcultured 4 times every 6 weeks on the same treatment medium; each time the branched shoots were cut into smaller clumps and microcuttings suitable for direct transplanting to soil (over 3.5 cm in length with at least 1 root). After the 3rd and 4th subculture the following data were collected: multiplication rate defined as the number of smaller clumps; number of microcuttings suitable for transplanting into the greenhouse; number of smaller shoots (over and below 1.5 cm); number of leaves manifesting necrosis. All treatments contained 4 replications, each consisted of 5 shoot clumps randomly selected. Final data were the means of 3rd and 4th subculture; the results of these subcultures did not differ significantly.

The effect of cytokinins (BAP and kinetin) and the growth retardant paclobutrazol on the growth and shoot branching was studied. Cytokinins and paclobutrazol were added separately or in combinations to BM containing 1 mg dm^{-3} NAA.

The effect of damage of apex by incising the shoot was examined in order to stimulate branching through elimination of apical dominance. Single microcuttings were incised 3 - 5 mm over a shoot base; the shoots were cultured on BM supplemented with 2.5 mg dm^{-3} BAP, 2.5 mg dm^{-3} kin and 1 mg dm^{-3} NAA.

For improvement of the shoot quality by decreasing the leaf hyperhydricity and necrosis the effect of a modification of the mineral salt composition of BM medium (MS1, MS2 and MS3, Table 1) and of saccharose concentration (3, 4.5 and 6 %) used in different media (BM, MS4 and MS5, Fig. 3) were studied. Ca raised

concentrations in media MS2, MS3 and MS4 were obtained by addition of $Ca(NO_3)_2 \cdot 4\ H_2O$ to BM medium containing 440 mg dm^{-3} $CaCl_2$.

Microcuttings over 3.5 cm in length were transplanted directly to compost in a greenhouse after the 4th subculture. The compost consisted of a 4:1 peat/perlite mixture in trays. The plants were placed for 5 weeks under a polyethylene tent maintaining high relative humidity. Their acclimation rate was evaluated 3 weeks later.

Table 1. Modification of mineral salt composition of BM medium [mg dm^{-3}].

	NH_4NO_3	$CaCl_2 \cdot 2\ H_2O$	$MgSO_4 \cdot 7\ H_2O$	$Ca(NO_3)_2 \cdot 4\ H_2O$	$MnSO_4 \cdot H_2O$	NaFeEDTA
MS1		880	740			
MS2	1400	440	740	700		
MS3	1400	440	740	700	33.8	80
MS4	1400	440	555	500	33.8	80

Results and discussion

Shoot branching was highly stimulated in the presence of BAP (Fig. 1). The highest total number of shoots was observed on a medium containing a combination of BAP and kinetin. Addition of paclobutrazol to the medium containing both cytokinins at 2.5 mg dm^{-3} did not influence significantly the shoot number, however, decreased leaf necrosis (Table 2, Fig. 2), and the shoots were darker green. Moreover, the total shoot number was markedly higher and the quality of shoots was similar when compared to those achieved on a medium with a raised concentration of Ca and Mg.

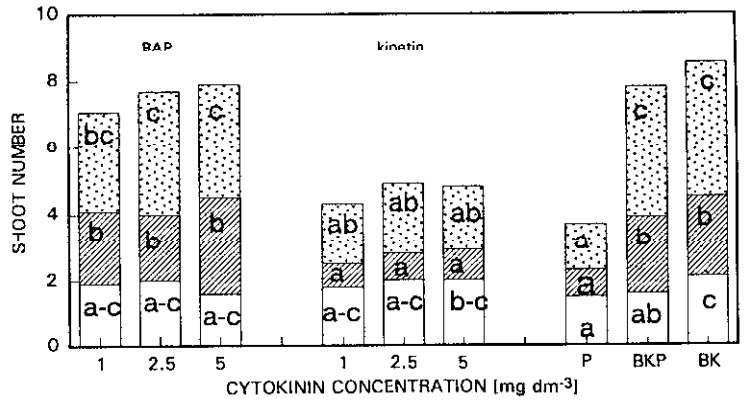

Fig. 1. Effect of cytokinins (BAP and kin) and paclobutrazol on the shoot growth and branching of *Calathea ornata*; shoot class: < 1.5 cm (*dotted columns*), > 1.5 cm (*hatched columns*) and > 3.5 cm (*empty columns*). *P* - 0.5 mg dm^{-3} paclobutrazol; BKP - 2.5 mg dm^{-3} BAP + 2.5 mg dm^{-3} kin + 0.5 mg dm^{-3} paclobutrazol; BK - 2.5 mg dm^{-3} BAP + 2.5 mg dm^{-3} kin. Means for each shoot class marked with the same letter do not differ significantly ($P < 0.05$; Duncan's multiple range test).

Chin (1982) was the first who report that the growth retardant ancymidol promoted shoot and root formation of an asparagus *in vitro* culture. Further study showed that ancymidol and paclobutrazol combined with cytokinins strongly induced shoot

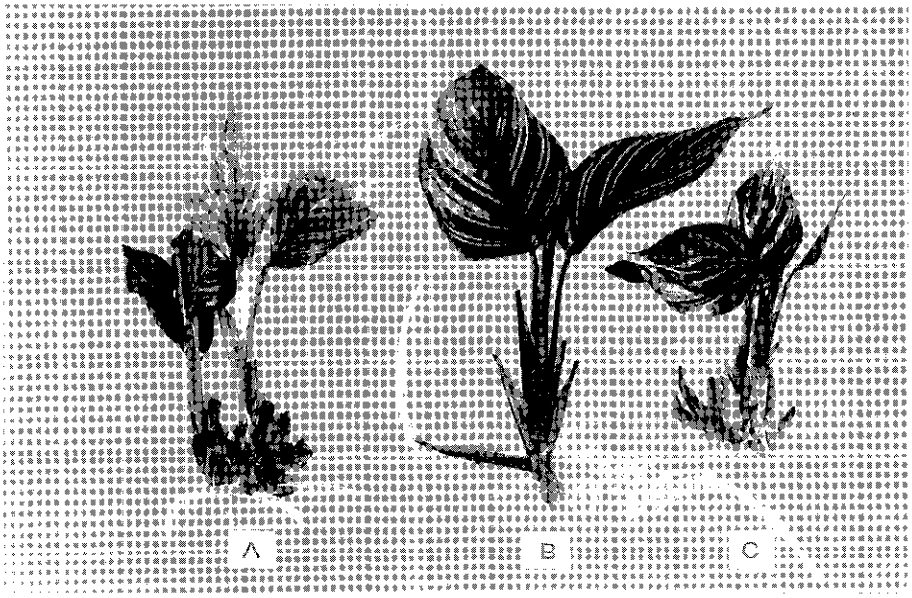

Fig. 2. Shoots of *Calathea ornata* cv. Sanderiana cultured on media: (*A*) BM + 2.5 mg dm^{-3} BAP + 2.5 mg dm^{-3} kinetin - leaves manifesting necrosis; (*B*) BM + 5 mg dm^{-3} kinetin - not branched shoot; (*C*) BM + 2.5 mg dm^{-3} BAP + 2.5 mg dm^{-3} kinetin + 0.5 mg dm^{-3} paclobutrazol - branched microcutting of the high quality.

proliferation of *Gladiolus* sp. and *Philodendron* sp. in liquid cultures (Ziv 1990, Ziv and Ariel 1991). There are also other reports on the improvement of plantlet survival after transplanting to soil by the addition of paclobutrazol to the rooting medium in apple, chrysanthemum, rosa and sugar beet (Ma *et al.* 1990, Smith *et al.* 1990, Ritchie *et al.* 1991 and Roberts *et al.* 1992) . This studies showed that the presence of paclobutrazol in the rooting medium decreased stomatal apertures, increased epicuticular wax, shortened stems and thickened roots. Each of these factors may improve resistance to desiccation. In addition, paclobutrazol markedly reduced hyperhydricity, increased the chlorophyll concentration in leaf tissue and promoted root formation. Our data showed that paclobutrazol improved shoot quality by reducing hyperhydricity and leaf necrosis (Table 2, Fig. 2). In the current study, thisgrowth retardant was used in experimental media continuously for 4 subcultures. This probably led to accumulation of paclobutrazol in the tissue resulting in bud and shoot deformation. Considering our data and the above reports, one can suggest that paclobutrazol can be used in the medium at a lower concentration or added sporadically every 2 - 3 subcultures in order to overcome its accumulation in plant tissue.

Table 2. Growth and quality of *Calathea ornata* shoots cultured *in vitro* on media containing paclobutrazol (PP333) in comparison to treatment with raised level of Ca and Mg. Means in each column followed with the same letter do not differ at $P < 0.05$.

Treatment [mg dm^{-3}]	Total shoots [plant^{-1}]	Multiplication rate	Necrotic leaves [plant^{-1}]	Hyperhydricity
BA 2.5 + kin 2.5	6.9 bc	2.0 b	0.90	all leaves
BA 2.5 + kin 2.5 + CaMg*	6.2 bc	1.9 ab	0.66	1 leaf
BA 2.5 + kin 2.5 + PP333 0.5	7.4 c	2.1 b	0.72	1 leaf
BA 1 + PP333 0.5	5.9 b	1.9 ab	0.94	2 leaves
Kin 1 + PP333 0.5	4.8 a	1.6 a	0.90	2 leaves

* CaMg - Ca and Mg concentration as in MS1 medium.

Strong stimulation of shoot branching was also obtained by shoot incisions (Table 3). This method of apex damage can be applied in mass production in order to increase the multiplication rate. Similarly, the elimination of apical dominance by apex removal strongly stimulated *in vivo* branching of *Strelitzia* sp. which belongs to the *Musaceae* family closely related to the *Marantaceae* including *Calathea* sp. (Van de Pol and Van Hell 1988). The excision of an apex was of importance for clonal propagation of valuable *Strelitzia* sp. plants with a naturally low rate of multiplication. However, the results of the previous study showed that the removal of *Calathea* sp. apex by its excision 3 - 4 mm above the shoot base caused a tissue decay (data not presented).

Table 3. Effect of apex damage by the shoot incising on the shoot growth and branching of *Calathea ornata*. Control - not incised shoots. Means for each shoot class marked with the same letter do not differ at $P < 0.05$.

Shoot class	Shoot number [plant^{-1}] < 1.5 cm	> 1.5 cm	> 3.5 cm	total
Control	0.4 a	0.4 a	1.0 a	1.8
Incised	0.9 b	0.9 b	1.3 b	3.1

The results of the study concerning the mineral composition of the medium showed that the best-quality microcuttings (darker green leaves with a distinct pattern characteristic to the cultivar) were obtained on medium MS3 with a raised level of Ca, Mg, Fe and Mn (Table 4). Generally, the reduced symptoms of leaf hyperhydricity and necrosis were observed on media containing a higher level of Ca and Mg (MS1, MS2 and MS3) (Table 4). Also Ziv *et al.* (1987a,b) and Kreutmeier *et al.* (1984) reported that elevated Ca was found to reduce hyperhydricity of herbaceous and woody species. In turn, Borkowska and Michalczuk (1987) did not find a correlation between Ca accumulation and vitreous leaves in sour cherry which showed an increased acid phosphatase activity. In the current study, the NH_4/NO_3 ratio in the MS3 and MS4 medium was lower than in BM medium and it could be

one of the factors improving shoot quality. Reducing the level of NH_4 in the medium increased lignification and reduced vitrification in several plants (Daguin and Letouze 1986, Leonhardt and Kandeler 1987). It is also interesting that a slightly better shoot quality was achieved on medium MS3 with a higher level of Ca, Mg and

Table 4. Effect of mineral salt composition on the growth and quality of *Calathea ornata* shoots grown *in vitro*. Means in each column followed with the same letter do not differ at $P < 0.05$.

Medium	Shoot number [plant^{-1}]	Multiplication rate*	Necrotic leaves [plant^{-1}]	Hyperhydricity
BM	6.3 a	2.2 a	0.9 b	all leaves
MS1	7.0 ab	2.2 a	0.7 ab	2 leaves
MS2	6.0 a	2.2 a	0.7 ab	2 leaves
MS3	6.1 a	2.1 a	0.6 a	1 leaf

* Shoot clumps number obtained after a division of a primary clump.

also Fe and Mn as compared with MS1 and MS2 (media with increased level of Ca and Mg only). Doubling the Mn and Fe concentration in a medium with raised Ca and Mg level seems to be well-founded, especially regarding the results of Singha *et al.* (1990). They found that increasing Ca concentration in the medium resulted in Mn decrease in plant tissue. Moreover, it is widely known that a high concentration of Ca and Mg inhibits the uptake of Fe and Mn; hence all these ions participate in mutual competition (Mengel and Kirkby 1978). Mn deficiency in the plant can decrease Ca and Mg concentrations and increase membrane permeability (Szkolnik 1980). Beneficial effect of higher concentrations of Ca, Fe and Mn on the shoot quality through the inhibition of leaf hyperhydricity and necrosis can also be related to their influence on activation of peroxidases (IAA-oxidase and ACC-oxidase). Gaspar *et al.* (1987) reported that peroxidases are very sensitive to changes in the levels of ions such as Ca and Mn. These enzymes affected the level of phenylalanine ammonia-lyase activity, ethylene and phenols, with resulting hypolignification and loss of cell wall rigidity in the plant tissue.

Table 5. Effect of medium composition (BM, MS4, MS5) and sacharose concentration (3, 4.5 and 6 %) on the number of necrotic leaves per plant in the shoot cultures of *Calathea ornata*.

BM			MS4			MS5		
3	4.5	6	3	4.5	6	3	4.5	6
0.44	0.34	0.38	0.34	0.26	0.34	0.70	0.36	0.26

The highest quality shoots with the most reduced symptoms of leaf hyperhydricity and necrosis were observed on medium MS4 with raised Ca, Mg, Fe and Mn (Table 5.). Raised concentration of saccharose up to 4.5 % also promoted shoot growth and branching and improved shoot quality (lower number of necrotic leaves)

(Table 5, Fig. 3). Similarly, elevated levels of sacharose up to 4.5 % together with a double dose of $MgSO_4$ decreased vitrification (Orlikowska 1987).

Unfortunately, when the MS3 or MS4 media containing higher level of Ca, Mg, Fe and Mn were used for prolonged mass production a reduction of the multiplication rate was noted (data not presented). Ca accumulation in plant tissue is probably responsible for inhibition of plant growth. This is in agreement with the observation of Podwyszyńska (1994), Sinha *et al.* (1990), Abousalim and Mantell (1994), who found that the increased Ca concentration prevented hyperhydricity and shoot-tip-necrosis, but also inhibited *in vitro* plant growth. Thus, the results of current study and the above data suggested that MS3 or MS4 media could be used every other subculture, and especially before transplanting the microcuttings into *ex vitro* conditions.

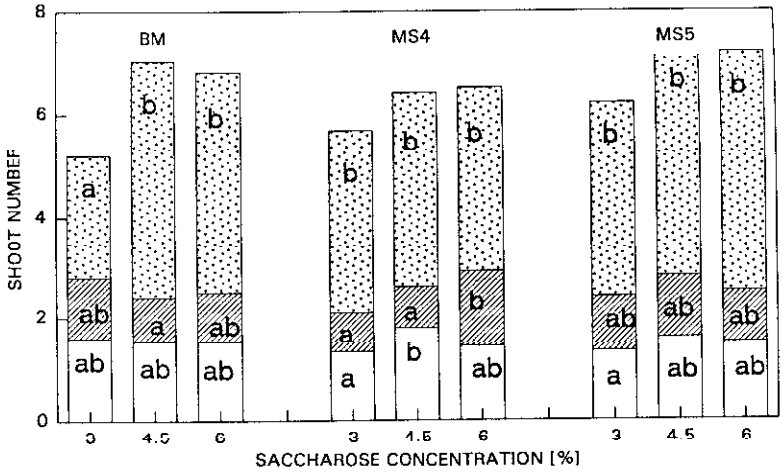

Fig. 3. Effect of saccharose concentration and medium composition on the shoot branching of *Calathea ornata*; shoot class: < 1.5 cm (*dotted columns*), > 1.5 cm (*hatched columns*) and > 3.5 cm (*empty columns*). 0.5 mg dm^{-3} paclobutrazol was added to BM (basal medium), MS4 (described in Table 1) and MS5 (BM + 20 mg dm^{-3} glycine + 10 mg dm^{-3} thiamine + 5 mg dm^{-3} pyridoxine + 200 mg dm^{-3} glutamine). Means for each shoot class marked with the same letter do not differ at $P < 0.05$.

All plants derived from each experiment survived and continued growth in a greenhouse. On the base of the results of the current study, an effective micropropagation method of *Calathea* sp. was worked out. The method also permits a propagation *in vitro* of another species belonging to *Marantaceae*: *Stromanthe*, *Ctenanthe* and *Maranta* sp.

References

Abousalim, A., Mantell, S.H.: A practical method for alleviating shoot-tip necrosis symptoms in *in vitro* shoot cultures of *Pistacia vera* cv. Mateur. - J. hort. Sci. **69**: 357-365, 1994.

Borkowska, B., Michalczuk, L.: The physiological disorder of sour cherry cultures: necrosis and vitrification. - Acta Hort. **212**: 235-237, 1987.

Chin, C.K.: Promotion of shoot and root formation in asparagus *in vitro* by ancymidol. - HortScience **17**: 590-591, 1982.

Daguin, F., Letouze, R.: Ammonium-induced vitrification in cultured tissues. - Physiol. Plant. **66**: 94-98, 1986.

Dunston, S., Sutter, E.: *In vitro* propagation of prayer plants. - HortScience **19**: 511-512, 1984.

Gaspar, T., Kevers, C., Debergh, P., Maene, L., Paques, M., Boxus, P.: Vitrification: Morphological, physiological and ecological aspects. - In: Bonga, J.M., Durzan, D.J. (ed.): Cell and Tissue Culture in Forestry. Vol. 1. Pp.152-166. Martinus Nijhoff, Dortrecht 1987.

Kreutmeier, C., Gebhardt, K., Paul, L., Feucht W.: The effect of $MgSO_4$ and $CaCl_2$ on regeneration of shoots tip cultures of *Prunus cerasus in vitro*. - Gartenbauwissenschaft **49**: 204-212, 1984.

Leonhardt, W., Kandeler, R.: Ethylene accumulation in culture vessels - a reason for vitrification. - Acta Hort. **212**: 223-229, 1987.

Ma, F.W., Wang, J.C., Rong, W.: Effect of plant growth regulators on *in vitro* propagation of apple cultivar Fuji. - J. Fruit Sci. **7**: 201-206, 1990.

Mengel, K., Kirkby, E.A.: Principles of Plant Nutrition. - International Potash Institute, Bern 1978.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol Plant. **15**: 473-497, 1962.

Orlikowska, T.: Vitrification problem in the *in vitro* culture of fruit tree rootstocks. - Acta Hort. **212**: 239-244, 1987.

Podwyszyńska, M.: Wpływ regulatorów wzrostu na ukorzenianie i aklimatyzacje róż miniaturowych mnożonych *in vitro*. [Effect of growth regulators on rooting and acclimatization of miniature rose cultured *in vitro*] - Ph. D. Thesis. Research Institute of Pomology and Floriculture, Skierniewice 1994. [In Polish.]

Ritchie, G.A., Short, K.C., Davey, M.R.: *In vitro* acclimatization of chrysanthemum and sugar beet plantlets by treatment with paclobutrazol and exposure to reduced humidity. - J. exp. Bot. **42**: 1557-1563, 1991.

Roberts, A.V., Walker, S., Horan, I., Smith, E.F., Mottley, J.: The effect of growth retardants, humidity and lighting at stage III on stage IV of micropropagation in chrysanthemum and rose. - Acta Hort. **319**: 153-158, 1992.

Singha, S., Townsend, E.C., Oberly, G.H.: Relationship between calcium and agar on vitrification and shoot-tip necrosis of quince (*Cydonia oblonga* Mill.) shoot *in vitro*. - Plant Cell Tissue Organ Cult. **23**: 135-142, 1990.

Smith, E.F., Roberts, A.V., Mottley, J.: The preparation *in vitro* of chrysanthemum for transplantation to soil. 2. Improved resistance to desiccation conferred by paclobutrazol. - Plant Cell Tissue Organ Cult. **21**: 133-140, 1990.

Szkolnik, M.J.: Mikroelementy w życiu roślin. [Microelements in Plant Life.] - PWRiL, Warszawa 1980. [In Polish.]

Van de Pol, P.A., Van Hell, T.F.: Vegetative propagation of *Strelitzia reginae*. - Acta Hort. **226**: 581-586, 1988.

Van Mil, A., Van Telgen, H.J.: Micropropagation of *Calathea* 'Ornata'. - In: Abstracts VIIth International Congress on Plant Tissue and Cell Culture. P. 117. Amsterdam 1990.

Ziv, M.: The effect of growth retardant on shoot proliferation and morphogenesis in liquid cultured gladiolus plants. - Acta Hort. **280**: 207-214, 1990.

Ziv, M., Ariel, T.: Bud proliferation and regeneration in liquid-cultured philodendron treated with ancymidol and paclobutrazol. - J. Plant Growth Regul. **10**: 53-57, 1991.

Ziv, M., Schwarts, A., Fleminger, D.: Malfunctioning stomata in vitreous leaves of carnation (*Dianthus caryophyllus*) plants propagated *in vitro*; implications for hardening. - Plant Sci. **52**: 127-134, 1987a.

Ziv, M., Schwartz, A., Fleminger, D.: Hardening aspects of micropropagated carnation plants having malfunctioning stomata. - In: Ducote, G., Jacob, M., Simeon, A. (ed.): Plant Micropropagation in Horticultural Industries. Pp. 47-54. Presses Universitaires, Liège 1987b.