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Short Communication

Androgenesis in *Citrus aurantifolia* (Christm.) Swingle*

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Abstract. Embryoids were differentiated from anthers of *C. aurantifolia* which were first floated on a modified Murashige and Skoog's liquid medium supplemented with 0.5 mg/l N^6-benzylaminopurine and 1 mg/l indole-3-acetic acid for 20–30 d, followed by 30 d culture in semisolid Schenk and Hildebrandt's medium having the same growth hormones. Embryoids originated from within the anther lobes. Initially, a few embryoids were formed by each anther; later, they multiplied rapidly by the production of new embryoids from the hypocotyl and cotyledon portions of the original embryoids. The embryoids could develop into plantlets, which were all diploid ($2n=18$). The androgenic plants grew normally in soil.

Key words: Androgenesis – Anther – *Citrus* – Embryoid.

There are only few reports of androgenesis in tree species, namely, in *Aesculus hippocastanum* (Radojević 1978), *Hevea brasiliensis* (Chen et al. 1979) and *Poncirus trifoliata* (Hidaka et al. 1979), in contrast to herbaceous plants (for a recent review, see Bajaj 1983). In *Citrus*, differentiation of shoots or embryoids in vitro has been obtained from various tissues and organs (Button and Kochba 1977; Spiegel-Roy and Kochba 1980), but so far there are, to the best of our knowledge, no reports on androgenesis in any *Citrus* species. We now report androgenesis and plantlet formation in *Citrus aurantifolia* (Christm.) Swingle (lime), a commercially important *Citrus* species which is normally d–i ploid (2n = 18).

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Abbreviations: BAP = N^6-benzylaminopurine; IAA = indole-3-acetic acid; MS = medium of Murashige and Skoog (1962); SH = medium of Schenk and Hildebrandt (1972)

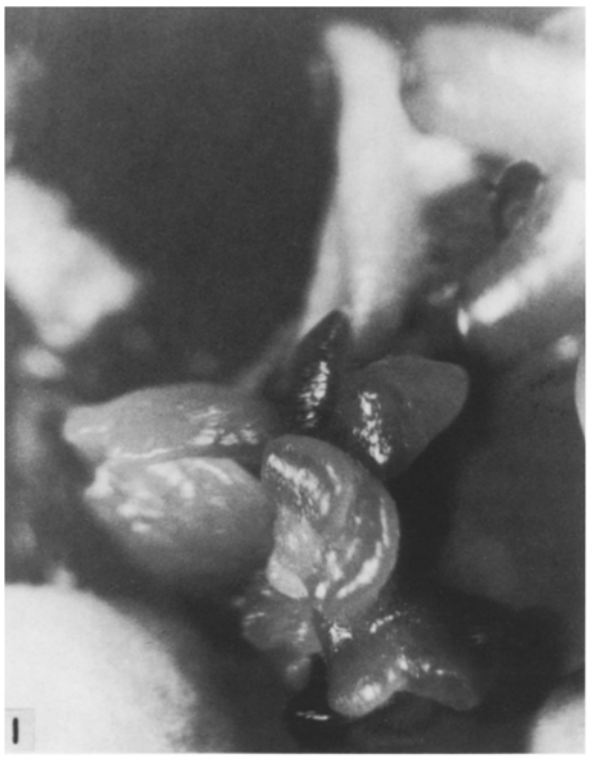
Closed flower buds of *C. aurantifolia* having pollen grains in tetrads at the uninucleate stage were collected from field-grown plants and surface-sterilized in chlorine-saturated water for 10 min. Anthers were excised and cultured in liquid as well as semisolid (7 g/l agar) modified Murashige and Skoog's (1962) medium (MS) as well as Schenk and Hildebrandt's (1972) medium (SH). The modified MS medium differed from the original MS in having, in mg/l: 500 NH_4NO_3; 200 each KNO_3, $CaCl_2 \cdot 2\,H_2O$ and $MgSO_4 \cdot 7\,H_2O$; 80 KH_2PO_4; 16.7 $FeSO_4 \cdot 7\,H_2O$ and 22.4 disodium ethylene diaminetetraacetate (Na_2EDTA); the trace elements of the Nitsch's (1951) medium; 2.5 thiamine-HCl; 0.625 each pyridoxine-HCl and nicotinic acid; 0.05 riboflavin; 0.1 each folic acid and biotin.

Using 0.5 mg/l N^6-benzylaminopurine (BAP) along with 1 mg/l indole-3-acetic acid (IAA), the effects of liquid and semisolid states of the media and their sequential use for incubating anthers were examined for causing androgenesis. Ten anthers per tube (2.5 cm diameter, 15 cm long; 20 ml of medium) were cultured and five replicates of each treatment were incubated. The pH of all nutrient media was adjusted to 5.8 and the media were sterilized by autoclaving at 1.08 kg/cm^2 for 15 min. The cultures were incubated under 3 klx light from fluorescent lamps for 15 h a day and at 27° ± 1° C.

For chromosome counts, the root tips of androgenic plantlets were hydrolysed in 1 N HCl for 10 min at 60° C, followed by staining in acetohaematoxylin solution.

The anthers remained more fresh in liquid than in semisolid medium. However, anthers submerged in liquid medium from the beginning remained quiescent and later turned brown. Anthers which remained floating on liquid medium or those cultured on semisolid nutrient agar, using both modified MS and SH as basal media, generally produced only callus, like cultured anthers of many other tree species, namely, sweet cherry, almond, peach, alder, aspen and *Cryptomeria* (Winton and Huhtinen 1976).

Non-callused anthers, which had been floating on the modified MS medium supplemented with 0.5 mg/l BAP and 1 mg/l IAA for 20–30 d, when transferred to semisolid SH medium having the same growth hormone supplement, produced embryoids in 25% of the cultures after 30 d (Fig. 1). Androgenesis did not take place if anthers were



Figs. 1, 2. Induced androgenesis in *Citrus aurantifolia*

Fig. 1. Differentiation of several heart-shaped embryoids from an in vitro-cultured anther unaccompanied by callusing. ×12.3

Fig. 2. Androgenic plants in soil after six months of transplantation. ×0.17

transferred from liquid to liquid, solid to solid, or solid to liquid medium of the same composition, or from liquid SH medium to semisolid modified MS medium. The role of sequential culture of tissues in media of two different physical states for differentiation of regenerants in *Daucus carota* and *Pelargonium* has been emphasized by Steward et al. (1958) and Chen and Galston (1967), respectively. Floating the anthers on liquid medium has also been found conducive to androgenesis (Wernicke and Kohlenbach 1976; Sunderland and Roberts 1977). However, the sequential culture of anthers first in liquid and then in semisolid medium for androgenesis has, as far as we are aware, not been reported.

During androgenesis, about eight to ten embryoids emerged from within the anther through the dehiscence line of the anther lobes. The group of embryoids from an anther, when excised and subcultured on the semisolid SH medium, proliferated further by giving rise to a total of fifty to sixty additional embryoids in 30 d. The young embryoids mainly differentiated from the radicle and the hypocotyl of the original embryoids. By subsequent subculturing of small groups of embryoids and their proliferation, a very large number of embryoids could be obtained from one anther within a period of a few months.

The mature embryoids developed into plantlets when transferred to the modified MS medium devoid of growth hormones. All androgenic plants examined (50) were diploid with $2n=18$ chromosomes.

The androgenic plants of *C. aurantifolia* were successfully acclimatized by first culturing them in 0.5-strength Knop's solution supplemented with the MS trace elements and 10.02 mg/l $FeSO_4 \cdot 7\,H_2O$ and 13.44 mg/l Na_2EDTA for 30 d, followed by transplantation in pots with soil. The plants were covered with polythene chambers for the initial 7-d period both in the nutrient and the pot cultures. The androgenic plants grew normally in soil (Fig. 2).

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References

Bajaj, Y.P.S. (1983) In vitro production of haploids. In: Handbook of plant cell culture, vol. 1: Techniques for propagation and breeding, pp. 228–287, Evans, D.A., Sharp, W.R., Ammirato, P.V., Yamada, Y., eds. Macmillan Publishing Co., New York

Button, J., Kochba, J. (1977) Tissue culture in the citrus industry. In: Applied and fundamental aspects of plant cell, tissue, and organ culture, pp. 70–92, Reinert, J., Bajaj, Y.P.S., eds. Springer, Berlin Heidelberg New York

Chen, C.-H., Chen, F.-T., Chien, C.-F., Wang, C.-H., Chang, S.-J., Hsu, H.-E., Ou, H.-H., Ho, Y.-T., Lu, T.-M. (1979) A process for obtaining pollen plants of *Hevea brasiliensis* Muell-Arg. Sci. Sinica **22**, 81–90

Chen, H.-R., Galston, A.W. (1967) Growth and development of *Pelargonium* pith cells in vitro. II. Initiation of organized development. Physiol. Plant. **20**, 533–539

Hidaka, T., Yamada, Y., Shichijo, T. (1979) In vitro differentiation of haploid plants by anther culture in *Poncirus trifoliata* (L.) Raf. Jpn. J. Breed. **29**, 248–254

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

Nitsch, J.P. (1951) Growth and development in vitro of excised ovaries. Am. J. Bot. **38**, 566–577

Radojević, L. (1978) In vitro induction of androgenic plantlets in *Aesculus hippocastanum*. Protoplasma **96**, 369–374

Schenk, R.U., Hildebrandt, A.C. (1972) Medium and technique for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. **50**, 199–204

Spiegel-Roy, P., Kochba, J. (1980) Embryogenesis in *Citrus* tissue cultures. In: Advances in biochemical engineering, vol. 16, pp. 27–48, Fiechter, A., ed. Springer, Berlin Heidelberg New York

Steward, F.C., Mapes, M.O., Mears, K. (1958) Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. Am. J. Bot. **45**, 705–708

Sunderland, N., Roberts, M. (1977) New approach to pollen culture. Nature **270**, 236–238

Wernicke, W., Kohlenbach, H.W. (1976) Investigations on liquid culture medium as a means of anther culture in *Nicotiana*. Z. Pflanzenphysiol. **79**, 189–198

Winton, L., Huhtinen, O. (1976) Tissue culture of trees. In: Modern methods in forest genetics, pp. 243–264, Miksche, J.P., ed. Springer, Berlin Heidelberg New York

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