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Vegetative propagation of Freesia through the isolation of shoots in vitro[1]

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Summary

Shoots from 4 *Freesia* cultivars, originating from excised flower-buds, were grown in vitro and were induced to form shoots on a modified Murashige and Skoog medium, containing PBA and IAA. The greatest number of shoots per excised shoot was obtained when the medium contained PBA at 5 mg/litre and IAA at 0.1 mg/litre. The number of shoots formed depended on the cultivar tested. Sub-cultured shoots, grown on a medium with IAA but without PBA could be rooted easily and viable plants were obtained.

Introduction

In previous papers (Bajaj & Pierik, 1974; Pierik & Steegmans, 1975) it was shown that *Freesia* plantlets can be regenerated from callus and from excised flower-buds. The method of *Freesia* propagation through excised flower-bud culture in particular has proved to be practically useful and has been applied commercially in the Netherlands on a large scale.

This paper briefly describes how *Freesia* plantlets can be produced through in vitro culture of shoots which orginated adventitiously from excised flower-buds.

Material and methods

The material consisted of 2-3 cm long shoots, obtained from experiments with excised flower-buds (Pierik & Steegmans, 1975). The following cultivars were used: 'Ballerina', 'Aurora', 'Rijnveld's Golden Yellow' and 'Rose Marie'. In most experiments the excised shoots were kept at 23 °C in continuous fluorescent light (Philips

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Table 1. The effect of various PBA and IAA concentrations (mg/litre) on the regereration of buds/sprouts on excised flower-buds of four Freesia cultivars.

Cultivar	Treatment		Bud/sprout formation (%)	Mean number of buds per flower-bud
	PBA (mg/litre)	IAA (mg/litre)		
Ballerina	1	0.01	88	2.6
	1	0.1	84	2.5
	1	1	94	3.6
	5	0.01	88	2.6
	5	0.1	95	4.6
	5	1	84	2.2
Aurora	1	0.01	87	3.2
	1	0.1	91	3.0
	1	1	84	3.5
	5	0.01	96	3.6
	5	0.1	100	3.4
	5	1	100	3.4
Rijnveld's Golden Yellow	1	0.01	86	4.9
	1	0.1	96	5.3
	1	1	92	4.1
	5	0.01	92	5.2
	5	0.1	92	4.6
	5	1	95	5.4
Rose Marie	1	0.01	96	6.5
	1	0.1	96	7.9
	1	1	96	7.4
	5	0.01	96	9.9
	5	0.1	95	10.6
	5	1	95	8.9

TL 40 W/57, 14 W m^{-2}). The experiments started on 21 July 1975 and were evaluated on 5 November 1975.

The basic culture medium contained the macro- and microelements and vitamins according to Murashige and Skoog (1962), NaFeEDTA 25 mg/litre, meso-inositol 0.1 g/litre, casein hydrolysate 500 mg/litre, saccharose 3 %, Difco Bacto-agar 0.8 % and pyrex-distilled water. The pH was adjusted to 6.0 before autoclaving. To obtain the formation of shoots, the medium was supplemented with various concentrations of the potassium salt of IAA (indoleacetic acid) and PBA or SD 8339 [(6-(benzylamino)-9-(2-tetrahydropyranyl)-9H purine)]. PBA was supplied by Shell International Research, The Hague, the Netherlands. The choice of the medium described above was based on our experience with *Freesia* (Bajaj & Pierik, 1974; Pierik & Steegmans, 1975).

Fig. 1. In vitro cultured shoots of Freesia cv. 'Aurora' after 6 weeks at 23 °C in continuous fluorescent light. Upper row: on a medium with only IAA at 0.1 mg/litre (only adventitious root formation). Lower row: on a medium with 0.1 mg IAA and 5 mg PBA per litre (only adventitious sprouts formation).

Results

In these experiments only the auxin/cytokinin interaction was studied as it has been shown (Pierik & Steegmans, 1975) that the regeneration of adventitious buds and/or sprouts in excised *Freesia* flower-buds requires a high concentration of both a cytokinin and an auxin. Table 1 shows the results obtained with four *Freesia* culivars. When considering the mean number of buds per flower-bud, we see that the number of buds/sprouts is determined primarily by the cultivar used. The percentage of bud/sprout regeneration is hardly affected by the auxin/cytokinin ratio. A comparison between PBA at 1 and 5 mg/litre shows that there is very little difference, except for 'Rose Marie', which requires a high cytokinin concentration to obtain the optimum number of buds. The IAA concentration has little effect. The mean number of buds is slightly increased at 0.1 mg IAA per litre in comparison to 0.01 or 1 mg/litre. In general the combination of 5 mg PBA and 0.1 mg IAA per litre is the most effective (see also Fig. 1).

Discussion and conclusions

The system of propagating *Freesia* through the isolation of shoots, as described in this paper, forms together with the flower-bud system (Pierik & Steegmans, 1975) a good method for growers and breeders to increase the propagation rate of newly

selected Freesia cultivars. The efficiency of the method is influenced more by the cultivar than by the auxin/cytokinin ratio. It is possible that, for example, a period of darkness (cf. Pierik & Steegmans, 1975) will increase the number of adventious buds of cultivars like 'Ballerina' or Aurora'. In preliminary experiments it was shown that 'Ballerina' reacts positively to a period of darkness, but 'Aurora' did not. Further research (e.g. a comparison of cytokinins) is necessary to see if a further increase in the number of buds is possible.

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