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Freesia plantlets from flower-buds cultivated in vitro[1]

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Summary

Flower-buds of 10 *Freesia* cultivars were grown in vitro and were induced to regenerate adventitious buds on a modified Murashige & Skoog medium, containing PBA and IAA. The formation of adventitious buds was strongly enhanced by growing the explants first in darkness and subsequently in light. Subcultured shoots, grown on a medium with IAA, could be rooted easily and viable plants were obtained.

Introduction

In a previous paper (Bajaj & Pierik, 1974) it was shown that explants of *Freesia* can regenerate shoots and/or roots in vitro. The formation of shoots was promoted by a high cytokinin/auxin ratio. This paper briefly describes how Freesia plantlets can be produced through in vitro culture of flower-buds.

Material and methods

Although in principle all types of organs of *Freesia* are capable to regenerate roots and shoots (Bajaj & Pierik, 1974) in this study only flower-buds were used. Young flower-buds (corolla not or just visible) have been isolated from the following cultivars: Aurora, Ballerina, Blue Heaven, Carmen, Fantasy, Golden Melody, Romany, Rose Marie, Royal Blue and Rijnveld's Golden Yellow. Before the excision of flower-buds inflorescences were sterilized in 1 % NaOCI for 20 minutes and subsequently washed three times for a total of 30 minutes in sterilized tap water.

During the first 16 weeks of culture, flower-buds were grown on the following medium: macro- and microelements and vitamins according to Murashige & Skoog (1962), NaFeEDTA 25 mg/litre, meso-inositol 100 mg/litre, casein hydrolysate 500 mg/litre, sucrose 3 %, the potassium salt of IAA 1 mg/litre, the cytokinin PBA 5 mg/litre, Difco Bacto-agar 0.8 % and pyrex-distilled water. The pH was adjusted to 6.0 before autoclaving. The culture medium for excised shoots after

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the regeneration and development of shoots was the same as described above; however, the cytokinin PBA was omitted, whereas IAA was added at 0.1 mg/litre instead of 1.0 mg/litre.

During the first 8 weeks of flower-bud culture, test tubes were kept at 25 °C in continuous darkness. During the second 8 weeks they were kept at 23 °C in continuous fluorescent light (Philips TL 40W/57). After 16 weeks the excised shoots were subcultured on another medium and then grown at 23 °C and in continuous fluorescent light.

Results

Preliminary experiments showed that the regeneration of adventitious buds was strongly enhanced by culturing the explants first for 8 weeks in darkness and then for 8 weeks in light. When test tubes were kept in continuous light throughout the 16 weeks, regeneration from flower-buds of most cultivars was drastically decreased. The light period after 8 weeks of darkness was essential for the regeneration of buds and for the normal growth and development of these buds.

A comparison of the cytokinins PBA, BA and kinetin showed that PBA was the most effective for induction of adventitious buds; BA was also favourable but kinetin was unsatisfactory. In comparison with IBA and NAA, IAA appeared to be the more effective auxin particularly in combination with PBA. The greatest number of buds per explant and the highest percentages of bud regeneration were obtained when PBA at 5 mg/litre was combined with IAA at 1 mg/litre. When

Fig. 1. *Freesia* plantlets from in vitro cultivated flower buds of the cultivar Aurora. Upper row, left: excised flower buds after 5 weeks at 25 °C in continuous darkness, parthenocarpic fruit set. Upper row, right: flower-buds, regenerating adventitious buds after 8 weeks at 25 °C darkness + 8 weeks 23 °C continuous light. Lower row, left: excised shoots without roots. Lower row, right: adventitious root formation after 8 weeks at 23 °C in continuous light.

the cytokinin/auxin ratio was decreased, bud formation decreased and root formation increased.

In a number of cultivars (Aurora, Blue Heaven, Golden Melody, Royal Blue and Rijnveld's Golden Yellow) bud formation already started during the first 8 weeks in darkness.

The other cultivars used generally reacted more slowly and formed their buds at the end of the dark period or during the light period (Fig. 1, upper row).

When flower-buds were grown according to the methods described above, all cultivars tested formed shoots but only a few formed roots. The mean number of shoots per explant and the percentage of bud regeneration after 16 weeks were strongly dependent on the cultivar. The highest number of shoots per explant was found with Rijnveld's Golden Yellow (9.4), Aurora (5.7) and Rose Marie (4.8). A very low number of buds per explant (0.6) and a low percentage of bud regeneration were observed in Romany. All cultivars tested showed at last some reaction; Schuring (personal communication) following our system and testing 14 other cultivars came to the same conclusions.

In subsequent experiments, approximately 16 weeks after the beginning of flower-bud culture, shoots of several cultivars were excised and subcultured on another medium (see methods). All cultivars regenerated 1-2 adventitious roots per shoot (Fig. 1, lower row, right). Complete plantlets were produced, which could be transferred to non-sterilized soil without any difficulty.

Discussion

As described previously (Bajaj & Pierik, 1974) *Freesia* plants of the cultivar Ballerina have a good regeneration capacity and can produce roots, shoots or both in vitro. However, this study showed that the regeneration capacity is strongly influenced by three factors: the cytokonin/auxin ratio, the cultivar and physical growth conditions, particularly darkness/light. That a relative high cytokinin/auxin ratio is necessary, has already been shown for many other plants (Pierik & Steegmans, 1975). The promotive effect of PBA on bud formation completely agrees with earlier results with *Anthurium andraeanum* (Pierik et al., 1974). The promoting effect of darkness on bud regeneration seems to be rather exceptional. The role of the cultivar is possibly correlated with its endogenous cytokinin and/or auxin level, as suggested in a previous paper (Pierik et al., 1975).

The low regeneration capacity of, for example, the cultivar Romany is certainly due to other factors which are presently unknown.

These experiments on the regeneration of *Freesia* flowerbuds in vitro point the way for *Freesia* breeders to increase the propagation (by corms) rate especially of new cultivars. In the future a new cultivar can be propagated much earlier by using in vitro techniques than by using the classical way of propagation by corms.

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