

SHORT COMMUNICATION

PRODUCTION OF VIRUS-FREE FREESIA'S BY MEANS OF MERISTEM CULTURE¹

Het verkrijgen van virusvrije freesia's door meristeeencultuur

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Methods developed to obtain virus-free plants by means of meristem culture have been known for some time (LIMASSET & CORNUET, 1949; MOREL & MARTIN 1952; QUAK, 1961; STONE, 1963). Using apical meristems of virus-diseased carnation plants QUAK (1957) succeeded in raising a number of carnation plants which reacted negatively when tested for the presence of the known carnation viruses. At present the production of virus-free carnations by means of meristem culture is carried out on a large scale by the Plant Protection Service at Wageningen. Carnations treated in this way proved to be superior in various qualities to the material commercially available (HAKKAART, 1963).

Considering the advantages of the meristem culture technique, its possible use for other horticultural crops was investigated. Preliminary work by VAN DORST (1957) with Freesia's indicated that the production of Freesia plants by means of this technique is possible.

In 1963 new investigations were carried out in the Phytopathological Laboratory at Baarn and by the Plant Protection Service³ at Wageningen. Samples of Freesia corms of the varieties 'White Swan', 'Oranje zon', 'Golden Yellow', 'Saffier' and 'Prinses Marijke' were provided by the Experimental Station for glasshouse vegetables and fruits at Naaldwijk and the N.A.K.S. (General Netherlands Inspection Service for Ornamental Plants).

When the corms had just started sprouting they were treated with a weak disinfectans. The lower part of the corm was cut off so that the upper part with the young shoot could easily be handled under a binocular microscope. With sterile dissecting needles and blades the meristematic vegetation point (0.8-1.0 mm) was excised (Fig. 1) and placed in upright position in a test tube containing a sterile nutrient medium.

The following media were used:

- (a) medium according to HELLER-BERTHELOT containing macro-elements of HELLER's solution (GAUTHERET, 1959), BERTHELOT's solution (MOREL, 1948) and, moreover, aneurin 10^{-6} , cystein 10^{-5} , Ca-pantothenate 10^{-6} , inositol 10^{-4} , folic acid 10^{-5} , nicotinic acid 10^{-6} , pyridoxin 10^{-6} , biotin 10^{-8} , MoO_3 15×10^{-12} , α -NAA 10^{-7} , glucose 2% and agar 1%,
- (b) same as (a) without biotin,
- (c) medium according to MOREL (1948),
- (d) medium according to MURASHIGE & SKOOG (1962).

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For 'Saffier' meristems medium (a) gave better results than (c). On medium (a) the meristems developed satisfactorily into plants while on media (b), (c) and (d) the isolations showed a vigorous development of roots in approximately 20–40% of the total number isolated. It was necessary to transfer the isolations to fresh media after five to six months. Ten small five-months old 'Saffier' plants were taken out of the culture tubes and planted in soil at Baarn. Seven survived. Four weeks later a second group of four plants were transferred of which three survived (Table 1). A serological test of the first group proved that in two plants out of seven virus could not be demonstrated. In the second group two out of three plants were virus-free.

At Wageningen four 'White Swan' plants were obtained grown on medium (c) (Fig. 2). Only two plants survived and proved to be free from virus after the first serological test. Although these experiments are still in progress it may be assumed that by using the technique of meristem cultures it will be possible to eliminate at least certain viruses from infested cultivars. Further research on a possible combination of culturing meristems with a heat-treatment preceding the meristem isolation is intended.

TABLE 1. Development of meristem cultures from five Freesia varieties on different media.
Groei van meristeeencultures, afkomstig van vijf Freesia-variëteiten, op verschillende media.

Medium:	(a)			(b)			(c)			(d)		
Freesia variety/ <i>variëteit</i>	Number/ <i>Aantal</i>											
	I	II	III	I	II	III	I	II	III	I	II	III
'White Swan'	—	—	—	6	—	—	168	44	4	—	—	—
'Saffier'	82	14	10	—	—	—	120	25	—	—	—	—
'Oranje zon'	—	—	—	—	—	—	48	9	—	—	—	—
'Golden Yellow'	—	—	—	—	—	—	48	28	—	144	20	—
'Prinses Marijke'	—	—	—	48	3	—	24	10	—	24	4	—

I: Number of isolated meristems/*Aantal geïsoleerde meristemen*

II: Number of growing meristems/*Aantal groeiende meristemen*

III: Number of plants transplanted in soil/*Aantal planten, dat in grond kon worden gezet*

SAMENVATTING

Door middel van meristeeencultures werd getracht virusvrije planten te verkrijgen van vijf Freesia-variëteiten. Hiertoe werden meristemen uitgeprepareerd en op verschillende voedingsbodems geplaatst (Fig. 1). Op één medium ontwikkelde zich een aantal meristemen tot normale planten, op de andere media vertoonden de cultures soms een overmatige wortelontwikkeling. Veertien meristemen groeiden uit tot planten (Tabel 1, Fig. 2), waarvan na serologische toetsing er zes virusvrij bleken te zijn.

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