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Virus elimination by meristem-tip culture from a range of Alstroemeria cultivars

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Abstract

A technique for virus elimination by meristem culture was developed for a range of *Alstroemeria* cultivars. Meristems were excised from the rhizome tips and placed on a medium with indole-3-butyric acid, the required concentration of which was cultivar dependent: Six to eight weeks after dissection a shoot had formed which was transferred to a medium without growth regulators. On filter paper bridges, in a liquid medium, root formation was better than on a solid medium. In many cases a new rhizome developed. If not, the plantlet eventually died. Transfer into soil was more successful with the plantlets rooted in liquid medium than with those rooted in solid medium. Virus elimination was cultivar dependent, but in most cultivars plants resulting negatively in serological tests could be obtained. After repeated testing and selection for horticultural properties these plants may be used to start high quality mother plots.

Introduction

As a consequence of intensive breeding several new *Alstroemeria* cultivars have been released into practice in the Netherlands. From the point of view of virus infection this is not always advantageous, because some of them may be completely infected with a virus at the very beginning of their commercial life. This may be due to the fact that *Alstroemeria* mosaic virus is seed transmitted, and because virus spread in the glasshouse may be rapid; these aspects are neglected during the breeding work.

In 1983 it was decided to establish an *Alstroemeria* clean stock program. To this aim the current as well as the new, infected cultivars, had to be cleaned if infected, so as to produce plants without viruses and use healthy parents for further breeding. The technique of meristem culture seemed promising and therefore a workable technique for virus elimination was developed.

There is one report on disease elimination from *Alstroemeria* by meristem culture with the cv. Orchid on Sheridan's medium (Quak, 1974). She produced 120 plants but the yellow flecks present in the original material reappeared in the plants produced. However, the etiology of the yellow flecks is still uncertain and they may be of physiological origin.

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Nowadays vegetative propagation of *Alstroemeria* by in vitro techniques receives much attention. However, little information is available on the subject. Ziv et al. (1973) regenerated adventitious plants from subapical sections of the inflorescence stem of Ligtu hybrids. Hussey et al. (1980) propagated *Alstroemeria* in vitro by culturing the rhizome tips on media with 1-4 $mg.l^{-1}$ 6-benzylaminopurine (BA) and subsequently dividing the rhizome into pieces. Lin and Monette (1987) working with cv. Alsaan cultured rhizome tips in media with indole-3-acetic acid (IAA) and a mixture of zeatin and kinetin (K) on solid and subsequently on liquid media.

A report on the viruses known from *Alstroemeria* and on some preliminary results of meristem-tip culture was published earlier (Hakkaart and Versluijs, 1985).

Materials and methods

Rhizome tips were trimmed to a size of about 3 mm, rinsed with tap water and submerged in 96% (v/v) alcohol for 1 min. Thereafter they were stirred for 20 min in a 5% (w/v) calcium hypochlorite solution and rinsed three times with sterile water. Axillary meristems were excised and placed on the surface of 15 ml of medium in glass tubes (2×15 cm) covered with polyethylene caps. The basal medium consisted of Murashige and Skoog (1962) macro and micro salts full strength, 25 $mg.l^{-1}$ FeNaEDTA, 2 $mg.l^{-1}$ glycin, 0.5 $mg.l^{-1}$ of both nicotinic acid and pyridoxine-HCl, 0.4 $mg.l^{-1}$ thiamine, and 100 $mg.l^{-1}$ m-inositol. Saccharose was 30 $g.l^{-1}$ and agar, if used, 6 $g.l^{-1}$. The growth regulators are mentioned at the separate experiments. The pH was adjusted to 5.8 before autoclaving during 20 min at 112 °C. The tubes were placed in a growth chamber at 17 °C with 12 h of light from fluorescent tubes (Philips 33) per day with 1200 lux at the explant level. When filter paper bridges (see Fig. 1) were used the explants stayed about 1 cm above the liquid level.

Rooted plantlets were transferred to steam-sterilized soil in boxes covered with plastic and grown until sufficiently large for virus testing. The serological virus tests were performed by the Netherlands General Inspection Service for Ornamental Plants (NAKS) at Roelofarendsveen applying ELISA with antisera to *Alstroemeria* mosaic virus and *Alstroemeria* carla virus.

Results

Shoot growth. Since the growth regulator requirements of the excised meristem tip were not well known, a range of preliminary experiments was set up with cv. Rosario. As growth regulators we used 2 $mg.l^{-1}$ K + 1 $mg.l^{-1}$ gibberellic acid, 0.1 $mg.l^{-1}$ K + 0.1 $mg.l^{-1}$ α-naphtalene acetic acid (NAA), 0.1 $mg.l^{-1}$ K + 0.3 $mg.l^{-1}$ NAA and a range of concentrations of 6-dimethylallylaminopurine from 0.01 $mg.l^{-1}$ to 10 $mg.l^{-1}$ combined with 0, 0.1 or 1 $mg.l^{-1}$ IAA. However, initial growth was not satisfactory. Then an experiment was performed with only auxins as growth regulators in the initial medium. Three auxins, viz. NAA, IAA and indole-3-butyric acid (IBA) were applied in the concentrations of 1, 3, 5 and 10 $mg.l^{-1}$. Meristem tips were dissected on August 15, 1984, the explants were transferred to the same medium on October 1, 1984, followed by a last transfer to a medium with 1 $mg.l^{-1}$ BA on November 8, 1984. Evaluation was on February 5, 1985. There were 15 explants for each treatment (Table 1). It can be concluded that for initial growth no cytokinin is

Table 1. Comparison of the effect of four concentrations of three auxins as the only growth regulator on the growth of meristems of cv. Rosario.

Concentration ($mg.l^{-1}$)	Number of explants with shoot growth	Callus clumps	Roots only	Dead explants	Contaminations
1 NAA[a]	2	8	0	4	1
3 NAA	1	10	1	3	0
5 NAA	2	10	1	2	0
10 NAA	1	10	0	4	0
1 IAA	3	6	0	6	0
3 IAA	2	7	0	4	2
5 IAA	3	8	0	4	0
10 IAA	1	4	0	10	0
1 IBA	1	3	0	10	1
3 IBA	7	3	0	5	0
5 IBA	4	6	0	5	0
10 IBA	3	6	0	6	0

[a] For each treatment 15 excised meristems were used.

needed. NAA gave rise to few good plantlets and many callus clumps, IBA caused the reverse effect and IAA held an intermediate position. The best results were obtained with 3 $mg.l^{-1}$ IBA.

Other cultivars were tested for IBA concentrations. Working with cvs Appelbloesem, Morning Star and Yellow King, concentrations of 0.5, 1 and 1.5 $mg.l^{-1}$ worked well. With cvs Flamengo, Luciana, Ohio, Olympic and Tango and concentrations of 0.5, 1 and 2 $mg.l^{-1}$ IBA with cv. Luciana only 2 $mg.l^{-1}$ proved satisfactory. The cv. Ontario required a relatively high IBA concentration of 5 $mg.l^{-1}$ which was better than 0.5 or 3 $mg.l^{-1}$. Therefore it may be concluded that many cultivars require IBA in the range of 0.5 $mg.l^{-1}$ to 5 $mg.l^{-1}$.

Rooting. After initial shoot growth further growth was still unsatisfactory because the plantlets did not root. Rooting could be induced by placing the shoot on a medium without any growth regulator. We compared an agar medium with a liquid one in which the explants were placed on filter paper bridges. The presence of growth regulators in the second medium was not only superfluous, but IBA sometimes even inhibited rooting. The results of experiments with nine cultivars are presented in Table 2.

Both on agar and on filter paper bridges rooting occurred. On filter paper bridges abundant root hair production took place, which was absent or very rare on roots formed in agar. Starting from equal numbers of explants of cvs Luciana, Ohio, Olympic, Ontario, Rosario, Tango and the botanical species *A. psittacina* 18 plants were obtained established in soil, originating from explants rooted on solid medium and 39 plants originating from explants rooted on filter paper bridges. Therefore the extra work in preparing the filter paper bridges seems worthwhile.

The process of shoot formation on the first medium took six to eight weeks and after about two months the rooted plantlets could be transferred into soil.

Table 2. Comparison of three indole-3-butyric acid (IBA) concentrations in the initial medium and a solid or liquid second medium.

Cultivar	IBA concentration in first medium ($mg.l^{-1}$)	Number of excised meristems tested	Number of rooted explants in solid agar	Number of rooted explants in liquid
Appelbloesem	0.5	4	1	1
	1	4	1	3
	1.5	4	2	0
Morning Star	0.5	8	4	1
	1	8	3	1
	1.5	8	3	3
Yellow King	0.5	4	2	2
	1	4	2	2
	1.5	4	1	1
Flamengo	0.5	12	2	3
	1	12	2	1
	2	12	0	3
Luciana	0.5	44	3	10
	1	44	3	7
	2	44	11	14
Ohio	0.5	18	5	5
	1	18	4	5
	2	18	7	5
Olympic	0.5	14	5	3
	1	14	2	4
	2	14	5	3
Rosario	0.5	12	0	0
	1	12	1	0
	2	12	3	3
Tango	0.5	25	10	9
	1	25	6	9
	2	25	10	11
Total		423	98	109

Rhizome formation. Many plantlets, thus obtained, formed a complete plant in vitro, i.e. one shoot, roots and a rhizome (Fig. 1). Rhizome formation proved to be essential for survival. Some explants did not form a rhizome and although some of these survived transplanting and reached a height of 10 cm, they eventually all died. This phenomenon may be due to the fact that rhizome formation of the explant is related with the site of the meristem on the mother rhizome. The rhizome of *Alstroemeria* is a sympodium. The first internode of the youngest shoot gives the lengthening of the rhizome. In the axil of the first phyllome a meristem forms which develops into the next shoot growth. In the axil of the second phyllome a meristem occurs which gives rise to a lateral rhizome, allowing branching. This situation is complicated by the fact that the terminal meristem may be subdivided into two meristems. Moreover several other meristems

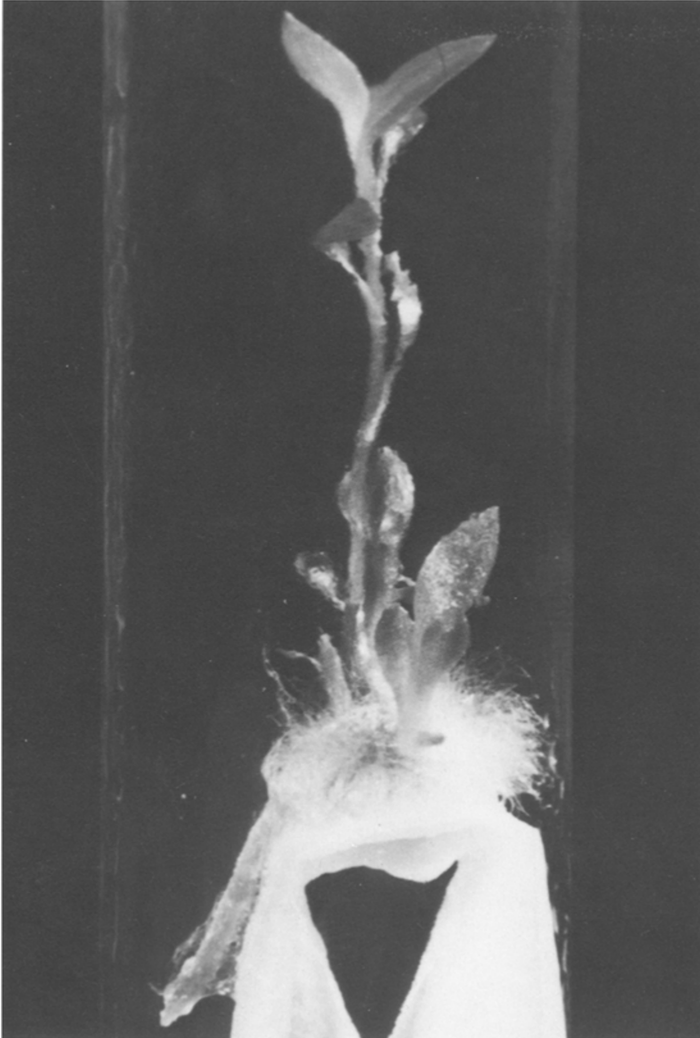

Fig. 1. Five months old plantlet of cv. Flamengo on filter paper bridge with shoots, rhizome, root hairs and roots.

can be observed. We discerned 23 different sites at a rhizome and all these sites were given a code. With 14 cultivars we followed 383 coded meristems during their culture. However, no mutual relationship could be discerned and the factor(s) governing rhizome formation by the explants remain(s) unknown. A certain loss of the explants, therefore, must be taken into account.

Virus elimination. From the boxes covered with plastic the plants were hardened and transplanted into pots. When the plants had reached a height of about 20 to 30 cm symptoms of *Alstroemeria* mosaic virus became visible in some of them. All plants

Table 3. Virus elimination by meristem culture in eight *Alstroemeria* cultivars.

Cultivars	Number of plant tested	Plants with virus	Plants without virus
Jubilee	11	0	11
Rosario	5	0	5
Luciana	14	6	8
Rita Groen	12	3	9
Rita Rood	9	4	5
Jumbo	3	3	0
Ohio	9	9	0
Olympic	8	8	0
Total	71	33	38

were tested by ELISA (Table 3). All cultivars mentioned in this table were previously infected with this virus.

Elimination of *Alstroemeria* mosaic virus seemed to be cultivar dependent: all plants of cv. Jubilee were free, with three others no healthy plants were obtained and sometimes there was a partial elimination (Fig. 2). This experiment should be repeated with a greater number of plants. *Alstroemeria* carla virus was not detected in these tests.

All plants that came into flowering were true to type. After repeated testing of the apparently virus-free plants they may be used as starting material.

Fig. 2. Stems of cv. Rosario. Right: stem with *Alstroemeria* mosaic virus. Left: stem from which virus has been eliminated by meristem culture.

Discussion

The results demonstrate the difference in growth regulator requirements both for a small meristem and for larger plant parts such as rhizome tips. Hussey et al. (1980) propagated *Alstroemeria* from rhizome tips with BA, whereas in our experiments complete plants were grown from meristem tips with IBA as the only growth regulator.

Some cultivars required one definite IBA concentration, but others grew well on IBA concentrations ranging from 0.5 to 5 $mg.l^{-1}$. So if an unknown or new cultivar has to be cultured a range of IBA concentrations is recommended.

Rooting occurred after withdrawal of the growth hormone. A similar situation is mentioned by Snir (1983) for *Prunus cerasus* where roots were initiated on shoots with 1 $mg.l^{-1}$ IBA, but root elongation was obtained on a medium without growth regulators. This, however, is not a general rule, because in many cases withdrawal of auxin is not necessary to induce rooting.

The rhizome formation by the shoot just grown from the meristem is an intriguing problem. During propagation this problem is not present because a rhizome is already there and only needs to grow and develop further. With meristem culture the situation is different because de novo formation is needed. In another rhizome forming species such as *Canna* Kromer and Kukulczanka (1985) experienced no difficulties when they grew plants from meristem tips. With *Alstroemeria* the conditions governing rhizome formation are not clear.

The whole in vitro procedure takes about four months and comprises two steps. Taking into consideration that a range of cultivars was involved in the experiments it can be concluded that new cultivars will probably respond if grown on a range of IBA concentrations as outlined above. The method may well be applied by commercial enterprises so as to free current and new cultivars from virus and use them for production and breeding work, thus further improving the quality of *Alstroemeria* plants.

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Samenvatting

Het elimineren van virus uit een aantal Alstroemeria cultivars door middel van meristeemcultuur

Een techniek werd ontwikkeld om door middel van meristeemcultuur virus te elimineren uit een aantal *Alstroemeria* cultivars. De meristemen werden uit de toppen van de rhizomen geprepareerd en op een voedingsbodem met IBA als auxine geplaatst. De IBA-concentratie nodig voor scheutvorming was afhankelijk van de cultivar. Na een periode van zes tot acht weken had het meristeem zich ontwikkeld tot een scheutje, dat vervolgens werd overgebracht op een voedingsbodem zonder groeistoffen. In ongeveer twee maanden vormden zich dan wortels. Deze wortelvorming was beter op een vloeibare voedingsbodem met papieren bruggetjes dan op een vaste voedingsbodem

van agar. In veel, maar niet in alle gevallen, vormde zich ook een nieuw rhizoom. Indien geen rhizoom werd gevormd stierf de plant. Gewortelde plantjes groeiden beter in grond indien de beworteling op papieren bruggetjes had plaatsgevonden. Het succes van het elimineren van het *Alstroemeria*-mozaïekvirus hing af van de cultivar. Na herhaalde toetsing kunnen de negatief reagerende planten worden gebruikt voor de opbouw van een partij gezonde moederplanten. Op deze manier kan de kwaliteit van het uitgangsmateriaal van *Alstroemeria* worden verbeterd.

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