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VIRUS-FREE PLANTS OF IRIS 'WEDGWOOD' OBTAINED BY MERISTEM CULTURE[1]

Virusvrije planten van iris 'Wedgwood,' verkregen door middel van meristeemcultuur

E. RHODA BARUCH and FREDERIKA QUAK
Laboratory of Virology, Agricultural University, Wageningen
Institute of Phytopathological Research (I.P.O.), Wageningen

Some commercial varieties of Dutch (bulbous) iris such as 'Wedgwood' and 'Imperator' are wholly infected with a virus called latent mosaic virus in Israel or iris mosaic virus in the Netherlands. The aim of the investigation was to obtain virus-free plants of these varieties by culture of meristem tips excised from bulbs kept in their vegetative stage by storage at 25.5°C. Several media were used; those based on the medium of MOREL (personal communication) gave the best results. About 100 meristems were placed on each medium and on the best media about 30% started to grow. By tranferring slowly growing meristems to the medium of MURASHIGE & SKOOG (1962), growth improved greatly, though root formation remained poor. Twenty meristems developed into plants, of which ten were tested serologically. Eight plants of the variety 'Wedgwood' proved to be virus-free.

INTRODUCTION

Enough evidence has now been obtained to support the assumption that the apical meristems of virus-infected plants may in some instances be free from virus. Meristem culture has now been used to obtain virus-free specimens from virus-infected clones of a number of vegetatively propagated species, valuable in horticulture and agriculture.

The method was developed by MOREL & MARTIN (1952) who cultivated virus-free plants from a wholly infected dahlia variety. Good results have also been obtained with potato varieties infected with viruses X, Y and A (MOREL & MARTIN, 1955), potato paracrinkle virus and potato virus S (KASSANIS, 1957). QUAK (1961) achieved successful results with potato varieties infected with potato virus X, combining meristem culture with the application of 2-thiouracil, 2,4-dichlorophenoxy acetic acid and indolyl acetic acid. She obtained virus-free carnations by means of a modified meristem culture applied to heat-treated carnations (QUAK, 1957). Results with monocotyledons are reported by VAN SLOGTEREN (1964) for certain varieties of hyacinth and by BRANTS & VERMEULEN (1965) for freesia.

Some commercial varieties of Dutch (bulbous) iris such as 'Wedgwood' and 'Imperator' are wholly infected with a commonly occurring aphid-borne virus causing a mild mosaic pattern and sometimes small dark blue streaks on the petals, particularly of 'Wedgwood'. This virus has been called latent mosaic virus (LOEBENSTEIN & ALPER, 1963), but is known in the Netherlands as iris mosaic virus (VAN SLOGTEREN, 1962). Notwithstanding the presence of this virus, the variety 'Wedgwood' is grown on quite a large scale, as the vigour of the plants is not markedly impaired. A great deal of damage can be effected, however, by the iris mosaic virus described by BRIERLEY & MCWHORTER (1936), also called yellow mosaic virus (LOEBENSTEIN & ALPER, 1963) or iris virus 1. In

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the Netherlands the syndrome caused by this virus (also aphid-borne) is called grey disease of iris. 'Wedgwood' and 'Imperator' are not wholly infected with this virus. By means of selection in the field and serological indexing stocks practically free from this virus are maintained. Both viruses can be detected and distinguished serologically.

In view of the above-mentioned successful results work was started in an attempt to produce virus-free plants of 'Wedgwood' and 'Imperator' by means of meristem culture applied to bulbs kept in their vegetative stage by storage at a temperature of 25.5 °C.

MATERIALS AND METHODS

The first two fleshy scales of the bulbs were removed with a knife. The remaining parts were disinfected in a 5% solution of calcium hypochlorite for 30 minutes and washed in distilled water for the same length of time. The bulbs were dried between sterile filter paper. Excising the vegetative tips was done using a binocular microscope (magnification × 20–60) in a culture room sprayed daily with a 3% solution of formaldehyde. The remaining scales of the bulbs were removed with sterile implements. These consisted of pieces broken from a thin safety-razor blade mounted in a rust-proof metal holder, and slightly blunt needles. Forceps were used to hold the stem or base of the bulb while the tips were excised. After dissecting each bulb, all implements were sterilized in 96% ethanol, rinsed in distilled water and dried between sterile filter paper. The size of the meristems varied between 0.1 and 0.5 mm; excising a tip with a leaf primordium, however small, was avoided.

The following media were used:

1. Four media based on the medium of WHITE (1963):
   White; White + indolyl acetic acid (IAA) 1 ppm; White + alpha naphtalene acetic acid (NAA) 1 ppm; White + Na_2Fe-chelate 10 ppm instead of $Fe_2(SO_4)_3$.
2. Embryo media according to RODRIGUES PEREIRA (1961) and TOMASZEWSKI (personal communication).
3. Four media based on the medium of MOREL (personal communication): Morel + IAA 1 ppm; Morel + NAA 1 ppm; Morel + kinetin 0.1 ppm; Morel + Na_2Fe-chelate 10 ppm.

Best results were obtained with media based on that of MOREL. Therefore the formula of that medium only is presented here: 1/2 concentration Knop solution 1000 ml; Berthelot solution 0.5 ml; cystein 1 mg; adenine 5 mg; hydrolysate of casein 200 mg; saccharose 20 g; agar 6 g; vitamin solution (containing calcium pantothenate 1 mg, inositol 100 mg, biotin 10 mg, nicotinic acid 1 mg, pyridoxin 1 mg, distilled water 100 ml) 1 ml. The media were adjusted to pH 6.

Small Pyrex tubes (length 5 cm, diameter 1 cm) were filled up to one third with nutrient agar and sterilized by autoclaving at a temperature of 110 °C for 20 minutes. After excision the tips were placed with the cut surface on the solidified medium, and the tubes were sealed with cottonwool and Parafilm. The tubes were placed under continuous light of 40 W fluorescent tubes at an air temperature of about 20 °C.

If neccessary the plantlets were transferred to a fresh medium, and when

large enough to a small plastic pot filled with sand. These plantlets were at first watered with a liquid medium of MOREL, and later with water.

Assaying for the presence of virus was done serologically using leaf material.

RESULTS

In January 1963 about 700 meristems were isolated, mainly of the variety 'Wedgwood'. Considerable differences were observed in the growth of the meristems on the media mentioned above. On the medium of WHITE and its modifications and on the embryo media very few tips grew and no plantlet survived transfer to sand. Only the three media based on MOREL's medium, viz. those with NAA, kinetin and NA_2Fe-chelate, gave good results. About 30% of the meristems started to grow, and after about four months some had produced a small leaf (Fig. 1). Root formation, however, was poor. Some of the slowly growing meristems were transferred to a medium according to MURASHIGE & SKOOG (1962); within a few weeks they showed much improved growth but still hardly any roots were formed (Fig. 2).

By the end of 1963 some forty plantlets were placed on sand. A few survived this treatment and in the summer of 1964 about 12 bulbs, 5 mm in diameter or less, had formed. During the following year the bulbs developed new roots and leaves and some produced new bulbs (offsets). By the formation of offsets the number of bulbs increased considerably.

In the summer of 1965 ten bulbs (diameter 5 mm, length 7 mm) had produced enough leaf material to be used for a serological test on the presence of virus. The test was repeated in January 1966. Eight of the bulbs proved to be virus-free and two were found to be infected. A second group of about ten bulbs have yet to be tested, including a few of the variety 'Imperator'. Close examination of the virus-infected plants revealed clear symptoms, even at this early stage (Fig. 3). Leaves of plants in which no virus could be detected were remarkably dark green in colour, whereas leaves of virus-infected ones were yellow-green and mottled, especially at the base of the leaves. The extent of growth of the healthy and virus-infected plants was identical up to this stage.

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SAMENVATTING

Enige variëteiten van de Hollandse (bol-)iris, zoals 'Wedgwood' en 'Imperator', zijn volledig besmet met irismozaïekvirus. Er werd getracht virusvrije planten te verkrijgen door middel van meristeemcultuur. Hiertoe werden de meristemen uitgeprepareerd van bollen, die in het vegetatieve stadium werden gehouden door bewaring bij 25,5°C. Verscheidene voedingsbodems werden gebruikt, waarvan die gebaseerd op het medium van MOREL (persoonlijke mededeling) de beste resultaten gaven (fig. 1). Per medium werden ongeveer 100 meristemen geïsoleerd. Op de beste media werd circa 30% groei verkregen.

Langzaam groeiende meristemen werden overgezet op het medium van MURASHIGE & SKOOG (1962). De groei op deze voedingsbodem was uitstekend, maar de wortelvorming bleef slecht (fig. 2). Twintig meristemen groeiden uit tot planten, waarvan er tien in 1965 en 1966 serologisch zijn getoetst. Hiervan bleken er acht virusvrij te zijn. De viruszieke planten onderscheidden zich van de gezonde planten door een duidelijke vlekkerigheid (fig. 3); in groei onderscheidden de virusvrije zich echter niet van de besmette planten.

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FIG. 1. Developing meristems of iris 'Wedgwood' (six months old).

Zich ontwikkelende meristemen van iris 'Wedgwood' (zes maanden oud).

FIG. 2. Plantlets of iris 'Wedgwood' (one year old) grown from meristems. Left: medium of MURASHIGE & SKOOG. Right: medium of MOREL.

Plantjes van iris 'Wedgwood' verkregen door meristeemcultuur (één jaar oud). Links: medium van MURASHIGE & SKOOG. Rechts: medium van MOREL.

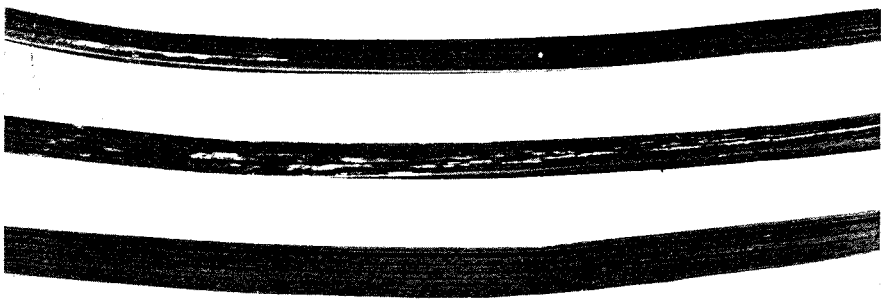

FIG. 3. Leaves from plants of iris 'Wedgwood' (three years old) grown from meristems. Two top leaves virus-infected, with symptoms of mottling; bottom leaf healthy.

Bladeren van planten van iris 'Wedgwood' verkregen door meristeemcultuur (drie jaar oud). Bovenste twee bladeren ziek, met symptomen van vlekkerigheid; onderste blad gezond.