SOMACLONAL VARIATION IN LILY AFTER *IN VITRO* CULTIVATION

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Abstract

Somaclonal variation occurring during cultivation of callus from lily has been determined. Scales of *L. longiflorum* 'Gelria' were induced to start callus formation. The undifferentiated callus was maintained for 3 years at 20 °C in the dark. After this period the callus was still able to regenerate. The regenerants were grown in the greenhouse and from the 400 regenerants obtained 14 plants differed visually. The variation was divers and consisted of coloured or malformed leaves, male sterility and a dwarf mutant. This variation, induced by in vitro cultivation, may be used in plant breeding as a tool to select for desirable plant characteristics such as resistence against diseases.

Introduction

Propagation of plants by tissue culture (micropropagation) is an accepted technique for reproduction of ornamentals, including lily. A recognized disadvantage of this technique is the possible arising of unwanted variation between plants obtained, the so-called somaclonal variation. The variation may be either genetic or epigenetic (De Klerk, 1990, De Klerk and Bouman, 1991). A few years ago, for example, the lily cultivar 'White Mountain' was extensively propagated *in vitro* in the Netherlands. However, in the first season of cultivation many of the in vitro obtained plants showed curling leaves. At first it was not clear whether this was a physiological or a genetic factor, but after a few cultivation seasons, the curling leaves had disappeared showing that the observed variation was not genetic.

However, variation is not always a disadvantage. Plant breeding highly needs the existence of genetic variation present in the existing assortment or wild species, as well as the genetic variation observed in plants after *in vitro* culture. Somaclonal variation may be a source of this induced variation. In general there is a positive correlation between the degree of disruption of an organized structure and the chances that Somaclonal variation will occur. Therefore, mutations can be expected to arise during callus, cell and protoplast cultures, certainly after repeated subculturing. Also the growth regulators used in tissue culture (auxins like 2,4-D and NAA, and cytokinins like BAP) may enhance the number of mutations (Pierik, 1987).

The advantages offered by cell and tissue culture to identify and exploit new sources of variation have been recognised (Larkin and Scowcroft, 1981, Evans, 1989, Phillips *et al.*, 1990). Exploitation of somaclonal variation as a tool for disease resistance breeding has been extensively reviewed (Wenzel, 1985, Daub, 1986, Van den Bulk, 1991).

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Somaclonal variation can be of specific interest in crops which show little variation after *in vitro* culture, as is the case with lily (Takayama *et al.*, 1991). The aim of this research was to study somaclonal variation in regenerants of lily, appearing after a long period of *in vitro* culture.

Material and Methods

Scales of *L. longiflorum* cv 'Gelria' were used to induce callus on a modified MS-medium containing MS+vitamins (4.6 g/l), myo-Inositol (0.1 g/l), thiamine-HCl (4 mg/l), sucrose (30 g/l), and the hormones NAA (0.1 μM) and BAP (0.1 μM). The callus was kept on this medium for 3 years in the dark at 20 °C. The medium was frequently refreshed and differentiated tissue (production of roots) was removed. After 3 years, the callus was transferred to shoot regeneration medium (modified MS medium, containing 0.5 μM NAA and 0.5 μM BAP) under light conditions. After 4 months, regenerated shoots were transferred to bulb production medium (modified MS medium in which the sucrose concentration was doubled to 60 g/l and the concentrations of NAA and BAP were returned to 0.1 μM). Temperature was 20 °C and the shoots were kept short to stimulate bulb production. After 2 months the material was placed in the cold (2-4 °C) for 10 weeks. Then the bulbs were planted in the greenhouse and grown to maturity. Mutants were scored visually during 3 growing seasons. Relative DNA content of leaf tissues was determined with a flowcytometer (Van Tuyl and Boon, 1997).

Results

After 3 years of callus cultivation normal regeneration of shoots was still feasible. In the first growing season (1993), 550 *in vitro* obtained bulblets measuring from 4 to 22 mm in diameter were grown in the greenhouse to 400 plants. Some of these plants already flowered during this first growing season and some mutants were observed. In 1994, all bulbs were planted again and this time most plants flowered. At least 95% of the plants was scored as being normal. A few "remarkable" plants and plants which had not yet flowered were planted once again and scored after the third growing season.

In total 14 visible mutants were observed. The types of mutation differed: some mutants showed partially coloured leaves (albinism), shorter and wider leaves or curled leaves. Six male sterile plants were found and one dwarf mutant (Table 1). Leaf samples from the mutants were analysed for DNA content. No major differences in DNA content were observed, indicating there was no change in level of ploidy. Nevertheless the DNA content of 2 plants was significantly lower than that of the control, so during a long period of callus cultivation followed by regeneration the DNA-content of the plant is not absolutely stable.

Table 1: Description of varying regenerants of 'Gelria' after 3 years of callus cultivation.

Description of the plant	male sterile	DNA content (relative)*
lightly striped chimera	-	78.8
moderately striped chimera	-	77.5
strongly striped chimera, small flower	-	78.4
strongly striped chimera, small flower	-	78.3
malformed leaves (thick, short, curly and dark)	-	77.6
malformed leaves (thick, short, curly and dark)	-	76.8
malformed leaves (thick, short, curly and dark)	+	76.6
malformed bud, late flowering, leaves broad	-	77.9
malformed bud, late flowering, leaves broad	+	75.6 **
malformed bud, late flowering, leaves broad	+	75.9 **
dwarf, with long pointed leaf	-	78.1
normal appearance	+	77.7
normal appearance	+	77.5
normal appearance	+	77.1
control		77.8
control		77.9

* Measured as described in van Tuyl and Boon, 1996.

** These plants showed significant lower DNA contents.

Conclusions

Callus of *L. longiflorum* 'Gelria' was able to regenerate normally after 3 years of cultivation. In general, only little variation was found after this long callus phase followed by regeneration. The appearance of a dwarf mutant, six male sterile plants and a few leaf mutants clearly show that somaclonal variation may be induced during *in-vitro* culture of lily. This variation might be used by plant breeders to select regenerants with desirable characteristics such as disease resistance.

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