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THE HIGH FREQUENCY OF VARIEGATED FORMS AFTER *IN VITRO* MUTAGENESIS IN *SAINTPAULIA IONANTHA* WENDL.

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ABSTRACT

The leaf-explants of *Saintpaulia ionantha* Wendl. var. 'miniature' were treated by different doses of MNH and cultured on shoot regeneration medium. A strong toxic effect of some MNH doses on explant survival during the first two subcultures was noticed. The explants surviving treatment regenerated shoots with the efficiency comparable to the control. The high number of shoots regenerated from mutagenised leaves showed chlorophyll chimerism (so-called variegated forms). The use of 5 mM MNH for 1.5 or 2 h was found very effective, as 100% of survived explants regenerated variegated shoots. Besides hundreds of variegated forms also leaf-shape and flower-colour variants were observed in MNH-treated culture. Somaclonal variation was not observed in the control culture. The results indicate the great efficiency of *in vitro* applied MNH for induction of morphological variants of *Saintpaulia*, and especially variegated forms.

KEY WORDS: African violet, MNH, leaf culture, chlorophyll deficient forms, organogenesis.

INTRODUCTION

African violet (*Saintpaulia ionantha* Wendl) is one of the most popular ornamental, vegetatively propagated plant and increase of its genetic variation is of economic value. Breeding of this species has only been achieved by intraspecific hybridization and sport selection (Grout 1990). Recent development of plant biotechnology can provide new methods for further improvement of *Saintpaulia* genotypes. Among these new techniques somatic hybridization and genetic transformation can be considered. However, these systems require efficient protoplast culture or transformation system which are not yet developed for *Saintpaulia* although promising results on establishment of African violet protoplast culture have been obtained recently (Winkelmann and Grunewaldt 1995; Hoshino et al. 1995). Another way to generate new variation is *in vitro* mutagenesis (Negrutiu 1990) which can be especially effective in plants easily propagated in *in vitro* culture as African violet. The high morphogenic potential of *Saintpaulia* leaf-explants cultured *in vitro* has been described (Kukłczanka and Suszyńska 1972) and micropropagation systems for this plant have been developed (Bilkely et al 1978; Smith and Norris 1983). The study was carried out to estimate the efficiency of *in vitro* mutagenesis in *Saintpaulia ionantha* leaf-culture in order to induce morphological variants.

MATERIALS AND METHODS

Leaf-explants from plants growing *in vitro* on the hormone free MS (Murashige and Skoog 1962) medium were used as explants for mutagenesis. Chemical mutagen, MNH, was applied in two concentrations: 1 and 5 mM. The mutagen was dissolved in water (1 mM) or in shoot inducing (SI) medium (5 mM) with a few drops of Tween 80. The small leaves with petioles or fragments of bigger leaves (about 1 cm²) were treated on a shaker during 2 or 3 h for 1 mM and 1.5 or 2 h for 5 mM. Following rinsing in water, the treated explants were cultured onto SI medium (MS basal medium; NAA – 0.1 mg/l; BA – 5.0 mg/l; sucrose – 20 g/l; Difco agar – 6 g/l; pH 5,8).

After 3 weeks of culture the number of surviving explants was scored and the growing leaves were subcultured onto a fresh SI medium. The procedure was repeated every four weeks. During a total of 8 months, the regenerating shoots were excised and after rooting on RI medium (MS basal medium; NAA – 0.01 mg/l; activated charcoal – 6 g/l; sucrose – 20 g/l; Difco agar – 6 g/l) the plants were planted in the soil.

RESULTS AND DISCUSSION

It was found that all MNH doses decreased the number of explants surviving on SI medium in comparison to the control combination (Table 1). After mutagenic treatment 42.8 to 75.0% of explants displayed growth during the first subculture. The toxic effect of the mutagen on African violet leaves and petioles was also observed in the second subculture when

Abbreviations:

MNH (N-nitroso-N-methyl urea), BA (benzyladenine), NAA (naphthaleneacetic acid)

TABLE 1. The effect of MNH on the survival of *S. ionantha* leaf-explants after *in vitro* mutagenesis.

Dose mM/h	No. of explants	Number and percentage (%) of surviving explants during	
		first subculture	second subculture
1/2	100	75 (75.0)	32 (32.0)
1/3	113	79 (65.5)	30 (26.5)
5/1.5	140	60 (42.8)	18 (12.8)
5/2	132	58 (43.9)	15 (11.4)
Control	52	49 (94.2)	43 (82.6)

the percentage of growing explants in the most harmful combination (5 mM x 2 h) dropped to 11.4% in comparison to 82.6 % in the control culture.

Contrary to the extremely strong effect of MNH on explant survival, no influence of the mutagen on regeneration ability of treated explants was noticed. The regeneration capacity in terms of shoot number developed from surviving explants was very high in all combinations. Shoots differentiation began after 6 weeks in control and after 8 weeks in treated culture from the cut ends of explants (Fig. 1). Shoots were regenerated by direct organogenesis as was observed by Ohki (1994). Finally, after 3 months, the entire surface of the explant became covered with multiple shoots unabling the correct estimation of the shoot number regenerated per explant (Fig. 2). Potentially hundreds of shoots could be excised from one explant but on average 40 to 60 shoots were excised, rooted and transferred to the soil in every combination.

The shoots regenerated from treated explants showed a high level of variation. The highest frequency of variant shoots was induced by the highest MNH doses (5 mM for 1.5 or 2 h) where every treated explant delivered variant shoots (Table 2). The great prevalence of variants exhibited changes in chlorophyll patterns in leaves, so-called "variegated forms" (Fig. 3). The variegated plants were also noticed after lower MNH

doses (1 mM for 2 or 3 h) but with much lower frequency (up to 6.6 %). Due to "bushy" organogenesis of shoots, the estimation of the number of variegated forms produced per explant was rough. The number of variegated shoots ranged from about 20 to 50% of the total shoot number produced by the treated explant.

The variegated shoots presented a great diversity in chlorophyll patterns of their leaves. Most of them showed different green-albino sectors (Fig. 4), but plain albino (Fig. 5) and light green plants were also found. Anthocyan variants were also observed but with lower frequency. All these variant types could be observed among regenerants from the same explant. Some mosaic leaves were used as explants. Shoots regenerating from them segregated into full spectrum of chlorophyll patterns representing both pattern of primary explant as well as new types of variegation or plain plants (albino, light or normal green).

The changes in chlorophyll pattern on leaves of some variegated plants during their growth were noticed suggesting the presence of heteroplastomatic cells in which sorting-out segregation of plastids resulted in different variegation patterns (Pohlheim 1981).

Besides the variegated plants, other morphological mutants of African violet, regenerated from different explants were

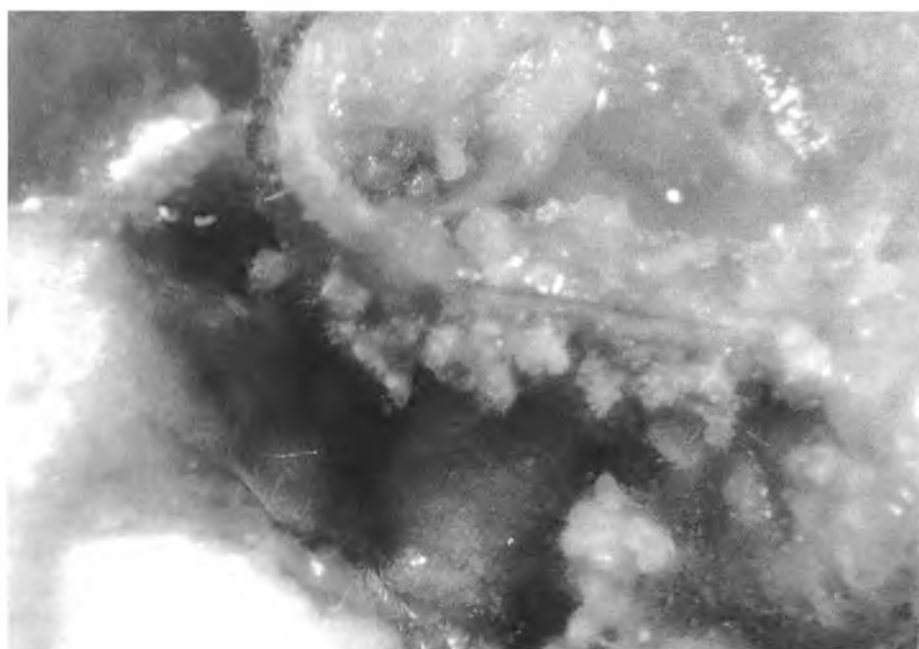


Fig. 1. Shoot regeneration from the cut end of *S. ionantha* leaf- explant in 7-week culture.

TABLE 2. The frequency of *S. ionantha* leaf-explants regenerating morfological variants after MNH induced in vitro mutagenesis.

Dose mM/h	No. of regenerating explants	Number and percentage (%) of explants regenerating variants	
		variegated	others
1/2	32	2 (6.2)	0
1/3	30	2 (6.6)	0
5/1.5	18	18 (100)	1 (5.5)
5/2	15	15 (100)	2 (13.3)
Control	36	0	0

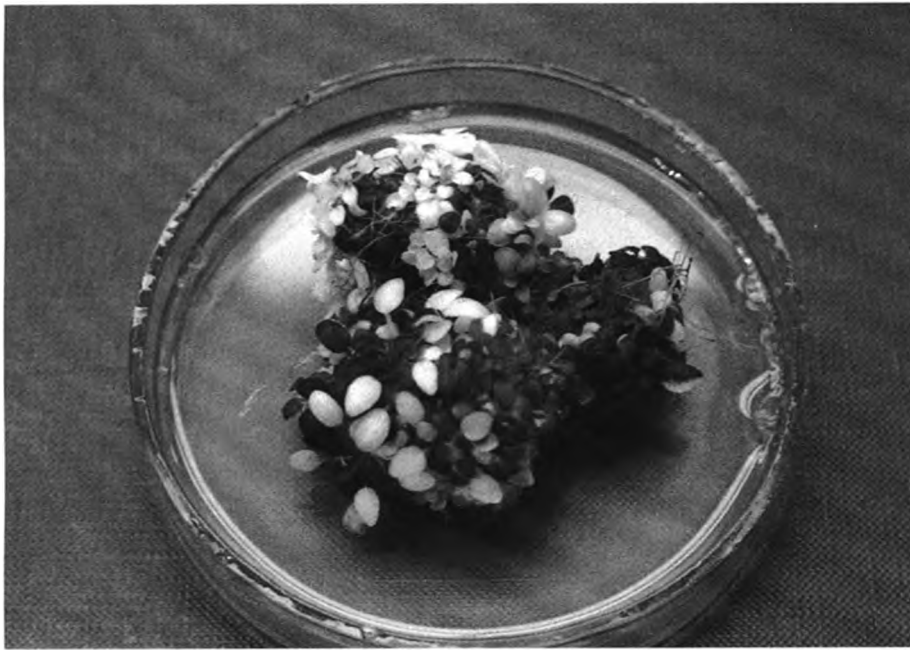


Fig. 2. MNH-treated *S. ionantha* leaf-explants entirely covered with regenerating shoots after 3 months of culture. Numerous albino shoots can be noticed.



Fig. 3. Variegated *S. ionantha* variant with chlorophyll-deficient sectors on leaves.



Fig. 4. *S. ionantha* plant regenerated from MNH treated leaf-explant presenting albino-green sectors on the leaves.



Fig. 5. Albino shoot of *S. ionantha* regenerated from MNH treated leaf-explant.

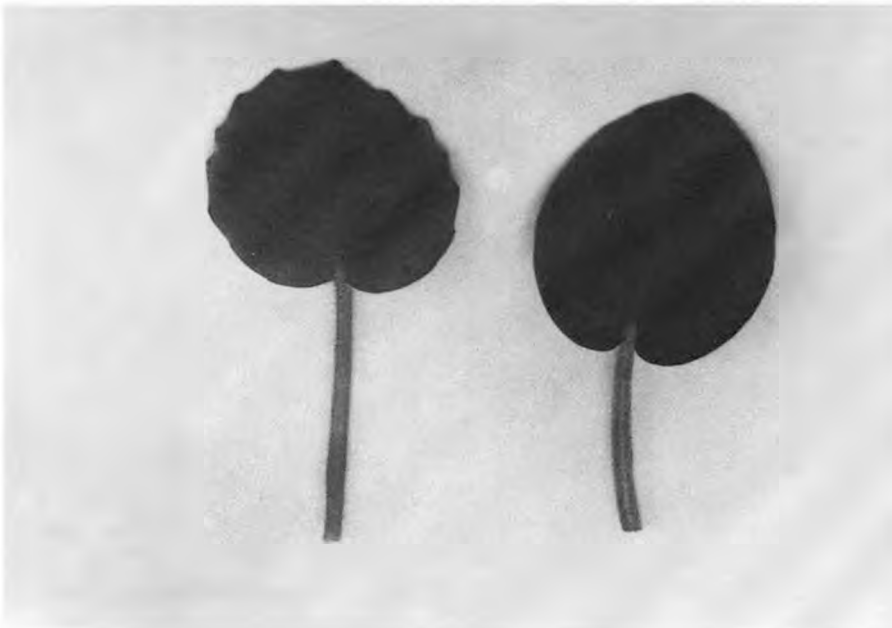


Fig. 6. MNH-induced *S. ionantha* variant with serrated leaves (left), control plant (right).

found with the frequency up to 5%. Variation presented by these plants included leaf shape, so-called serrated leaves (Fig. 6), flower colour (light pink-plain or sectors of albino and pink colour) and plant size. It should be stressed that all variants were found only in mutagenised culture. Contrary to Jain (1993) suggesting that BA can be responsible for variation in *Saintpaulia ionantha* plants regenerated in leaf-disc culture, in the presented experiment non somaclonal variants were observed in the control culture.

The genetic determination of induced variants is not proved but the high frequency of variegated and other forms found only after mutagenic treatment indicates their genetic background. Considering MNH as a very efficient plastome mutagen (Hagemann 1982) and suggestion of Pohlheim and Berger (1974), cytoplasmatic determination of variegated forms can be assumed.

It was indicated that from one MNH-treated leaf-explant dozen of variant plants could be obtained. The results showed the great efficiency of *in vitro* mutagenesis in *Saintpaulia ionantha* and advantages of this method over mutagenesis of *in vivo* formed adventitious buds of African violet (Warfield 1973; Pohlheim 1974).

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WYSOKA CZĘSTOTLIWOŚĆ WARIANTÓW CHLOROFILOWYCH *SAINTPAULIA IONANTHA* WENDL. INDUKOWANYCH NA DRODZE MUTAGENEZY *IN VITRO*

STRESZCZENIE

Eksplantaty liści *Saintpaulia ionantha* Wendl. var. "miniature" traktowano różnymi dawkami (1 mM przez 2 i 3 godz. oraz 5 mM przez 1,5 i 2 godz.) mutagenu chemicznego MNH (N-nitroso, N-metylo-mocznik). Do regeneracji pędów zastosowano pożywkę agarową Murashige i Skooga (1962) zawierającą 0,1 mg/l NAA i 5,0 mg/l BAP. W ciągu dwóch kolejnych subkultur zaobserwowano silny wpływ wszystkich zastosowanych dawek mutagenu na przeżywalność traktowanych eksplantatów. Podczas gdy w kulturze kontrolnej ponad 80% eksplantatów regenerowało pędy, to w kulturze traktowanej zdolność tę wykazywało od 11 do 32% fragmentów liści. Pędy regenerowane z traktowanych eksplantatów charakteryzowały się wysoką częstotliwością zmian morfologicznych, wśród których najczęściej występowały formy o mozaikowych, chlorofilowych zmianach na liściach (tzw. warianty pstrokatolistne) oraz formy albinotyczne i antocjanowe. Najbardziej efektywne w indukowaniu wariantów chlorofilowych były dawki 5 mM MNH x 1.5 lub 2 godz., po zastosowaniu których wszystkie przeżywające traktowanie eksplantaty regenerowały pędy o zmianach w zabarwieniu liści. Otrzymane na drodze mutagenezy *in vitro* warianty morfologiczne obejmowały ponadto formy o zmienionym kształcie blaszki liściowej, barwie kwiatów oraz rozmiarach roślin. W kulturze nietraktowanej nie zaobserwowano żadnych wariantów morfologicznych.

SŁOWA KLUCZOWE: MNH, mutageneza *in vitro*, *Saintpaulia ionantha* Wendl., organogeneza pędów, warianty chlorofilowe.