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ВЛИЯНИЕ НА ХИМИЧНИ МУТАГЕНИ ВЪРХУ МОРФОЛОГИЧНИ БЕЛЕЗИ В M_3 ПОКОЛЕНИЕ НА ПЕТУНИЯ (*PETUNIA x ATKINSIANA* D. DON)

THE INFLUENCE OF CHEMICAL MUTAGENS ON MORPHOLOGICAL TRAITS IN M_3 GENERATION OF PETUNIA (*PETUNIA x ATKINSIANA* D. DON)

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Резюме

Целта на представеното проучване беше да се определи влиянието на химическите мутагени върху морфологичните характеристики в M_3 поколение на петуния. Морфологичните промени се изразяват главно в промяна на цвета на пигментите в цветовете, както и в промените в хлорофилното съдържание в листата. Резултатите показват, че най-ефективни за индуциране на мутации са EMS и DES с доза 0,5 mM. За оценка на промените на нивото на ДНК е използвана ISSR-PCR техника. Най-високото разнообразие сред изследваните генотипове на M_3 поколение на петуния е получено след амплификация на $(GA)_8A$, $(AC)_8G$, $(GA)_8T$ и $(AC)_8C$ (3) праймери.

Abstract

The aim of the presented study was to determine the effect of chemical mutagens on the morphological characteristics in M_3 generation of petunia. The morphological changes referred principally to: discoloration of pigments in the flowers, as well as chlorophyll changes on the leaves and a return of crown-shaped petals. The results showed that EMS and DES at a dose of 0.5 mM were the most effective for inducing mutations. ISSR-PCR techniques were used to assess changes at the DNA level. The highest diversity among the analyzed genotypes of M_3 generation of petunia were obtained after the amplification $(GA)_8A$, $(AC)_8G$, $(GA)_8T$ and $(AC)_8C$ (3) primers.

Ключови думи: петуния, мутация, химични мутагени, ISSR-PCR.
Key words: petunia, mutation, chemical mutagens, ISSR-PCR.

INTRODUCTION

Mutagenesis is known as mutation, that is, uncontrolled changes in the genetic material of plants exposed to the mutagen. It can be used either to induce changes in plants propagated vegetatively and generatively. The frequency of spontaneous mutations, which arise naturally in the environment is relatively low. Therefore, to increase this process chemical or physical mutagens were used. With their application various kinds of changes can be seen (both positive and negative). The appropriate choice of a measure and concentration of a mutagenic can create specified mutants with high frequency (Ahloowalia and Maluszynski, 2001; Berenschot et al., 2008).

Changes that occur in the genes of plants treated with mutagen have a recessive nature and do not always reveal the phenotype of M_1 generation. Fission occurs in the generation characteristics of M_2 - M_3, although these relationships differ significantly from mendelian relationships (Sakin and Yildirim, 2004). Therefore, the aim of this study was to determine the frequency of phenotypic and genetic changes in plants induced M_3 generation of petunia by ethyl methanesulphate (EMS), methyl methanesulphate (MMS), diethylsulphate (DES) and sodium azide (SA).

MATERIALS AND METHODS

The objective of this study was to analyze mutagenesis of the M_3 generation of petunia after application of SA, EMS, MMS and DES. Mutagen doses selected were 1.0 and 1.5 mM of SA, 0.5 and 1.0 mM DES, 0.5 and 1.5

mM EMS,1.5 and 2.0 mM MMS. These solutions were diluted with a sterile 0.025 mM phosphate buffer (pH = 4). As a control plants not treatment by chemical mutagens were used. The procedure of mutagenic treatment was described previously by Krupa-Malkiewicz (2009).

The M_3 generation was raised from 100 seeds of the each mutagen used. Plants were grown in a glass greenhouse. The plants that obtained were screened for chlorophyll changes, different shape and number of flower petals, discoloration of pigment in flowers. During the experiment germination energy of seeds, number of buds and flowers, as well as shoot height was determined. The results were analyzed by using Tukey's test.

Plants with phenotype variability and control were examined using the ISSR-PCR technique (Zietkiewicz et al., 1994). Each fragment that was amplified using 17 ISSR primers was coded in binary form by '0' or '1' or absence or presence in each plant, respectively.

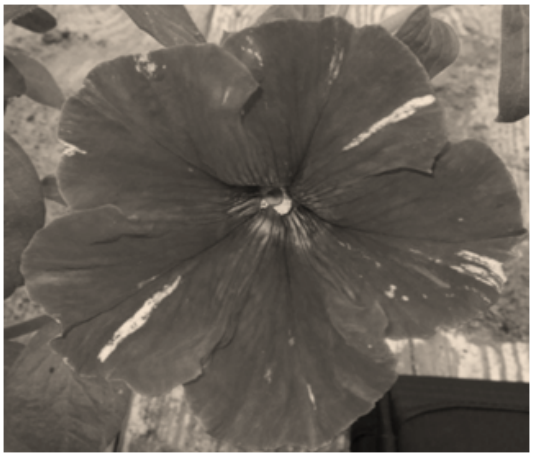
RESULTS AND DISCUSSION

Induced mutations have been applied for the past 70 years to produce mutant cultivars by changing the plant characteristic for a significant increase in plant production among both seed and vegetatively propagated crops. The most common chemical mutagens used are EMS and SA (Jain, 2006). EMS has been widely used in plants because it causes a high frequency of gene mutations and a low frequency of chromosome aberrations (Bhagwat and Duncan, 1998; Van Harten, 1998; Bhate, 2001; Koh and Davies, 2001; Latado et al., 2004; Vagera et al., 2004).

In our study the population of petunia M_3 consisted of 100 seeds representing each concentration of mutagens used in the experiment. A similar size of population was used by Krupa-Malkiewicz (2009) – with 150 seeds (*Petunia* x *atkinsiana* D. Don 'Flash Red') in any combination of mutagens, Bhagwet and Duncan (1998) used from 105 to 225 shoot apices of banana and Bhate (2001) - 250 morning

a) control

b) 1.5 mM EMS



d) 0.5 mM DES

d) 0.5 mM EMS

Фот. 1. *Цветовете на петуния M_3 поколение с фенопип вариации (b-d) и контрола (a)*
Phot. 1. *Flowers of petunia M_3 generation with phenotype variations (b-d) and control (a)*

glory seeds (*Ipomea purpurea*) at each concentration selected for testing mutagens. While, Koh and Davies (2001) used to induce mutations in 1000 seeds *Tillandisia faciculata* for any combination of experiment.

The chemical mutagens applied in the experiment, significantly reduced the germination of seeds of M_3 generation of petunia. The lowest germination energy of seeds were observed after a treatment of 1.0 mM solution of SA (16%), the highest (59%) by using - 1.5 mM EMS. The weakening of germination on very similar level (averaging 12%) was also observed in the M_2 generation of petunia by Krupa-Malkiewicz (2009). However, in studies made by Rzepka-Plevnes et al. (1998, 2004) germination of seeds were 84% in M_1 and 73% M_2 generations of *Petunia* x *hybryda*.

Chemical mutagens used to induced mutation had a stimulating effect on the plant height and number of flowers. Differences between petunia M_3 mutants and control were proved statistically (Tab. 1). The longest shoots (220% control) were obtained in mutants of petunia M_3 generation, which grew from seeds treated with EMS solution at a concentration of 0.5 mM, the shortest (115% of control) - AS solution at a concentration of 1.5 mM. However, the greatest number of flowers were obtained in petunia mutants derived from seeds treated solution of 0.5 mM EMS (520% of control), and the smallest - 1.5 mM AS (210% of control). Similar results were obtained for the petunia by Napoli and Ruehle (1996), Rzepka-Plevnељ et al. (2004) and Krupa-Maikiewicz (2009).

SA, EMS and MMS mutagens have a negative influence on the number of flower buds (Tab. 1). However, plants derived from seeds treated with DES showed a clearly higher amount compared to the control number of flower buds, regardless of the concentration (142-175% controls).

In addition to morphological change caused by the chemical mutagens, discoloration of pigments in flowers, as well as chlorophyll changes on the leaves and a return of crown-shaped petals were also observed (Phot. 1). Their frequency were respectively 53%, 27% and 20% and were lower than in the M_1 (73.6%, 5.4%, 21%) and M_2 generation (50%, 5.5%, 44.5%). Most of these changes were observed in mutant M_3 grown from seeds treated with 0.5 mM solution of EMS (30%) and DES (27% of total phenotypic changes).

Phenotypic changes obtained in petunia may have a dominant character. Plants with similar characteristics were also observed in the M_1 and M_2 generations (Krupa-Maikiewicz, 2009). Similar changes in the colour of flowers

Таблица 1. Височина на растенията (cm), брой цветни пъпки и цветове на растение от петуния M_3 поколение след третиране с различни концентрации от мутагени

Table 1. Plant height (cm), number of flower buds and flowers per plant of petunia M_3 generation after treatments of different concentration of mutagens

		Morphological traits					
mutagen	concentration (mM)	plant height (cm)		number of flower buds		number of flowers	
control	0.0	15.0	ab*	5.5	b	2.0	b
DES	0.5	30.0	a	9.5	a	10.5	a
	1.0	22.5	ab	8.0	ab	6.0	ab
	NIR	14.6		3.2		5.3	
control	0.0	15.0	ab	5.5	a	2.0	b
MMS	1.5	29.0	a	3.5	a	11.0	a
	2.0	24.4	ab	5.4	a	5.5	b
	NIR	14.6		3.2		5.3	
control	0.0	15.0	b	5.5	b	2.0	b
EMS	0.5	32.7	a	4.0	a	10.6	a
	1.5	20.0	ab	6.8	a	8.1	a
	NIR	14.6		3.2		5.3	
control		15.0	a	5.5	a	2.0	ab
AS	1.0	20.0	a	6.5	a	8.0	a
	1.5	17.2	a	3.4	a	4.4	ab
	NIR	14.6		3.2		5.3	

*a, b – difference significant at α = 0.05

were noted after physical mutagenesis in chrysanthemum by Mandal et al. (2000) as well as Latado et al. (2004), after chemical mutagenesis in kalanchoe by Krupa-Malkiewicz (2010 in press). The effect of EMS and gamma radiation on the formation of chlorophyll mutants or chimeras were described in *Tilandsii fasciculata* var. fasciculata *(Bromeliaceae)* by Koh i Davies (2001).

The genetic differences between control and mutants of petunia were determined using the ISSR-PCR technique (Zietkiewicz et al., 1994). Among 15 primers, 7: $(GA)_8A$, $(GA)_8T$, $(AG)_8G$, $(AC)_8C$, $(AC)_8G$, $(GGGTG)_3$, $(GA)_8GT$, amplified the ISSR-PCR products. In total within the ISSR-PCR reaction, 403 ISSR products were amplified. The highest number of polymorphic bands (80, 66, 60) were obtained by using $(GA)_8A$, $(AC)_8G$ and $(GA)_8T$ primers (respectively), while accession-specific products were observed by means of $(GA)_8T$ (4), $(GA)_8A$ (3) and $(AC)_8C$ (3).

CONCLUSIONS

1. The highest frequency of phenotypic changes in the M_3 generation of petunia was obtained after 0.5 mM EMS (30%) and DES (27% of the total phenotypic changes) treatments.
2. The frequency of morphological changes observed after mutagenesis was 53% - in the case of pigment changes in flowers, 27% - of chlorophyll changes on the leaves and 20% of the return of crown-shaped petals.
3. Chemical mutagens used to induce mutation had a stimulating effect on the plant height and number of flowers. SA, EMS and MMS mutagens have a negative influence on the number of flower buds.
4. The analysis of DNA polymorphism carried out by ISSR-PCR technique, showed significant differences within the microsatellite sequence from the M_3 petunia mutants and the control.

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