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INFLUENCE OF CHEMICAL MUTAGENS ON MORPHOLOGICAL TRAITS IN KALANCHOE (*KALANCHOE HYBRIDA*)

WPŁYW MUTAGENÓW CHEMICZNYCH NA CECHY MORFOLOGICZNE U KALANCHOE (*KALANCHOE HYBRIDA*)

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Streszczenie. W pracy określono zmiany fenotypowe i genotypowe u kalanchoe (*Kalanchoe hybryda*), wywołane azydkiem sodu (AS), siarczanem etylowo-metylowym (EMS), siarczanem metylowo-metylowym (MMS) i siarczanem dietylowym (DES), w stężeniach: 0,5; 1,0; 1,5 i 2,0 mM. Do oceny zmian genotypowych na poziomie DNA wykorzystano technikę ISSR-PCR. Otrzymane u kalanchoe zmiany to: rozrośnięte dno kwiatowe, różny kształt i liczba płatków kwiatu, przebarwienia na płatkach kwiatów od żółtego do pomarańczowego. Częstotliwość zmian zależała od zastosowanego mutagenu i jego stężenia w roztworze. Największą częstotliwość zmian otrzymano, stosując do indukowania mutacji DES w stężeniu 2,0 mM.

Key words: chemical mutagens, DNA, ISSR-PCR, kalanchoe, mutation.
Słowa kluczowe: DNA, ISSR-PCR, kalanchoe, mutacje, mutageny chemiczne.

INTRODUCTION

Mutations are a natural process occurring in the genome of living organisms. Induced by various chemical and physical factors, they constitute a source of new genes which is indispensable in plant breeding. The obtained effect depends on the used mutagen, its dose, conditions, plant susceptibility and is measured by the frequency of new phenotypes in the progeny populations (Privalov and Yakovleva 1991; Rybiński 2001; Berenschot et al. 2008).

In vitro culture methods have facilitated the use of mutation techniques for the improvement of both seed and vegetative propagated plants. In many vegetative propagated crops, mutation induction in combination with *in vitro* culture techniques may be the only effective method for plant improvement. The combination of micropropagation and induced mutations can develop and multiply elite mutants in a short period of time in most of the ornamental plants (Małuszyński et al. 1995; Mandal et al. 2000; Rout et. al. 2006; Patade and Suprasanna 2008).

The characteristic of genetic relations between the compared plants was obtained using the ISSR-PCR (inter simple sequence repeat – polymerase chain reaction) method, which is considered by many authors to be both precise and reliable (Kochieva et al. 2002; Rzepka-Plevneš et al. 2004). It combines the simplicity of analyses comparable with that of the RAPD method with the reliability or results from the AFLP method (Pradeep et al. 2002).

The presented studies aimed to determine the effect of chemical mutagens sodium aside, EMS, MMS and DES on phenotype and genetic changes in kalanchoe (*Kalanchoe hybryda*).

MATERIAL AND METHODS

To induce mutation in kalanchoe (*Kalanchoe hybrida*) plant were used sodium aside (AS), ethyl methano-sulfonate (EMS), methyl methano-sulfonate (MMS) and diethyl sulphate (DES) in different concentrations 0.5; 1.0; 1.5 and 2.0 mM, at the presence of buffer orthophosphoric acid at the concentration 0.025 mM.

Mutagenic treatment

Explants sampled from auxiliary buds were plant material. After initiation and rooting the obtained plantlets were treated with AS, EMS MMS and DES at four concentrations (0.5; 1.0; 1.5 and 2.0 mM). Filter-sterilized solutions of AS, EMS, MMS and DES were prepared in deionized water. These solutions were diluted with a sterile 0.025 mM phosphate buffer (pH4) to give working solutions of each mutagen. The concentration of the applied chemical mutagen and the method of mutation initiation was developed on the basis of available literature (Bhagwat and Duncan 1998; Rzepka-Plevneš et al. 1998, 2004). Dissected plantlets were submerged in mutagen solution (0.5 ml/apex) for periods of 60 min. Plantlets submerged in sterile deionized water and 0.025 mM phosphate buffer for 60 min were served as control. All treatments were carried out at 25^{o}C. After treatments, plantlets were rinsed three times with sterile water, removed from agar, planted into pots and taken to the greenhouse. After 12 weeks under greenhouse conditions the first observations of phenotypic changes (color of flowers and their structure) were made and essential biometric measurements taken (plants height, number of flower buds and flowers).

ISSR-PCR amplification and electrophoresis

The genetic differences between control and mutants of kalanchoe were determined using the ISSR-PCR technique (Ziętkiewicz et al. 1994). The total DNA from about 100 mg of fresh leaf material was extracted using the A&A Biotechnology kit (DNA Prep Plus).

DNA was amplified using a Mastercycler (Eppendorf) thermal cycler. The amplified products were analyzed by electrophoresis on a 2% agarose gel, at a constant voltage of 60 V for 1 hour, using 1X TBE buffer at room temperature. The PCR products were visualized with ethidium bromide (0.5 mg · ml^{-1}) on a UV-21 transilluminator (Fotodyne). Gels were photographed (Polaroid DS-34). Each fragment that was amplified using ISSR primers was coded in binary form by ‘0’ or ‘1’ or absence or presence in each species, respectively. To infer genetic relationships, the 0/1 matrix was used to calculate genetic similarity and then UPGMA (Nei and Li 1979) was employed by arithmetic means – dendrogram using software packages Diversity one 1.3 (Pharmacia LKB). Bootsrapping was done using the softwar program TREECON to determine the confidence limits of the UPGMA based dendrogram (van de Peer and de Wachter 1994). Two-thousand bootstrap replications were carried out as suggested by Felsenstein (1985) and the values were obtained in terms of the percentage of the number of times a group would be found in the bootstrap replications.

RESULTS AND DISCUSSION

Chemical and ionizing radiation mutagenesis have been routinely used to generate genetic variability for breeding research and genetic studies, especially in ornamental crops (Mandal et al. 2000; Jain 2006). Mutants induced by gamma radiation are often generated by deletion of large DNA fragments, whereas chemical mutagens induce the formation of *O*-alkyl adducts of nucleotides leading to mispairing that preferentially cause C/G to T/A transitions. As compared with physical mutagens, chemicals may give rise to relatively more gene mutations rather than chromosomal changes (Berenschot et al. 2008).

Among the chemical mutagens used to induce mutation in kalanchoe most of the phenotype changes (sturdy receptacle, different shape and number of flower petals and discoloration of pigments in flowers from yellow to orange) were obtained after 1.5 and 2.0 mM DES treatment (Fig. 1). Similar results obtained in the present work have been described by Rzepka-Plevneš et al. (1998, 2004) and Krupa-Małkiewicz (2009) in petunia, Mandal et al. (2000) and Latado et al. (2004) in chrysanthemum and Koh and Davies (2001) in tillandsia.

a) control (kontrola) b) 2.0 mM DES

c) 1,5 mM EMS d) 1.5 mM DES

Fig. 1. Kalanchoe flowers with phenotype variations
Rys. 1. Kwiaty kalanchoe z widocznymi zmianami fenotypowymi

The higher frequently occuring changes in kalanchoe mutants phenotype were obtained after DES (50%) and AS (25%) treatment, the lowest frequently – EMS and MMS (12%). The results of analysis presented here are in concordance with results obtained by Bhagwat and Duncan (1998). Similar results were presented for a banana genome cv. Highgate (*Musa* spp., AAA Group).

Chemical mutagens used to induced mutation had a stimulating effect on the plant height, number of flower buds and flowers (Fig. 2). Differences in plant height between kalanchoe mutants and control were proved statistically. Mutants were noted to be higher by 20 and 28% than control were observed after treatment of DES and AS in 1.5 and 2.0 mM concentrations, higher by 22% than control – 2.0 mM EMS treatment, and higher by 30% than control – 2.0 mM MMS. The greatest number of flower buds in mutants resulted from the application of 1.5 mM of DES (140% of control), 1.5 mM of EMS (180% of control) and 1.5 mM of MMS (170% of control) for mutagenesis. Mutants treated 1.5 mM concentration of EMS and MMS had more flowers than control (respectively: 175% and 160% of control).

For the estimation of the genetic similarity between kalanchoe mutants and control 30 microsatellite primers were used, differing in length and sequence of the repeated block, as well as a specific sequence at end 3'. Among them 9 (805, 804, 801, 840, 818, 834, 820, 810 and 819) amplified products of the ISSR-PCR reaction, were visualized on agarose gel. In total in the ISSR-PCR reaction 184 ISSR loci were amplified, of which 95 (51,6%) were polymorphic and 6 (3,3%) were accession-specific. In reaction with one primer a mean of 5 ISSR loci were amplified from ~2600 to ~360 bp long. The longest product (~2600 bp) was obtained when using primers 805, the shortest (~360 bp) – primer 834. The highest number of polymorphic loci (19), from ~1000 to ~360 bp long, were obtained with the use of primer 834. The least (3) polymorphic products (~1380 – ~508 bp) visible on agarose gels were obtained when primer 840 was used for the ISSR-PCR.

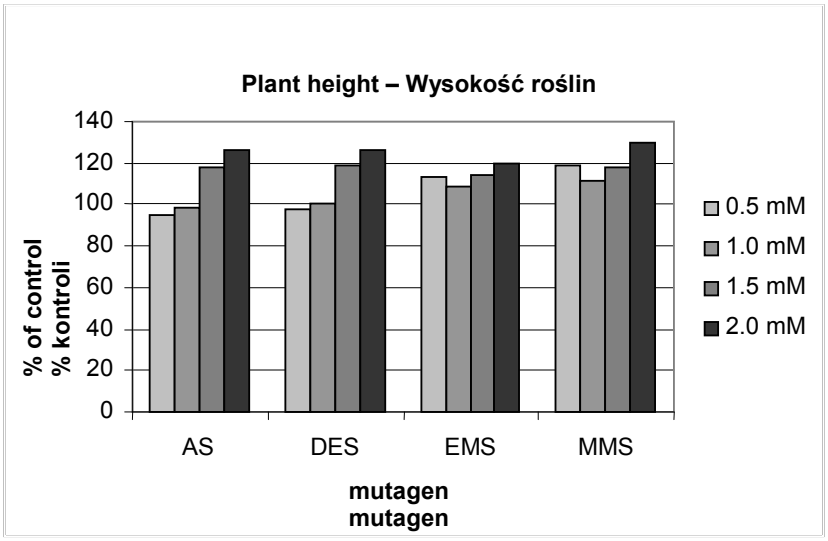
Basing on the ISSR amplification products obtained for kalanchoe mutants and control, a dendrogram of genetic similarity was constructed, using the UPGMA method (Fig. 3). The genetic similarity between mutants and control tested in the present studies amounted to 31,9 – 93,6%. The most similar genetically to control proved to be kalanchoe treated with 2.0 mM concentration of MMS and the least – 2.0 mM DES.

On the basis of the drawn tree of genetic similarity four groups were separated, marked on Figure 3 as a, b, c and d.

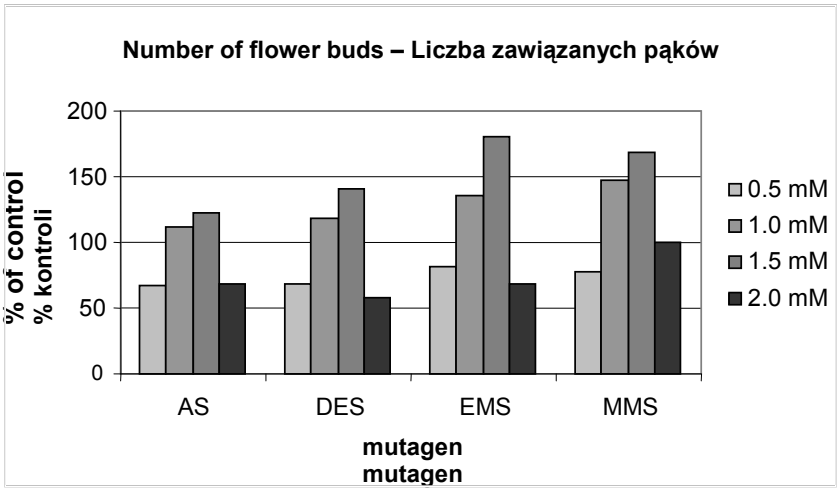
The obtained results are compliant with those of other authors who confirm the value of this method for studies on genetic relations between plants (Nagaoka and Ogihara 1997; Joshi et al. 2000; Fernàndez et al. 2002; Rzepka-Plevneš et al. 2004; Smolik et al. 2006).

CONCLUSIONS

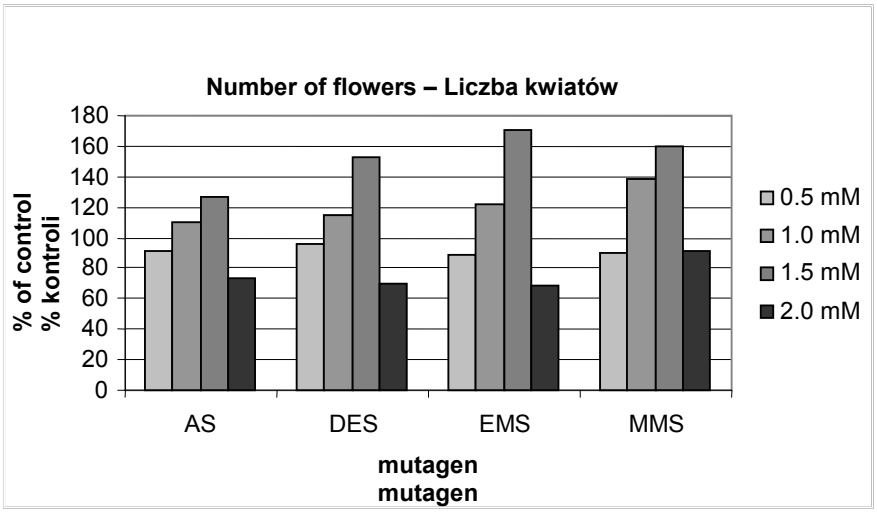
1. Among chemical mutagens (AS, EMS, MMS, DES) used to induced mutation in kalanchoe plantlets the most frequently changes (50%) were obtained after DES treatment in concentration 2.0 mM.



$\bar{x}$ = 29.41 cm = 100%



$\bar{x}$ = 9 = 100%



$\bar{x}$ = 11.26 = 100%

Fig. 2. Plant height, number of flower buds and number of flowers of kalanchoe depending on the mutagen, expressed as percentage of the control

Rys. 2. Wysokość roślin, liczba zawiązanych pąków kwiatowych i kwiatów kalanchoe w zależności od zastosowanego mutagenu chemicznego, wyrażona w procentach kontroli

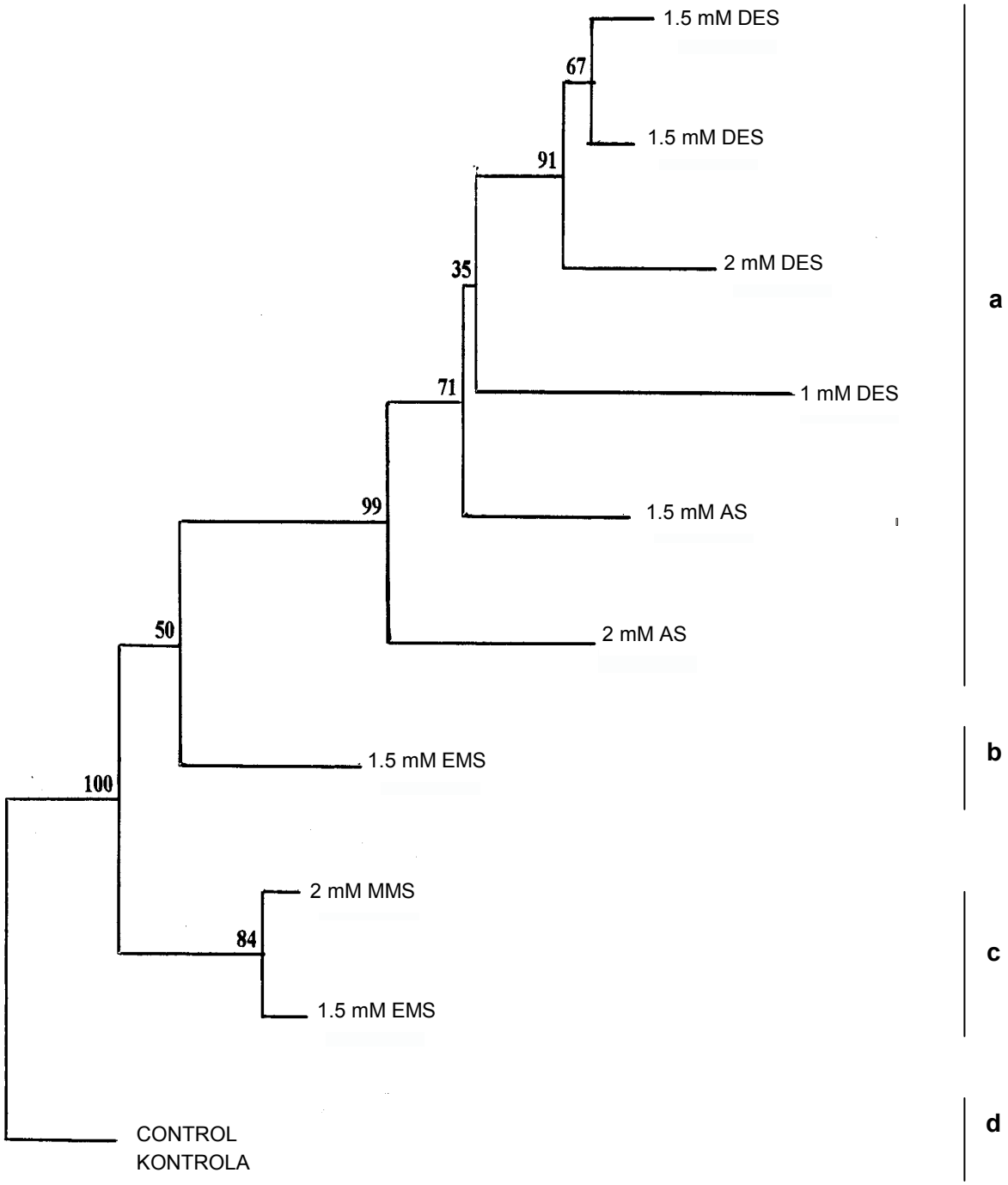


Fig. 3. UPGMA dendrogram representing genetic relationship among mutants and control of kalanchoe. The numbers at the forks indicate 2000 bootstrap replications expressed in percents (van de Peer and de Wachter 1994).

Rys. 3. Drzewo podobieństwa filogenetycznego wykreślone metodą UPGMA dla mutantów i kontroli kalanchoe. Liczby wskazują 2000-krotne „próbkowanie" metodą bootstrap, wyrażone w procentach (van de Peer i de Wachter 1994).

2. Chemical mutagens used to induced mutation had a stimulating effect on the plant height used in concentrations 1.5 and 2.0 mM, number of flower buds and flowers used in concentration 1.5 mM.

3. The results obtained in the present work have revealed a high usefulness of ISSR markers for characterization of variability in kalanchoe mutants. The obtained differences consisted in different number and weight of PCR products in all obtained plant population.

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Abstract. The objective of this study was to induce mutation in kalanchoe (*Kalanchoe hybrida)* using sodium azide (AS), ethyl methano-sulfonate (EMS), methyl methano-sulfonate (MMS) and diethyl sulphate (DES) at different concentrations 0.5; 1.0; 1.5 and 2.0 mM. The genetic variation of kalanchoe was investigated by the ISSR-PCR method.

Morphologic changes, observed in mutants referred principally to: sturdy receptacle, different shape and number of flower petals, discoloration of pigment in flowers from yellow to orange. Frequency of new phenotypes in the progeny populations dependent on the used mutagen and its dose. The most effective were DES in concentration 2.0 mM.