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IN VITRO INDUCTION OF MUTATIONS IN *DIEFFENBACHIA PICTA* CV. TROPICA

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ABSTRACT

In vitro **proliferated shoots of *Dieffenbachia picta* cv. Tropica were subjected to chemical mutagenic treatment with N-nitroso-N-methylurea (NMU) through either immersion of explants in different concentrations (0.0, 0.1, 0.2, 0.3 and 0.4 %) or by supplementation MS medium with different concentrations (0.0, 0.01, 0.02, 0.03, 0.04 and 0.05 %). The obtained results showed that survival percentage, number of shoots/explant and number of leaves/shoot were decreased with increasing the concentration of NMU agitation concentration. In this experiment, the lethal dose causing 50% reduction in explant survival (LD_{50}) ranged between 0.3 - 0.4% concentrations of NMU. Increasing of NMU concentration in MS medium decreased number of shoots/explant and number of leaves/shoot without affecting the survival percentage. Screening of the second mutated generation showed that the highest NMU agitation concentration (0.4%) was efficient in increasing the number of shoots/explant. Supplementation of medium with NMU did not significantly affect the second mutated generation shoot characters except the shoot length which increased significantly by addition of 0.03% NMU to the culture medium. Successfully acclimatized plantlets will be subjected to further screening for selection any mutated plants.**

Keywords: ***Dieffenbachia picta*****,** ***in vitro*****, chemical mutagen, N-nitroso- N- methylurea (NMU).**

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INTRODUCTION

Dieffenbachia is one of the most important ornamental tropical foliage plant genera. It ranks among the top five most popular ornamental foliage plant genera. It is highly prized for its decorative value, ease of growth and tolerance to interior environments (Henny *et al.*, 2000). Due to its naturally occurring dichogamy and limited seed production, development of a new Dieffenbachia cultivar through traditional breeding usually requires 7–10 years (Henny and Chen, 2003). Induced mutations technique is a valuable tool in ornamental plant breeding. Tissue culture makes it more efficient by allowing the handling of large populations and by increasing mutation induction efficiency, possibility of mutant recovery and speediness of cloning selected variants. Mutagenesis offers the possibility of altering only one or few characters of an already first-rate cultivar, while preserving the overall characteristics (Jain and Häggman, 2007). The combination of *in vitro* culture and mutagenesis is relatively inexpensive, simple and efficient (Ahloowalia, 1998).

The mutagenic agent, nitroso methylurea (NMU), is a potential inducer of mutations in chloroplast DNA (cp DNA). Some authors explained the wide spectrum of NMU mutagenic effect by the fact that this agent is involved in not only alkylation but also nitrozation and carbomyelation reactions, i.e. has a complex effect on molecules (Serebryannyi and Randalu, 1977). It is believed that the main contribution to the mutagenic effect is made by the interaction of the products of degradation of the mutagen with biopolymers rather than by reaction of NMU proper with target molecules (Serebryannyi *et al.*, 1998).

In the present investigation the effect of different concentrations and application methods of nitroso methylurea (NMU) on *in vitro* grown shoots of *Dieffenbachia picta* cv. Tropica growth has been studied. The main objective of this study was to induce mutations in this plant by using chemical mutagen (NMU), so that obtained regenerated plantlets were screened *in vitro* and *ex vitro* following mutagenic treatment. To best of our knowledge, this is the first time to study the effect of chemical mutagenesis on *in vitro* induction of mutation in dieffenbachia plant.

MATERIALS AND METHODS

This study was carried out in Plant Tissue Culture Laboratory of Horticulture Department, Faculty of Agriculture, Zagazig University, throughout the period of 2007-2010.

Lateral buds with small pieces (about 1×1 cm) of stem were excised from vigorously growing *Dieffenbachia picta* cv. Tropica plants grown in greenhouse. Buds were surface sterilized by immersion for 30 min. in fungicide solution of Rhizolex (2 g/l), followed by soaking in 0.3 % mercuric chloride solution for 20 min., then transferred to sodium hypochlorite solution at 1.0 % for 30 minutes. Explants were thoroughly rinsed three times with sterile distilled water after each previous step. Explants were inoculated in glass tubes (25 ×150 mm) containing MS (Murashige and Skoog, 1962) basal medium with 30 g/l sucrose combined with 16 2ip + 2 IAA + 1 DPU mg/l. After 8 weeks the obtained shoots (about 2.0 cm length) were subcultured on the same medium for further 12 weeks. Lateral shoots with about 3 cm length without leaves were immersed in different concentrations (0.0, 0.1, 0.2, 0.3 or 0.4 %) of filter-sterilized N-nitroso-N-methylurea which was diluted in 1% dimethyl sulfoxide (DMSO). Explants were removed after 90 min and thoroughly rinsed three times with sterile distilled water before transferring to the above mentioned medium. In another experiment, explants with the same characters were transferred to the above mentioned medium supplemented with different concentrations (0.0, 0.01, 0.02, 0.03, 0.04 or 0.05 %) of filter-sterilized N-nitroso-N-methylurea which diluted in 1% DMSO. Each treatment was consisted of 10 jars and each one contained about 50 ml medium. Three explants were cultured in each jar. Cultures of both experiments were incubated at 25 ±2 °C under 16 h photoperiod for 12 weeks before the following data were recorded; survival percentage, number of shoots/ explant and number of leaves/ shoot.

New proliferated shoots on each explant (first mutated generation, M_1) were excised and recultured on the above mentioned medium for further 12 weeks in order to obtain the second mutated generation (M_2). The following data were recorded; number of shoots/explant, shoot length (cm) and number of leaves/shoot. Rooted shoots were acclimatized

by transferring them to plastic pots (9 x 7 cm) containing peat moss and sand (1:1, v/v). The cultured pots were covered with polyethylene bags for four weeks before removing them. The plantlets were hold in greenhouse at about 26 ±2°C. The statistical layout of all experiments was simple completely randomized design. The recoded data were statistically analyzed, and the means were compared using Duncan multiple range test according to Little and Hills (1978).

RESULTS AND DISCUSSION

Effect of Immersion in Different Concentrations of N-Nitroso-N-Methylurea on Growth of Dieffenbachia Explants After 12 Weeks

From data presented in Table 1 it was observed that survival percentage was gradually decreased when NMU concentration was increased to 0.2% or more. This inhibition reached its maximum effect with the highest concentration (0.4%). There was no difference in survival percentage between control and low concentration (0.1%), since both of them attained 100% survival percentage. In this experiment, the lethal dose causing 50% reduction in explant survival (LD_{50}) was ranged between 0.3 - 0.4% concentrations of NMU. Similar trend was recorded concerning the number of shoots/ explant. The depressive effect of NMU on shoot proliferation was recorded when the concentration was increased to 0.2% or more.

Number of leaves/shoot was gradually decreased with increasing NMU concentration. There was no significant difference between 0.1 and 0.2% or between 0.3 and 0.4% concentrations, however there was a significant difference between both groups. These results are in harmony with those obtained by treating chrysanthemum explants (Latado *et al.*, 2004) and banana explants (Bhagwat and Duncan, 1998) with ethyl methane sulphonate.

Effect of Addition of Different Concentrations of N-Nitroso-N-Methylurea to the Medium on Growth of Dieffenbachia Explants After 12 Weeks

Survival percentage of explants was not affected by addition of NMU to the medium, since all treatments gave 100% survival percentage (Table 2). The inclusion of NMU in the culture

medium showed decreasing in the number of proliferated shoots on explant. It is also cleared that increasing the MNU concentration resulted in decreasing the number of shoots/explant without significant difference between either low concentrations (0.1 and 0.2%) or among higher concentrations (0.3, 0.4 and 0.5%). It was obvious that addition of NMU at any tested concentration had an adverse effect on leaf formation without significant differences between different NMU concentrations in most cases. This inhibitory effect of NMU on explant growth has been previously described with some chemical mutagens such as NMU (Singh *et al.*, 2002), ethyl methane sulphonate (Bhagwat and Duncan, 1998; Singh *et al.*, 2000 ; Latado *et al.*, 2004), diethyl sulphate and sodium azide (Bhagwat and Duncan, 1998). This detrimental effect of high dose of NMU may be due to that the increase in the dose of chemical mutagens caused damaging effects on biological activities of plants which may be due to inactivation of cells because of mitotic disturbances/ chromosomal aberrations at higher doses of chemical mutagens, leading to poor growth of the plants (Zargar *et al.*, 1994).

Effect of Immersion in Different Concentrations of N-Nitroso-N-Methylurea on Growth of Second Mutated Generation (M_2) of Dieffenbachia Explants

Data in Table 3 illustrate the response of second mutation generation shoot growth to immersion in different concentrations of NMU during first mutated generation production. Concerning number of shoots per explant, it was found that there was no significant difference between the control and the different NMU treatments except for the highest concentration (0.4%) which produced the highest significant shoot number (5.55 shoots/explant). This may be due to the increased activity of enzymes involved in biosynthesis of hormones like auxins, cytokinins, etc. in cell at lower doses of mutagen (Vagera *et al.*, 1976), which increases the growth of cell and ultimately whole plant resulting in the increase in number of shoots. On the other hand, using of NMU at any concentration resulted in significant decrease in shoot length. In most cases, there was no significant difference in shoot length among different concentrations of NMU. Number of leaves was not affected by treating with NMU at any concentration.

Table 1. Effect of immersion in different concentrations of nitroso methylurea on growth of dieffenbachia explants after 12 weeks

Concentrations (%)	Survival %	Number of shoots /explant	Number of leaves/ shoot
0.0	100.00	3.07 a	3.58 a
0.1	100.00	2.94 a	2.36 b
0.2	96.29	1.95 b	2.40 b
0.3	78.26	1.88 b	1.72 c
0.4	36.66	1.75 b	1.58 c

* Means having the same letter(s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability

Table 2. Effect of addition of different concentrations of nitroso methylurea to the medium on growth of dieffenbachia explants after 12 weeks

Concentrations (%)	Survival %	Number of shoots /explant	Number of leaves/ shoot
0.00	100	3.07 a	3.58 a
0.01	100	2.56 b	2.40 bc
0.02	100	2.50 b	2.35 bc
0.03	100	1.70 c	2.66 b
0.04	100	1.72 c	2.53 b
0.05	100	1.73 c	2.25 c

* Means having the same letter(s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability

Table 3. Effect of immersion in different concentrations of nitroso methylurea on growth of the second mutated generation of dieffenbachia explants after 12 weeks

Concentrations (%)	Number of shoots /explant	Shoot length (cm)	Number of leaves/ shoot
0.0	3.18 b	2.29 a	2.93 a
0.1	4.14 b	1.27 c	2.32 a
0.2	3.30 b	1.29 bc	2.37 a
0.3	3.19 b	1.17 c	2.27 a
0.4	5.55 a	1.54 b	2.85 a

* Means having the same letter(s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability

Table 4. Effect of addition of different concentrations of nitroso methylurea to the medium on growth of the second mutated generation of dieffenbachia explants after 12 weeks

Concentrations (%)	Number of shoots /explant	Shoot length (cm)	Number of leaves/ shoot
0.00	3.18 a	2.29 d	2.93 a
0.01	2.91 a	4.52 c	3.21 a
0.02	2.78 a	4.78 bc	3.16 a
0.03	2.61 a	6.92 a	3.59 a
0.04	2.70 a	5.10 b	2.90 a
0.05	2.68 a	5.15 b	3.35 a

* Means having the same letter(s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability

Effect of Addition of Different Concentrations of N-nitroso-N-methylurea to the Medium on Growth of Second Mutated Generation (M_2) of Dieffenbachia Explants After 12 Weeks

Data in Table 4 show that addition of NMU to the medium at different concentrations during the first mutated generation production had no significant effect on number of shoots/explant and number of leaves/shoot in the second mutated generation. On the other side, addition of NMU had a significant positive effect on shoot length. Shoot length was gradually increased with increasing NMU concentration up to 0.03%, while this increase was declined by increasing the concentration beyond that; i.e, 0.04 and 0.05%. It is also clear that, both low and moderate concentrations of NMU had a stimulatory effect on shoot growth. A similar result was reported by Singh *et al.* (2000) who reported that low dose of ethyl methane sulphonate showed stimulatory effect on *in vitro* growth of carnation shoot.

REFERENCES

Ahloowalia, B. S. (1998). *In-vitro* techniques and mutagenesis for the improvement of vegetatively propagated plants. In: S.M. Jain, D.S. Brar and B.S. Ahloowalia (Eds) Somaclonal Variation and Induced Mutations in Crop Improvement Kluwer Acad. Pub., Dordrecht, 293–309.

Bhagwat, B. and E.J. Duncan (1998). Mutation breeding of banana cv. Highgate *Musa* spp., AAA Group/ for tolerance to *Fusarium oxysporum* f. sp. cubense using chemical mutagens. Scientia Horticulturae, 73 : 11–22.

Henny, R.J. and J. Chen (2003). Cultivar development of ornamental foliage plants. Plant Breed Rev., 23:245–289.

Henny, R.J., L. Goode and W. Ellis (2000). Plant Tissue Culture Concepts and Laboratory Exercises. CRC Press LLC, USA.

Jain, S.M. and H. Häggman (2007). Protocols for Micropropagation of Woody Trees and Fruits, Springer, USA. 323–333.

Latado, R.R., A.H. Adames and A.T. Neto (2004). *In vitro* mutation of chrysanthemum (*Dendranthema grandiflora* Tzvelev) with ethylmethanesulphonate (EMS) in immature floral pedicels. Plant Cell, Tissue and Organ Culture, 77: 103–106.

Little, T.M. and F.J. Hills (1978). Agriculture Experimentation Design and Analysis. John, Wiley and Sons, Inc.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant., 15: 473- 497.

Serebryannyi, A.M. and K.K.A. Randalu (1977). Carbomoylation of bases in DNA by Nitrosomethylurea. Bioorg. Khim., 3 (5): 633–638.

Serebryannyi, A.M., L.E. Salnikova and L.M. Bakhitova (1998). Specificity of the mutagenic effect of Nitrosomethylurea and association of its mutagenicity and carbomoylating action. Genetika (Moscow) 24 (10): 1786–1793.

Singh, K.P., B. Singh, S.P.S. Raghava and C.S. Kaua (2000). Induced flower colour mutations in carnation through *in vitro* application of chemical mutagen. Indian J. Genet., 60 (4): 535-539.

Singh, K.P., M.L. Choudhary and K.P. Ananda (2002). Characterization of *in-vitro* induced mutants of carnation by means of electrophoretic protein analysis. Indian Journal of Horticulture, 59 (4): 427-430.

Vagera, P., F.J. Novak and B. Vysko (1976). Anther cultures of *Nicotiana tabacum* L. mutants. Theor. Appl. Genet., 47: 109-114.

Zargar, G.H., N.A. Zeerak, H.J. Ahanger and A. Wani Shafiq (1994). Mutagenic effect of ethyl methane sulphonate and their combinations in top onion (*Allium cepa* var. *viviparum*). J. Nuclear Agric. Biol., 23: 249-250.

استحداث طفرات في نبات الدفينباخيا من خلال مزارع الأنسجة

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عوملت أفرخ نبات الدفينباخيا بكتا صنف تروبيكا الناتجة من مزارع الأنسجة بالمطفر الكيماوي نتروزو ميثيل يوريا من خلال نقع هذه المنفصلات النباتية في تركيزات مختلفة (صفر ، ١,. ، ٢,. ، ٣,. ، ٤,.٪) من هذا المطفر أو بإضافته إلى بيئة الزراعة بتركيزات مختلفة (صفر ، ٠١,. ، ٠٢,. ، ٠٣,. ، ٠٤,. ، ٠٥,.٪). أظهرت النتائج المتحصل عليها أن النسبة المئوية للبقاء ، و عدد الأفرخ/منفصل نباتي ، وعدد الأوراق/فرخ قد انخفضت بزيادة تركيز المطفر الذي تم النقع فيه ، كما ثبت أن الجرعة التي تسببت فى موت ٥٠٪ من المنفصلات النباتية (LD_{50}) فى هذه التجربة تراوحت ما بين تركيزي ٣,. – ٤,.٪ من المطفر. ثبت أيضا أن زيادة تركيز المطفر في البيئة قد أدي لانخفاض عدد الأفرخ/منفصل نباتي و عدد الأوراق/فرخ دونما تأثير على النسبة المئوية لبقاء المنفصلات النباتية حية بعد المعاملة. باستعراض نتائج الجيل الطفري الثاني تبين أن النقع في المطفر بتركيز ٤,.٪ كان له فاعلية على زيادة عدد الأفرخ/منفصل نباتي. بينما وجد أن إمداد البيئة بالمطفر بتركيزاته المختلفة لم يؤثر معنويا على مواصفات أفرخ الجيل الطفري الثاني إلا فيما يتعلق بطول الفرخ ، حيث زاد معنويا عند إضافة المطفر للبيئة بتركيز ٠٣,.٪، ويجدر الإشارة إلى أن النباتات التي ستنتج في الأقلمة سيتم متابعتها لاكتشاف أي طفرات قد تظهر بها.