www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 3/3 (2010) 1-5

Somaclonal variations through indirect organogenesis in eggplant (*Solanum melongena* L.)

Ely ZAYOVA [1], Roumiana VASSILEVSKA – IVANOVA [*1], Boris KRAPTCHEV [1], Daniela STOEVA [1]

[1] Institute of Genetics "Prof. D. Kostov", Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Abstract

Somaclonal variations in eggplant (*Solanum melongena* L.) were observed among plants regenerated from cotyledon-derived callus. Variations included certain morphologic deviations, frequency of *in vitro* propagation and rooting. They were detected in 9 to 12 passage culture and occurred irrespective of the type or concentration of plant growth regulator used for callus induction or plant regeneration. Based on morphological similarities, regenerated plants were classified into five phenotypes designated as LNR1 - LNR5. The plants were propagated by node and shoot tip explants on Murashige and Skoog medium supplemented with 1.0 mg/L^{-1} BAP and rooted on the same medium but without growth hormones. All cloned lines exhibited lower frequency of micropropagation and root formation than the control plants, except the dwarfed plants, which do not multiplies on the used medium for *in vitro* propagation. Future research will determine the importance of new somaclonal lines for genetic variability of eggplant.

Abbreviations: BAP = 6-benzylaminopurine; 2, 4-D = 2, 4-dichlorophenoxyacetic acid; NAA = α-naphthalene acetic acid; TDZ = thidiazuron; PGR=plant growth regulator.

Key Words: Eggplant, Solanum melongena L., Indirect Organogenesis, Micro-Propagation, Somaclonal Variation

1. Introduction

Somaclonal variation, defined as the genetic variation induced by *in vitro* techniques (Larkin and Scowcroft, 1981) has been demonstrated among tissue culture regenerants of many species including eggplant (*Solanum melongena* L.), a vegetable of high economic value. The phenomenon offers an opportunity to uncover the natural variability in plants and to use this genetic variability for new product development and crop improvement (Collonier et al., 2001; Kashyap et al., 2003). Many factors influence the frequency of somaclonal variation, such as plant species, the genotype and the type of explants involved, the culture protocol applied during the *in vitro* process, particularly the hormone composition of the medium as well as the number of subcultures (Cai et al., 1990; Ducos et al., 2003; Magioli and Mansur, 2005).

The variability observed in regenerated plants might be triggered of the growth regulators during indirect organogenesis. It has been shown that the somaclonal variations in eggplant are caused by the hormonal concentrations in tissue culture medium (Rotino et al., 1991). Although the effect of specific growth regulators on variation remains unclear, most scientist agree that variation rates are increased as the overall concentrations of growth regulators rise. High growth regulator concentrations also can alter the frequency of ploidy changes vs. point mutations (Perschke et al., 1991). For example, Hitomi and Amagaki (1998) studied the impact of different growth regulators such as NAA and 2, 4-D on somaclonal variations in eggplant. The study reported high frequency of morphological variants in plants regenerated by somatic embryogenesis. The effect of somaclonal variations on agronomic traits of embryogenic and androgenic colchicine - treated double haploid lines was also investigated (Rotino et al., 1991). However, practical application of somatic embryogenesis and plant regeneration for isolation of somaclonal variation has lagged due to the non-availability of mass scaling techniques and effective field delivery systems (Kantharajan and Golegaonkar, 2004).

In our laboratory we have developed an *in vitro* system of producing callus culture and regenerated plants therefrom, through indirect organogenesis with potential for inclusion in plant breeding programs in the eggplant (*S.*

* *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +359 2 9746229; E-mail: ru_vas_bg@yahoo.com

melongena). The aim of the present study was to evaluate the morphological variability that occurred among *S. melongena* plants regenerated through this protocol and to characterize somaclonal variation that could be potentially selectable *in vitro*.

2. Materials and methods

2.1. *Plant material*

Mature seeds of commercial eggplant cultivar Larga Negra (LN) were obtained from Institute of Plant and Genetic Resources, Agricultural Academy, Sadovo, Bulgaria. Plants were regenerated from cotyledon derived calluses. The protocol for callus induction and subsequent plant regeneration of *S. melongena* was previously described (Zayova et al., 2008).

2.2. *Morphological analysis*

For root induction**,** plants were transferred on Murashige and Skoog (1962) (MS) medium without growth regulators. Both, parent plants and somaclonal lines were evaluated by nine morphological traits: length of plants (cm), length of leaves (cm), petiole length (cm), internode length (cm), length of roots (cm), leaf width (cm), number of leaves, number of nodes, and number of roots per plant. Morphological traits were measured in laminar-box after 4 weeks of culture, and each measure was repeated tree times at regular intervals.

2.3. *Micropropagation and rooting of regenerated plants from callus*

Nodal and shoot tip explants were excised from *in vitro* regenerated plants. The explants were cultured individually on MS medium containing BAP and TDZ with 3 % sucrose (w/v) and 0.7 % (w/v) agar. The multiple shoots were rooted on MS medium without PGRs. The pH of the medium was adjusted to 5.8 before sterilization by autoclaving at 121°C for 20 min. The cultures were incubated at 22 ± 2°C with a 16-h photoperiod and light intensity of 40 µM $m^{-2}s^{-1}$ provided by cool-white fluorescent light. Each experiment was repeated twice; for each treatment 10 replicates were used. All the data were analyzed using routine statistical analysis (Lidansky, 1995).

3. Results

Plants of eggplant (*S. melongena*) were regenerated trough indirect organogenesis following the protocol described previously (Zayova et al., 2008). During 2 to 8 passages, the regenerants exhibited the same morphological phenotype as control (cultivar LN); however, significant variation in morphological traits became apparent after 9 to 12 passages, and also in frequency of *in vitro* propagation and rooting. Based on these variations, the regenerated plants from the last passages were divided into five deviant morphological phenotypes:

LNR1 – refers to plants with green stem, smooth and narrow leaves with thin petioles (Fig. 1B); LNR2 – corresponds to plants with dark-red stem and smooth, pale green leaves (Fig. 1C); LNR3 – corresponds to plants with an abnormal leaves, i. e., they are larger, curly, and light-green when compared with parent plants (Fig. 1D); LNR4 – corresponds to plants with thick stem, short leaf petioles and larger, curly leaves accumulated in the top of the plant stem and forming an area resembling the inflorescence (Fig. 1E); LNR5 – refers to variation in plant height. Plants have normal morphological appearance but remain smaller, i. e., they could be classified as dwarf with significantly reduced plant stature compared with control plants (1.5 vs. 6.7 cm) (Fig. 1F).

Table 1 shows the mean values of studied morphological traits of all five variant phenotypes. Generally, these characteristics had lower means than the parent cultivar. The plant height, leaf petiole and internodes length, number of leaf nodes, root length and number of roots/plant were highly reduced (above 50 %). Only two exceptions that concern leaf characteristics (width and number of leaf) were recorded in LNR4 phenotype (Table 1).

The reduction of the average mean values could be due to the effect of NAA in the induction medium in spite of its low concentration. The changes in plant height and leaf size among micro-propagated plants have been reported to be the effect of somaclonal variation in some plant species (Hitomi and Amagaki, 1998; Ravindra et al., 2004). In this sense, many factors leading to the reduction of morphological parameters could be considered. One of them seems to be the regeneration procedure applied. For instance, it was found that frequencies of somaclonal variations in eggplant in the NAA experiments were higher than those in the 2, 4-D experiment (Hitomi and Amagaki, 1998); however, it causes more negative changes in regenerants obtained trough indirect organogenesis including reduction of morphological parameters. In our case, the results also confirmed that the more effective plant regeneration occurred by the presence of NAA in culture medium; than, similar negative influences were noticed. An additional explanation for the reduced morphological parameters of plants could be prolonged maintenance of the morphogenic callus (long-term culture cycles) at regeneration medium. Finally, mutations in the genes could be another possible reason (De Klerk, 1990).

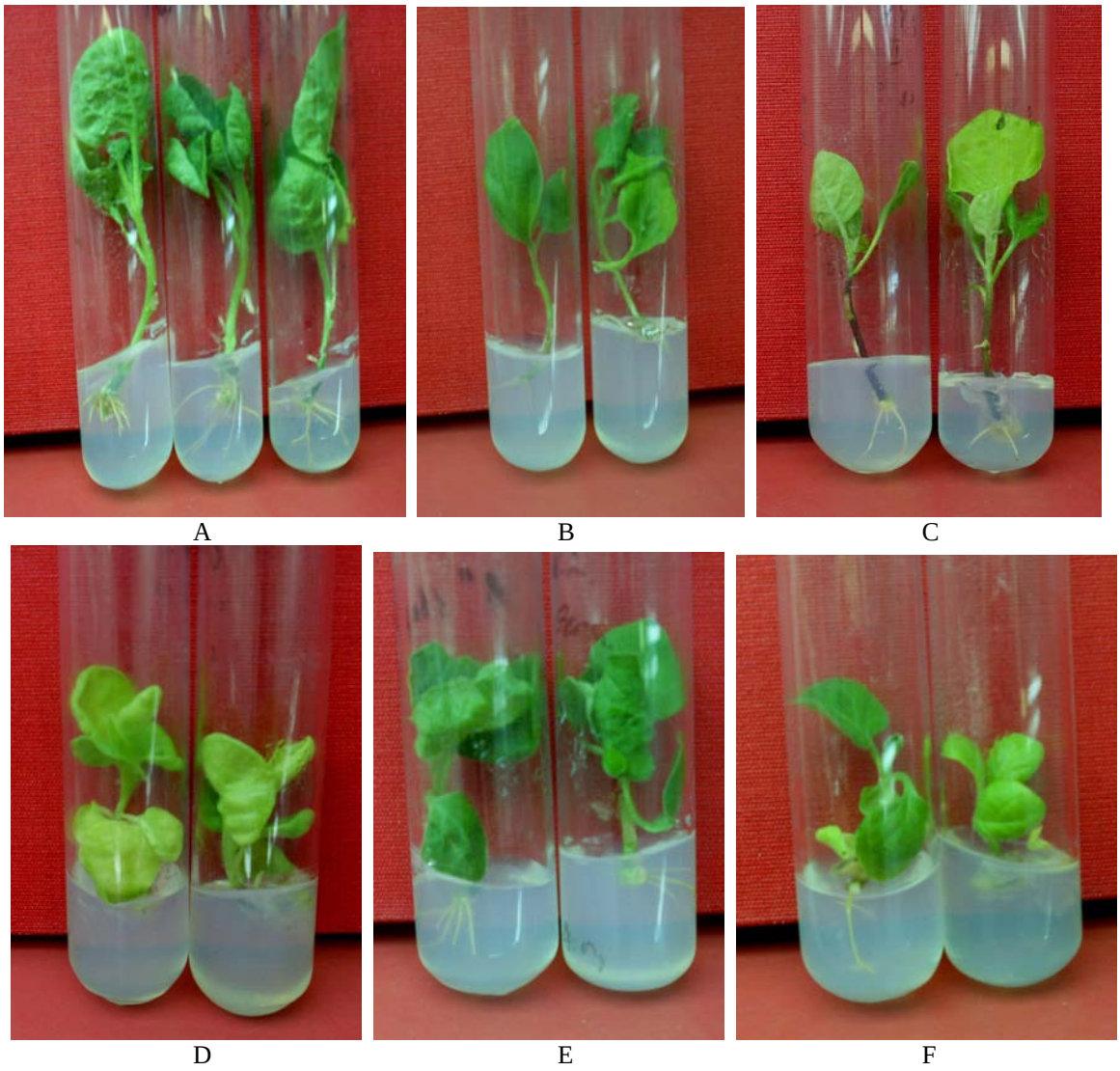

Figure 1. Morphological variations in plants regenerated from callus cultures of eggplant (*Solanum melongena* L.): A - Control plants - cultivar Larga Negra – (LN) ; Phenotypes: B - LNR1, C – LNR2, D – LNR3, E – LNR4 and F – LNR5

Table 1. Comparison of morphological characters between regenerants and control plants

Character	cv LN (control)	Phenotypes				
		LNR1	LNR2	LNR3	LNR4	LNR5
Plant height, cm	6.7 ± 0.46	3.7 ± 1.12	3.8 ± 1.53	2.8 ± 0.73	3.4 ± 1.08	1.5 ± 0.67
Leaf length, cm	3.5 ± 0.51	1.4 ± 0.21	1.8 ± 0.49	2.3 ± 0.28	2.2 ± 0.28	1.0 ± 0.36
Leaf width, cm	2.7 ± 0.57	1.3 ± 0.47	1.8 ± 0.42	2.4 ± 0.31	3.0 ± 1.24	1.2 ± 0.41
No of leaves	7.0 ± 2.42	3.0 ± 1.28	3.0 ± 0.75	3.5 ± 1.16	8.0 ± 2.06	2.4 ± 0.37
Petiole length, cm	2.2 ± 0.45	1.2 ± 0.19	1.6 ± 0.34	0.8 ± 0.42	1.0 ± 0.37	0.8 ± 0.16
Internode length, cm	0.9 ± 0.34	0.4 ± 0.05	0.5 ± 0.15	0.4 ± 0.14	0.2 ± 0.11	0.1 ± 0.04
No of leaf nodes	7.0 ± 0.63	2.7 ± 0.83	3.3 ± 0.75	2.5 ± 0.78	2.0 ± 0.56	1.3 ± 0.38
Root length, cm	3.5 ± 0.52	1.4 ± 0.54	1.7 ± 0.21	1.2 ± 0.39	1.1 ± 0.38	1.0 ± 0.26
No of roots/plant	5.6 ± 1.23	1.0 ± 0.41	1.4 ± 0.55	0.9 ± 0.18	2.6 ± 0.74	1.1 ± 0.29

Significant differences at P≤0.05; cv LN - cultivar Larga Negra

To obtain multiple shoots, nodal and shoot tip explants from *in vitro* regenerated plants were inoculated on MS supplemented with BAP and TDZ (Table 2).

Table 2. Effect of BAP and TDZ on in vitro propagation of regenerants from Larga Negra eggplant cultivar

Growth regulator, mg/ L	Nodal explants		
	Shoot induction (%)	No shoots/culture x ± SE	Length of shoots, cm x ± SE
MS hormone free	10	1.0 ± 0.98	1.3 ± 1.12
0.5 BAP	70	1.5 ± 1.21	2.5 ± 1.56
1.0 BAP	90	3.6 ± 1.87	3.2 ± 1.77
0.5 TDZ	40	1.3 ± 1.12	1.9 ± 1.36
1.0 TDZ	55	2.5 ± 1.56	2.1 ± 1.43
	Shoot tip explants		
MS hormone free	non	non	non
0.5 BAP	30	1.2 ± 1.08	1.9 ± 1.36
1.0 BAP	55	2.4 ± 1.53	2.1 ± 1.43
0.5 TDZ	20	1.0 ± 0.98	1.5 ± 1.21
1.0 TDZ	35	1.7 ± 1.29	2.0 ± 1.40

After four weeks, the proliferation efficiency of nodal explants was significantly higher than that of shoot tip. Maximum proliferation in 90 % of cultured explants resulted in BAP in concentration of 1.0 mg L^{-1}, and the nodal explants produced the highest number of shoots/culture (Table 2). On the same medium, shoot tip explants produced shoots in 55 % of the cultures. When the nodal explants were cultured on MS medium with TDZ (1.0 mg L^{-1}) only 55 % of them responded to proliferation. The results presented here showed that shoot proliferation of the explants was affected by the composition of the culture medium (Table 2), since the medium supplemented with BAP was more effective than TDZ. Effect of BAP singly, or in combination with kinetin on multiple shoot regeneration from cotyledon-leaf explants was documented (Sarker et al., 2006; Taha and Tijan, 2002).

Table 3 presents the micro-propagation frequency of tested lines on medium supplemented with 1.0 mg L^{-1} BAP, and root formation on hormone free medium. The highest rate of multiplication (90 %) and the highest number of shoots/culture were found in parent plants (Table 3). Also, it was established that the number of roots/ plant in LNR4 was higher than that of cultivar LN. The rest lines revealed lower frequency of micropropagation and root formation than the parent plants, except of regenerants having dwarfed phenotype (LNR5); they do not multiplies on the used medium. These results illustrated that for *S. melongena*, 1.0 mg/l BAP is effective PGR for shoot proliferation from nodal explants. The best one for root development occurred at MS medium without PGRs.

Table 3. Frequency of micropropagation through nodal explants (MS + 1.0 mg/L BAP) and rooting of the eggplant regenerants (MS hormone free)

Clone	Shoot induction, (%)	Number of shoot/culture x ± SE	Rooted plants, (%)	Number of roots/plant x ± SE
Control*	90	3.5 ± 1.85	100	2.6 ± 1.59
LNR1	65	1.8 ± 1.32	75	2.2 ± 1.46
LNR2	60	1.4 ± 1.17	70	1.5 ± 1.21
LNR3	55	1.0 ± 0.98	60	1.8 ± 1.32
LNR4	70	2.4 ± 1.53	80	3.7 ± 1.90
LNR5	non	non	non	non

*Control-cv Larga Negra

4. Conclusions

Our work shows that somaclonal variation can be found in *S. melongena* plants regenerated trough indirect organogenesis. Several morphological variations resulting from somaclonal variation were assessed by analysis of phenotype. Our work emphasizes the fact that phenotypic evaluation should not be neglected as a tool to assess the genetic variability of the plant regeneration process. In the absence of reliable genetic markers of somaclonal variation and considering the long time for confirmation the inheritability of abnormal phenotypic traits, phenotype still represents the easiest and fastest way to identify putative mutants. The overall high variation frequency of variant plants observed shows that, at least for eggplant, somaclonal variation is a frequent phenomenon and should constitute a tool for producing new valuable eggplant genotypes. Thus, the natural variability associated with tissue culture represents a pool upon which selection pressure can be imposed to isolate unique forms of a clone. Also, our results indicate that the regenerated plants from five phenotypes were morphologically different to the parent plants-cultivar Larga Negra. It may conclude that the latter one is mutable, but the stable characteristics could be obtained by formation of adventitious, callus-derived shoots, only. In summary, several somaclones exhibiting useful variation would be evaluated to be proposed as initial plant material for breeding programs. Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species (Karim et al., 2009). Further investigations are needed to elucidate the nature of morphological variants described here and to characterize its genetic nature.

References

Cai, T., Ejeta, G., Axtell, J.D., Butller, L.G. 1990. Somaclonal variation in high tannin sorghums. Theor. Appl. Genet. 79/6. 737 – 747.

Collonier, C., Mulya, K., Fock, I., Mariska, I., Servaes, A., Vedel, F., Siljak-Yakovlev, S., Souvannavong, V., Ducreux, G., Sihachakr, D. 2001. Source of resistance against Ralstonia solanaceraum in fertile somatic hybrids of eggplant (*Solanum melongen*a L.) with Solanum aethiopicum L. Plant Sci. 160. 301–313.

De Klerk, G.J. 1990. How to measure somaclonal variation. Acta Bot. Neerl. 99. 129-144.

Ducos, J.P., Alenton, R., Reano, J.F., Kancnanomai, C., Deshayesl, A., Petiard, V. 2003. Agronomic performance of *Coffea canephora* P. trees derived from large-scale somatic embryo production in liquid medium. Euphytica. 131. 215-223.

Hitomi, A., Amagaki, H. 1998. The influence of auxin type on array of somaclonal variants generated from somatic embryogenesis of eggplant, *Solanum melongena* L. Plant Breeding. 117. 379-383.

Kantharajan, A.S., Golegaonkar, P.G. 2004. Somatic embryogenesis in eggplant. Sci. Horticult. 99/2. 107-117.

Karim, K., Rawda, A., Hatem,C-M. 2009. Genetic diversity in barley genetic diversity in local Tunisian barley based on RAPD and SSR analysis. Biological Diversity and Conservation (BioDiCon). Volume 2/1. 27-35.

Kashyap, V., Kumar, S., Collonier, C., Fusari, F., Haircour, R., Rotino, G.L., Sihachakr, D., Rajam, M.V. 2003. Biotechnology in eggplant Sci. Horticult. 97/1. 1–25.

Larkin, P.J., Scowcroft, W. R. 1981. Somaclonal variation-a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60. 197-214.

Lidansky, T. 1995. Statisticheski metodi v biologiyata i selskoto stopanstvo. Sofia, Zemizdat, (in Bulgarian).

Magioli, C., Mansur, E. 2005. Eggplant (*Solanum melongena* L.): tissue culture, genetic transformation and use as an alternative model plant. Acta Bot. Bras. 19/1. 139-148.

Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15. 473–497.

Perschke, V.M., Phillips, R.L. Gengenbach, B.G. 1991. Genetic and molecular analysis of tissue culture derived Ac elements. Theor. Appl. Genet. 82. 121-129.

Ravindra, N. S., Kulkarni, R. N. Gayathri, M.C., Ramesh, S. 2004. Somaclonal variation for some morphological traits, herb yield, essential oil content and essential oil composition in an Indian cultivar of rose-scented geranium. Plant Breed. 123. 84-86.

Rotino, G.L., Schiavi, M., Vicini, E., Falavigna, A. 1991. Variation among androgenetic and embryogenetic lines of eggplant (*Solanum melongena L.*). J. Genet. Breed. 45. 141-146.

Sarker, R.H., Yesmin, S., Hoque, M.I. 2006. Multiple shoot formation in eggplant (*Solanum melongena* L.). Plant Tissue Cult. & Biotech. 16/1. 53-61.

Taha, R.M., Tijan, M. 2002. An *in vitro* production and field transfer protocol for *Solanum melongena* plants. South African J. Bot. 68. 447-450.

Zayova, E., Nikova, V., Ilieva, K., Philipov, Ph. 2008. Callusogenesis of eggplant (*Solanum melongena* L.). Compt. Rend. ABS. 61/11. 1483-1488.

(Received for publication 26 May 2010; The date of publication 01 December 2010)