INDIRECT SHOOT ORGANOGENESIS OF EGGPLANT (*SOLANUM MELONGENA* L.)

ИНДИРЕКТЕН ОРГАНОГЕНЕЗИС ПРИ ПАТЛАДЖАНА (*SOLANUM MELONGENA* L.)

Ely ZAYOVA, Roumiana VASSILEVSKA-IVANOVA*, Boris KRAPTCHEV and Daniela STOEVA

Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria, ru_vas_bg@yahoo.com, Tel: +359 2 9746229; Fax: +359 2 987551 *correspondence

ABSTRACT

A protocol for indirect shoot organogenesis of *Solanum melongena* 'Larga Negra' and 'Black Beauty' was established using hypocotyl and cotyledon derived calluses. The maximum morphogenic callus induction was observed from cultured cotyledons of 30-days old seedlings on Murashige and Skoog (MS) medium containing 2.0 mg/l α-naphthalene acetic acid and 0.5 mg/l 6-benzylaminopurine. The highest percentage of shoot regeneration and the highest mean number of shoots/callus were obtained on hormone-free MS medium. In terms of callus induction and subsequent plant regeneration, cotyledon explants were more responsive than hypocotyl explants. Regenerated shoots (2-3 cm) were rooted on MS hormone-free medium or medium containing 0.1 mg/l indole-3-butyric acid. About 90% of regenerated plantlets survived under field conditions after hardening in the glasshouse. Several somaclones exhibiting useful variation would to be proposed as initial plant material for eggplant breeding programs.

Keywords: callus tissue, eggplant, explants, growth regulator, plant regeneration, somaclonal variation

Abbreviations: BAP (6-benzylaminopurine), IAA (indole acetic acid), IBA (indole-3-butyric acid), Kn (kinetin), NAA (α-naphthalene acetic acid), PGRs (plant growth regulators), TDZ (thidiazuron), 2,4-D (2,4-Dichlorophenoxyacetic acid)

РЕЗЮМЕ

Разработена е ефективна система за растителна регенерация чрез индиректен органогенезис при два сорта патладжан (*Solanum melongena),* която представлява източник на нови форми растения, ценни като изходен материал за селекционни програми. Подробно са описани всички етапи и условия на култивиране, които водят до получаването на растителни регенеранти и успешно развитие в полски условия.

Ключови думи: експланти, калусна тъкан, патладжан, растежен регулатор, регенерация на растенията, сомаклонално вариране

РАЗШИРЕНО РЕЗЮМЕ

Разработен е протокол за индиректен органогенезис при *Solanum melongena* от калусни култури, получени от два вида експланти (хипокотили и котиледони). В изследването са включени два сорта патладжан - Larga Negra и Black Beauty. Индуциране на морфогенетичен калус е наблюдаван при котиледони от 30-дневни кълнове, култивирани върху среда Murashige and Skoog (MS), съдържаща 2.0 mg/l α-нафтилоцетна киселина и 0.5 mg/l 6- бензиламинопурин. Определени са цвета, консистенцията и структурата на калуса. Преобладаващата част от експлантите формират калус на 14-15 ден от култивирането. Най-висок процент на регенерация и среден брой пъпки от калус са получени на MC среда без фитохормони. Установено е, че отговорът на котиледоните към формиране на калуси и последваща растителна регенерация е по-висок от този на хипокотилите. Растенията-регенеранти са вкоренени на MC среда без растежен регулатор или на среда, съдържаща 0.1 mg/l индолилмаслена киселина. Степента на преживяемост на регенерантите при полски условия е около 90 %. Получени са сомаклонални вариации, които биха могли да бъдат използвани като изходен растителен материал в селекционни програми.

INTRODUCTION

Application of biotechnological aspects of eggplant (*Solanum melongena* L.) improvement has been used as powerful tools for solving many target tasks in vegetable breeding. Tissue culture protocols for organogenesis, somatic embryogenesis, anther culture and protoplast culture have been well established (Collonier et al., 2001; Kashyap et al., 2003; Khatun et al., 2006; Magioli et al., 2001; Saxena et al., 1992). However, the regeneration efficiency reported in different systems of *S. melongena* was relatively low (Sharma, Rajam, 1995). The type and concentration of a given growth regulator can cause significant differences in the callus formation and morphogenic responses of eggplant. For example, Kamat and Rao (1978) reported shoot regeneration from hypocotyl segments of *S. melongena* in presence of indole acetic acid (IAA). In response to different α-naphthalene acetic acid (NAA) and thidiazuron (TDZ) concentrations using the same explant both embryogenesis and organogenesis were reported (Magioli et al., 1998; Matsuoka, Hinata, 1987). Macchia et al. (1983) induced callus and root formation from leaf explants of F_1 hybrids in eggplants on MS medium containing indole-3-butyric acid (IBA). Induction of organogenic calluses from cultured roots and subsequent differentiation into shoot buds were observed on MS medium in the presence of TDZ and 6-benzylaminopurine (BAP) (Franklin et al., 2004).

Plant tissue culture is recognized as a source to generate useful genetic variability (somaclonal variation) for crop improvement (Brar, Jain, 1998; Larkin, Scowcroft, 1981). The somaclonal variations in eggplant are caused by the hormonal concentrations in culture medium (Rotino et al., 1991). The effect of the growth regulators NAA and 2,4-Dichlorophenoxyacetic acid (2, 4-D) on somaclonal variations in eggplant were studies (Hitomi, Amagaki, 1998). However, practical application of plant regeneration for isolation of somaclonal variation has lagged due to the non-availability of mass scaling techniques and effective field delivery systems (Kantharajan, Golegaonkar, 2004).

The aim of this study was to establish a protocol for inducing indirect shoot organogenesis of *S. melongena* under influence of different combinations of plant growth regulators.

MATERIALS AND METHODS

Two eggplant commercial cultivars, 'Larga Negra' ('LN') and 'Black Beauty' ('BB') used in the experiment were obtained from Institute of Plant and Genetic Resources, Agricultural Academy, Sadovo, Bulgaria. The surface sterilization was carried out with 70% (w/v) ethanol for 2 min and by dipping into 15% (w/v) commercial bleach solution for 10 min. Sterilized seeds were rinsed three times in sterile distilled water to remove bleach. They were then placed into tubes containing culture medium (40 seeds per treatment). For seed germination *in vitro*, the MS (Murashige, Skoog, 1962) hormone - free medium supplemented with 1.0% sucrose and solidified with 0.8% agar was used. The percentage of germination was determined after 20 days of culture. All media used in the experiments were adjusted to pH 5.8 before autoclaving. MS medium was used as a culture medium. Hypocotyls and cotyledons obtained from *in vitro* seedling cultures served as explant sources for callus induction.

Collected cotyledons and hypocotyls from 30-days old seedlings were cut into pieces and placed into 20/160 mm culture tubes containing 10-ml MS medium supplemented with NAA and Kn or BAP; there were three explants per tube and ten replicate tube per treatment as well as for cotyledons and hypocotyls.

The number of explants forming calluses was scored after 14-15-days of culture. Callus formation frequency was calculated as the percentage of explants forming calluses. The consistency, color and structure of the obtained calluses were monitored. Induced calluses were sub-cultured into fresh MS medium for further growth and proliferation.

Within 28 days of inoculation, shoot primordia were formed from each callus. For shoot induction, friable callus with primordia was transferred on diverse MS media to select the most effective combination: MS0 - hormones-free medium serving as the control and MS1, MS2, MS3 and MS4 containing BAP, NAA and GA_3 for 21 days. The number of calluses producing shoots and total number of shoots were counted for each treatment. Mean number of adventitious shoots per callus was calculated. Shoot elongation was promoted by the addition of 0.4 mg/l GA_3 to the all five MS media.

Maintenance and regeneration experiments were conducted under physical conditions as described in Table 1. Unless otherwise indicated, regeneration media differed from callus induction medium only in concentration of hormones employed. Organogenic calluses were sub-cultured into fresh media after 21-day intervals till 12 passages for cotyledons-derived callus and till 9^{th} passages for hypocotyls-derived callus. Brown and dead portions of calluses were discarded during every subculture. Friable, organogenic callus was selected for maintenance and regeneration. The frequency of shoot formation was determined after 21-days of culture for every one passage.

Shoots of 2-3 cm were separated from callus and transferred to either hormone-free MS regeneration medium or medium containing IBA with concentration 0.1 mg/l for rooting. There were 15 regenerants per treatment. Three parameters were recorded: percentage of rooted regenerants, length of the roots and number of roots per explants.

For *in vivo* acclimatization, plantlets were planted individually in pots containing 2:1:1 (v/v/v) soil less mixture of Bulgarian peat: perlite: sand and moved to a greenhouse to reach their subsequent development. Each experiment was repeated twice. Data were statistically analyzed using Sigma Stat computer package (Sigma Stat 3.1, Systat Software, San Jose, California, USA). Confidence intervals were given for $P \leq 0.05$ using *t*-test.

RESULTS

The cultural conditions and used media for each experimental phase (callus induction, callus subculture, shoot regeneration, shoot elongation, root formation and acclimatization) are given in Table 1. These conditions were found to be optimal after preliminary testing of different media compositions (data not shown).

Table 1. Cultural conditions and used media for each experimental phase for indirect organogenesis in *S. melongena*

Stages	Culture media	Culture conditions
Callus induction	MS + 2mg/l NAA + 0.5 mg/l BAP	42 days in darkness, 25ºC ± 1ºC
Callus subculture	MS + 0.1mg/l NAA + 1 mg/l BAP + 0.2 mg/l GA_3	28 days, 16h light/8h dark, 25ºC ± 1ºC
Shoot regeneration	MS hormone-free	21 days, 16h light/8h dark, 25ºC ± 1ºC
Shoot elongation	MS with 0.4 mg/l GA_3	21 days, 16h light/8h dark, 25ºC ± 1ºC
Root formation	MS hormone-free or with 0.1 mg/l IBA	28 days, 16h light/8h dark, 25ºC ± 1ºC
Acclimatization	Peat:Perlite:Sand 2:1:1 (v/v/v)	18-20 days, 16h light/8h

pH =5.8; all types of media were autoclaved at 120 C for 20 min; light intensity – 40 μmol m-2s-1

Seed germination capacity of both eggplant cultivars was high and sufficed for 100% for 'LN' and 85% for 'BB', respectively. No visually symptoms of contamination were detected during germination indicating the efficacy of the surface sterilizing procedures applied. The seedlings 30-days old were a good source for taking the

explants for inducing undifferentiated tissues. The influence of combinations NAA/ Kn and NAA/BAP on callus formation for both types of explants is shown in Table 2. These hormonal supplements were selected because they were optimum for callus formation among many tested combination, described by us previously (Zayova et al., 2008). Callus was initiated within a period of 14-15 days of culture and a mass of callus was formed within 42 days. A relatively high frequency of callus induction was observed from both the explants in both cultivars and cotyledon was more responsive than hypocotyl in 'LN' than in 'BB'. Previous reports (Franklin et al., 2004; Sharma, Rajam, 1995) on *S. melongena* show that the type of explants used as callus source and the developmental stage of the seedlings significantly affect callus- and organogenesis.

Table 2. Influence of PGRs on callus induction of seedling explants of *S. melongena*

Cultivar	Nutrient media	Frequency of formed calli, %		Callus characteristic	
		Cotyledons	Hypocotyls	Cotyledons	Hypocotyls
'LN'	MSC1 + 2 mg/l NAA + 0.5 mg/l Kn	83.3	54.5	White Compact	White Friable
'LN'	MSC2 + 2 mg/l NAA + 0.5 mg/l BAP	90	63.3	Pale-yellow Friable	Pale-yellow Friable
'BB'	MSC1 + 2 mg NAA + 0.5 mg/l Kn	60	50	White Compact	White Friable
'BB'	MSC2 + 2 mg/l NAA + 0.5 mg/l BAP	62.5	53.3	Pale-yellow Friable	Pale-yellow Friable

MSC – MS Callus medium

A mass of callus was formed by two type explants on medium MSC2 where BAP was added instead of kinetin. Two types of calluses were produced: compact white with hard appearance and pale-yellow with friable structure. In our study, successful induction of potentially organogenic callus (friable structure – Figure 1a.) from hypocotyls and cotyledons was achieved using a combination of BAP, NAA and GA_3. Shoot primordia (Figure 1b.) were produced on MS medium different from those for callus induction and in presence of GA_3. The plant growth regulators (PGRs) combination and used concentrations were totally effective for inducing shoot formation. Organogenic calluses, sub-cultured on five nutrient media developed shoot buds. Highest percentage of regeneration of cotyledon-derived callus occurred on MS0 in absence of phytohormone (Table 3.). A number of small shoot buds occurred on the surface of cotyledon–derived calluses within 21 days of culture on MS0 (Figure 1c.). The type, concentration, and combination of plant growth regulators (auxins and cytokinins) are the key factors influencing indirect shoot organogenesis in eggplant (Macchia et al., 1983; Picoli et al., 2002; Rahman

et al., 2006). In our case, the optimum response in terms of regeneration frequency and the number of shoots per callus was recorded on MS medium without hormones (Table 3.). Our results indicated that BAP at concentrations 0.5, 1, and 2 mg/l increases regeneration frequency, especially in 'LN'. However, higher BAP level (MS4) lead to the development of vitrificated buds- like structures (aberrant tissues) instead (Table 3.). In addition, cotyledon -derived calluses from 'LN' showed higher shoot formation ability compared to 'BB' at respective concentrations. The predominance of 'LN' over 'BB' proved genotypic variation for this trait. Similar results were obtained for hypocotyl-derived callus. Also, the number of shoots/callus of hypocotyls was lower than that of cotyledons cultured on MS1, MS2 and MS3 media. Calluses with small buds were transferred onto MS media supplemented with 0.4 mg/l GA_3 for further development. Leaf formation and shoot elongation occurred in the following three weeks (Fig. 1d.). The age of callus also affected shoot regeneration in eggplant. The callus maintained its regeneration capacity up to 12^{th} passages for cotyledons-derived callus of 'LN' and up to 9^{th} passages for 'BB' (each passage of three weeks of culture).

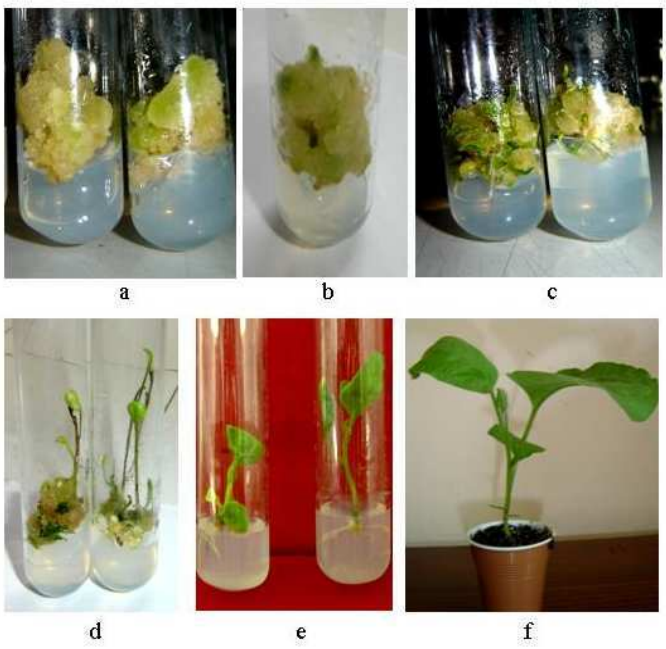

Figure 1. Indirect shoot organogenesis of eggplant: a) Friable callus on MS medium supplemented with 2 mg/l NAA and 0.5 mg L^{-1} BAP; b) Shoot primordia on MS with 0.1 mg/l NAA, 1 mg/l BAP and 0.2 mg/l GA_3 ; c) Shoot buds from cotyledon- derived callus after transferring on hormone-free MS; d) Shoot elongation and plantlets on MS medium supplemented with 0.4 mg/l GA_3; e) Rooted plantlets on hormone-free MS medium; f) Adapted *ex vitro* regenerant.

Table 3. Effect of plant growth regulators on shoot regeneration from cotyledon-, and hypocotyl-derived callus

Medium	Plant growth regulators, mg/l			Shoot regeneration, %		No shoots/callus × ± SE	
	NAA	BAP	GA_3	'LN'	'BB'	'LN'	'BB'
				a/ from cotyledon-derived callus			
MS0	0	0	0	80	60	14.3 ± 0.97*	7.5 ± 0.50*
MS1	0.1	0.5	0.2	60	40	5.8 ± 0.30	3.6 ± 0.24
MS2	0.1	1	0.2	60	46.7	6.4 ± 0.43	5.2 ± 0.35
MS3	0.1	2	0.2	73.3	53.3	8.6 ± 0.58	6.0 ± 0.41
MS4	0.1	3	0.2	vitrification		vitrification	
				b/ from hypocotyl-derived callus			
MS0	0	0	0	66.7	60	9.7 ± 0.65*	5.3 ± 0.36*
MS1	0.1	0.5	0.2	40	53.3	3.4 ± 0.23	2.8 ± 0.19
MS2	0.1	1	0.2	33.3	40	4.3 ± 0.29	4.0 ± 0.27
MS3	0.1	2	0.2	40	46.7	5.5 ± 0.37	4.2 ± 0.28
MS4	0.1	3	0.2	vitrification		vitrification	

*Significant at P≤0.05

Three-week-old callus showed the maximum shoot regeneration frequency (Figure 2.). The shoot regeneration frequency decreased gradually with an increase in callus age from 3 to 36 weeks for 'LN' and from 3 to 27 weeks for 'BB'. Among the tested cultivars, the morphogenic potential was most rapidly lost with age for callus of 'BB'.

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.

Based on these variations, the regenerated plants from the last passages were divided into five morphological phenotypes (Figure 3.). Both, parent plants and somaclonal lines were evaluated by nine morphological traits detailed in an earlier study (Zayova et al., 2010). Roots were observed as early as two weeks after placing the microshoots (2-3 cm) on rooting media. Most of the shoots developed roots by week four (Figure 1e.). The influence of two tested media on the percentage of root formation, mean length of the roots and roots/plant is summarized in Table 4. Higher number of roots was induced on MSR0. Similar results for rooting in PGRs-free medium have been reported in *S. melongena* (Sarker et al., 2006; Taha, Tijan, 2002).

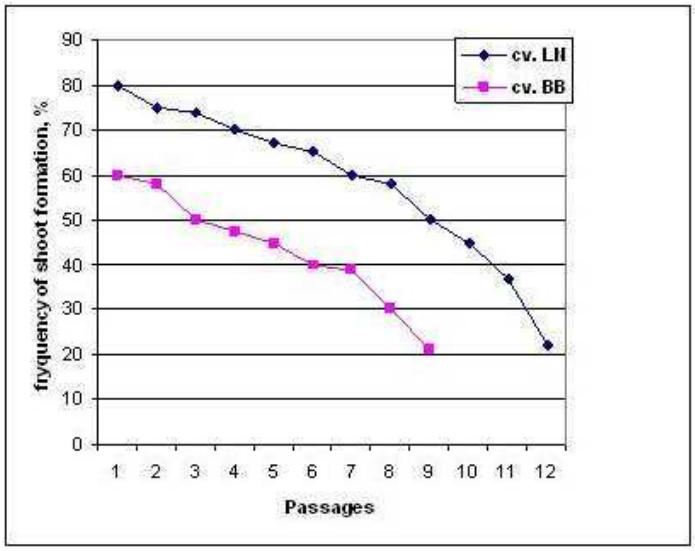

Figure 2. Influence of callus age on frequency of shoot formation of eggplant cultivars 'Larga Negra' ('LN') and 'Black Beauty' ('BB')

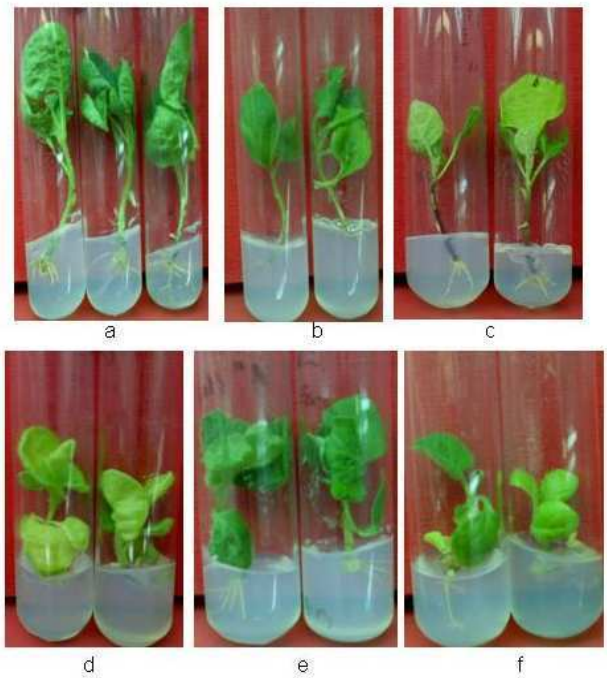

Figure 3. Morphological variations in plants regenerated from cotyledon-derived callus of eggplant (*S. melongena)*: a) Control plants 'LN' ; Phenotypes: b) LNR1 - plants with green stem, smooth and narrow leaves with thin petioles, c) LNR2 - plants

with dark-red stem and smooth, pale green leaves, d) LNR3 - plants with abnormal leaves, curly and light-green, e) LNR4 - plants with thick stem, short leaf petioles and large, curly leaves accumulated in the top of the plant stem and forming an area resembling the inflorescence and f) LNR5 - plant dwarf.

Table 4. Rooting response in *S. melongena* after cultivation on basal-, and supplemented with auxin (IBA) MS media

Medium	'LN'			' BB'		
	Root formation %	Root length, cm × ± SE	No roots/plant × ± SE	Root formation %	Root length, cm × ± SE	No roots/plant × ± SE
			a/ from cotyledon -derived callus			
MS (control)	86.7	3.2 ± 0.21	2.6 ± 0.17*	80	2.9 ± 0.19	2.8 ± 0.18*
MS + 0.1 mg/l IBA	66.7	1.4 ± 0.91	5.2 ± 0.34*	53.3	1.0 ± 0.67	4.1 ± 0.27*
			b/ from hypocotyl-derived callus			
MS (control)	73.3	2.0 ± 0.13	2.4 ± 0.16*	60	2.2 ± 0.15	1.7 ± 0.11*
MS + 0.1 mg/l IBA	53.3	1.2 ± 0.80	4.6 ± 0.31*	46.7	1.5 ± 0.93	4.8 ±0.32*

*Significant at P≤0.05

The potential of *in vitro* regenerated plantlets to be used for *in vivo* establishment was investigated with plantlets transferred to pots under culture-room conditions (Figure 1f.). Almost 90% of these regenerants survived under *ex vitro* conditions.

DISCUSSION

The present study was made to evaluate the morphogenic potential of two explant types excised from *in vitro* grown seedlings. Calluses of *S. melongena* could be successfully induced from *in vitro* germinated seedlings when explants from cotyledons and hypocotyls were cultured on MS media added with NAA and BAP. Levels and NAA/BAP ratio supplemented to MS medium caused dedifferentiation of cells in the used cotyledon- and hypocotyl-derived tissues. Also, callus induction response may be affected by the specificity of explants used for shoot regeneration; thus, explants from 30-days old seedlings revealed better callus induction frequency and subsequent regeneration compared with explants from *in vitro* developed older plants. Further, the differences in callus and organ induction may be a result of genotype or cultural conditions (Franklin et al., 2004; Magioli et al., 1998; Mir et al., 2008; Scoccianti et al., 2000; Sharma, Rajam, 1995). In our case, duration of callus proliferation on cultural media was very important for shoot regeneration. When callus was sub-cultured for proliferation before being transferred to media for organogenesis, the regeneration frequency significantly decreased

structures or shoot bud initials. The development of primordia from friable callus (regardless of the explant type) was observed after four weeks of culture on MS medium supplemented with BAP, NAA, and GA_3. This combination of growth regulators stimulated cell proliferation but it cannot initiate shoot induction. It was established that the calluses of two cultivars generated a great number of shoot primordia but only a small number of shoots initiated plant regeneration. To identify the best combination which would initiate shoot regeneration in callus of eggplant, a variety of growth regulators were tested (Table 3.). In the present study, we used a range of BAP levels; the maximum multiple adventitious shoot regeneration and elongation was observed by culturing calluses on MS media without growth regulators. It was established that BAP promoted organogenesis from each callus in both cultivars, as the frequency of shoot regeneration and number of shoot per callus increased when compared MS1, MS2 and MS3 media (Table 3.). However, higher BAP level (MS4 medium) had negative effect on organogenesis leading to shoots vitrification. Similar results were reported in *S. melongena* by several authors (Collonier et al*.,* 2001; Kashyap et al*.,* 2005; Sharma, Rajam, 1995). The interaction of NAA, BAP and GA_3, and also MS0 supplemented with GA_3 resulted in significant shoot elongation throughout stimulation of their growth and development. Comparison of different explants types and cultivars in terms of shoot regeneration revealed a genotypic variation between the types of explants and cultivars, with 'BB' being less productive than 'LN'. As to the comparison of explants type, cotyledon was more responsive for shoot induction than hypocotyl.

IBA is widely used PGR for root induction (Mir et al., 2008; Rahman et al., 2006), although several authors demonstrated rooting in PGR-free medium during organogenesis (Sarker et al., 2006; Taha, Tijan, 2002). In the present study, a good rooting response was established in both tested media: hormone-free and medium supplemented with IBA (Table 4.). The first one induced small number of long roots while the second stimulated the production of numerous short roots. In both cases, the roots were tightly attached to the base of plants. *S. melongena* shoots regenerated *in vitro* proved to be easily adaptable to *ex vitro* plant establishment.

In summary, this work results in a valuable contribution to establish *in vitro* culture conditions for inducing indirect organogenesis in eggplant. In this sense different NAA/BAP ratio resulted in the best phytoregulator supplementation to establish *S. melongena* callus. The efficient system for regeneration shoots from callus culture described here could be useful for developing an efficient method for regenerating of plants indirectly from an intervening callus stage. Indirect regeneration can lead to *S. melongena* improvement and even to incorporation of valuable and desirable traits into eggplant cultivars. Several somaclones exhibiting useful variation would to be proposed as initial plant material for breeding programs in this economically important plant species.

REFERENCES

Brar, D.S., Jain, S.M., (1998) Somaclonal variation: Mechanism and application in crop improvement. In: S. M., Jain, D. S., Brar, B. S., Ahloowalia, eds. (1998) Somaclonal variation and induced mutations in crop improvement. London: Kluwer Academic Publishers, pp.15-37.

Collonier, C., Fock, I., Kashyap, V., Rotino, G.L., Daunay, M.C., Lian, Y., Mariska,

of biotechnology in eggplant. Plant Cell Tissue & Organ Culture, 65, 91–107.
Franklin, G., Sheeba, C., Sita, L., (2004) Regeneration of eggplant (*Solanum melongena* L.) from root explants. In Vitro Cellular & Developmental Biology - Plant, 40, 188-191.
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.
Kamat, M.G., Rao, T.S., (1978) Vegetative multiplication of eggplants (*Solanum melongena* L.) using tissue culture techniques. Plant Sciences Letters, 13, 57-65.
Kantharajan, A.S., Golegaonkar, P.G., (2004) Somatic embryogenesis in eggplant. Sci. Horticult., 99, No 2, 107-117.
Kashyap, V., Kumar, V., Collonier, C., Fusari, F., Haircour, R., Rotino, G.L., Sihachakr, D., Rajam, M.V., (2003) Biotechnology in eggplant. Sci. Hort., 97, 1-25.
Khatun, F., Meah, M.B., Nasiruddin, K.M., (2006) Regeneration of eggplant through anther culture. Pakistan Journal of Biological Sciences, 9, 48-53.
Larkin, P.J., Scowcroft, W.R., (1981) Somaclonal variation: A novel source of variability from cell cultures for plant improvement. Theoretical and Applied Genetetics, 60, 197-214.
Macchia, F., Scaramuzzi, F., Porcelli, S., (1983) Organogenesis and propagation *in vitro* of F_1 hybrid of *Solanum melongena* L. from vegetative segments. ISHS Acta Horticulturae, In Vitro Culture, XXI IHC, p. 131.
Magioli, C., Rocha, A.P.M., de Oliveira, D.E., Mansur, E., (1998) Efficient shoot organogenesis of eggplant (*Solanum melongena* L.) induced by thidiazuron. Plant Cell Reports, 17, 661– 663.
Magioli, C., Rocha, A.P.M., Tarré, E., Santiago-Fernandes, L.D., de Oliveira, D.E., Krul, W.R., Mansur, E., (2001) Effect of morphological factors, antibiotics and Agrobacterium co-cultivation in the efficiency of somatic embryogenesis of eggplant (*Solanum melongena* L.). Journal of Plant Biotechnology, 3, 19–25.
Matsuoka, H., Hinata, K., (1987) NAA-induced organogenesis and embryogenesis in hypocotyls callus of eggplant (*Solanum melongena* L.). The Journal of Experimental Botany, 30, 363–370.
Mir, K.A., Dhatt, A.S., Sandhu, J.S., Gosal, S.S., (2008) Genotype, explant and culture medium effects on somatic embriogenesis in eggplant (*Solanum melongena* L.). Horticulture, Environment and Biotechnology, 49, 182-187.
Murashige, T., Skoog, F., (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15, 473–497.
Picoli, E.A., Cecon, P.R., Fari, M.G., Otoni, W.C., (2002) Organogenesis in eggplant (*Solanum melongena* L. cv. Embu) as affected by antibiotics and growth regulators. Intern. Journal of Horticultural Science (Hungary), 82, 76–82.
Rahman, M., Asaduzzaman, M., Nahar, N., Bari, M.A., (2006) Efficient plant regeneration from cotyledon and midrib derived callus in eggplant (*Solanum melongena* L.). Journal of Bio-Science, 4, 31–38.
Rotino, G.L., Schiavi, M., Vicini, E., Falavigna, A., (1991) Variation among androgenetic and embryogenetic lines of eggplant (*Solanum melongena* L.). Journal of Genetics and Breeding, 45, 141-146.
Sarker, R., Yesmin, S., Hoque, M., (2006) Multiple shoot formation in eggplant (*Solanum melongena* L.). Plant Tissue Culture & Biotechology, 16, 53-61.

Saxena, P., Malik, C., Gill, R., (1992) Plantlet formation from isolated protoplasts to *Solanum melongena*. Planta, 187, 421-424.

Scoccianti, V., Sgarbi, E., Fraternale, D., Biondi, S., (2000) Organogenesis from *Solanum melongena* L. (eggplant) cotyledon explants is associated with hormone-modulated enhancement of polyamine biosynthesis and conjugation. Protoplasma, 211, 51–63.

Sharma, P., Rajam, M.V., (1995) Genotype, explant and position effects on organogenesis and somatic embryogenesis in eggplant (*Solanum melongena* L.). Journal of Experimental Botany, 46, 135–141.

Taha, R., Tijan, M., (2002) An *in vitro* production and field transfer protocol for Solanum melongena L. plants. South African Journal of Botany, 68, 447-450.

Zayova, E., Nikova, , Ilieva, K., Phylipov PH., (2008) Callusogenesis of eggplant (Solanum melongena L.). Comptes rendus de l'Academie bulgare des Sciences, 61, 1485-1488.

Zayova, E., Vassilevska–Ivanova, R. , Kraptchev, B., Stoeva, D., (2010) Somaclonal variations through indirect organogenesis in eggplant (*Solanum melongena* L.). Biological Diversity and Conservation (BioDiCon), 3, No 3, 1-5.