2009

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Shimaa Bahgat

Omer A. Shabban

Osama El-Shihy

David A. Lightfoot
Southern Illinois University Carbondale, ga4082@siu.edu

Hany A. El- Shemy

Published in *Current Issues in Molecular Biology*, (2009) 11 (Suppl. 1): i47-54

Establishment of the Regeneration System for *Vicia faba* L.

Shimaa Bahgat[1], Omer A. Shabban[1], Osama El-Shihy[2], David. A. Lightfoot[3] and Hany A. El-Shemy[1,3]*

[1]Faculty of Agriculture Research Park (FARP) and Biochemistry Department, Faculty of Agriculture, University of Cairo, 12613 Giza, Egypt
[2]Plant Physiology Department, University of Cairo, Faculty of Agriculture,12613 Giza, Egypt
[3]Center of Excellence: The Illinois Soybean Center, Department of Plant, Soil and Agriculture Systems, SIUC, Carbondale, IL 62901, USA

Received 4 August 2008
Revised 13 September 2008
Accepted 11 October 2008

Abstract

A reliable regeneration system for faba bean has been difficult to establish and therefore, the genetic improvement of *Vicia faba* L. was delayed. The paper describes a method of somatic embryo induction in callus of *V. faba*. Two Egyptian faba bean cultivars 'Giza 2' and '24 Hyto' were used. Callus was induced from epicotyls and shoot tips cultured on MS or Gamborg medium supplemented with 3% sucrose and 0.025% (w/v) for each of ascorbic and citric acid, 0.8% agar and different concentrations of 10 mg/l BAP, 0.5 mg/l of each NAA and 2,4-dichlorophenoxyacetic acid (M1) and 1 mg/l BAP and 0.5 mg/l NAA (M2) . The media with BAP, NAA and 2,4-D were optimal for embryogenic callus induction. Somatic embryos developed after transfer of the callus to 1/2 B5 medium with no plant growth regulators. There were various stages of somatic embryo development present including globular, heart-shaped, torpedo, and cotyledonary stages. Embryos developed into plantlets and plants were regenerated. RAPD analyses were performed to investigate the genetic stability of the regenerated plants obtained from different treatments and different explants. The cultivar Giza 2 exhibited more genetic stability than cultivar 24 Hyto. In conclusion, a regeneration system was established suitable for both gene transformation and the isolation of somaclonal mutants. The regeneration system will be used in order to improve the nutritional value of faba bean.

Introduction

Legumes are the third largest family of dicotyledons, *Leguminosae*. Examples include soybean (*Glycine max)*, pea (*Pisum sativum*), faba bean (*Vicia faba*), mung bean (*Vigna radiata*), chickpea (*Cicer arietinum*) and common bean (*Phaseolus vulgaris*). Faba bean (*Vicia faba* L.) is one of the most important grain Legumes in the world. Because Faba bean is an excellent source of protein, it is an important pulse crop used for human consumption and animal feeding, faba bean also plays a great role in the biological fixation of aerial nitrogen (Duke, 1981; Jelenić *et al*., 2000). This species alone occupies nearly 3.2×10^6 ha worldwide in 1991 (FAO statistics, 1992) with a world production close to 4.5 million tons in 2004 (Gutierrez, 2006). Even though faba bean has been cultivated in many countries, 49% of the total production comes from China followed by Egypt (10, 8%), Ethiopia (8.5%), and Australia by 5.8% in 2000 (FAO, statistics 2001). Unfortunately, its susceptibility to environmental conditions, biotic and abiotic stress and instability of the yield made this crop less attractive. Also, difficulties in pollination control and the limited genetic pool has led to slow down progress in varietal improvement (Bond, 1987; Selva *et al*., 1989). Today to improve faba against biotic and abiotic stress and to improve its nutritional value with sulphur amino acids, traditional breeding programs could be supplemented by gene transfer technology which requires an efficient and suitable regeneration system, which occurs via organogenesis or embryogenesis, either directly from the excised tissue or indirectly after formation of callus depending on internal and external plant factors (Kuchuk, 2001). In tissue culture faba bean appears to be recalcitrant towards *in vitro* regeneration (Khalafalla and Hattori, 2000), due to difficulties in regeneration from callus, and the releasing of phenolic compounds, so studies on *in vitro* culture of faba bean are therefore difficult to carry out (Böttinger *et al*., 2001). Regeneration system of faba bean through indirect somatic embryogenesis was reported by Griga *et al.* (1987) they were used the cotyledons on media supplemented with 2,4D. Somatic embryos at globular stage to early torpedo stage were released, but their development was terminated at the late-torpedo stage. In tissue culture, the regenerated plants from cultured cells are known to exhibit genetic and epigenetic changes collectively called somaclonal variations (Razdan, 1994). In this study we described a new method for regeneration of faba bean through somatic embryogenesis, with a development to complete plants. RAPD-PCR molecular markers were performed to identify the genetic variability between the control plants and the regenerated plants of each cultivar.

Materials and methods

Plant material

Two cultivars of faba bean Giza 2 and 24 Hyto (cv. Giza 2 was obtained from Agricultural Research Center, Egypt and cv. 24 Hyto was obtained from Agronomy Department,

*For correspondence: helshemy@hotmail.com

Faculty of Agriculture, Cairo University, Egypt) were used for this study. Segments of shoot tips and epicotyls were the sources of *in vitro* experimental materials.

Preparation of explants
Seeds of faba bean were sterilized in 70% (v/v) ethanol for 30 second, followed by 20% (v/v) commercial bleach for 15 min and washed 3–4 times with sterile distilled water. Sterilized seeds were germinated on half-strength MS medium (Murashige and Skoog, 1962) supplemented with 15 g/l sucrose and solidified with 8 g/l agar. The seeds were germinated in darkness at 25ºC. After a week shoot tips and epicotyls were cut with scalpel blade into segments of 5–7mm before cultivation on callus induction media.

Culture media
Two different treatments (M1& M2) were used in this study. The MS medium and Gamborg medium (Gamborg *et al*.1968) were used for plant tissue culture. All of callus and regeneration media were supplemented with 3% (w/v) sucrose and 0.025% (w/v) for each of ascorbic and citric acid (Sigma, USA). The pH of all cultures was adjusted to 5.7 then solidified with 0.8% (w/v) agar and autoclaved at 121ºC for 25 min. The cultures were incubated at 25ºC ± 1 under a 16/8 h light/dark photoperiod regime provided by white fluorescent light at 716 Lux.

M1 treatment
Epicotyls and shoot tips of the two cultivars were placed in callus induction medium CIM1 which contained MS medium with 10 mg/l BAP, 0.5 mg/l of each NAA and 2, 4D (Sigma, USA), and sub-cultured every 3–4 weeks, after two months calluses were transferred to a regeneration medium RM1 containing Gamborg medium or MS medium supplemented with B5 vitamins and 2 mg/l BAP. When globular structures appeared on the surface of callus with a white-greenish brilliant color, or a single embryo with a long structure had formed, they were separated from the callus and transferred to RM1 medium supplemented with 20 mg/l of glutamine (Sigma, St Louis, MO, USA). After 4–6 weeks, Nemours somatic embryos were formed (at globular stages) on an embryogenic callus, they were transferred to hormone free medium 1/2 B5 to encourage the development of the somatic embryos into plants. They were sub-cultured every 4 weeks. The hormone-free medium caused the formation of hundreds of somatic embryos. Each was separated and allowed to develop into a plant. Normally they formed shoots and roots in the same medium. Such normal plantlets were transferred to the soil, covered at first for adaptation to the glasshouse. Covers were gradually removed to allow hardening. Plants were allowed to flower and self fertilize.

M2 treatment
Epicotyls and shoot tips of the faba bean cvs. were placed in the callus induction medium CIM2 which contained MS medium with 1 mg/l BAP and 0.5 mg/l NAA. Within 6–7 weeks calluses were formed and embryo-like structures started to be formed. The embryo-like structures were excised and transferred to the regeneration medium RM2, containing MS medium supplemented with B5 vitamins, 1.2 mg/l BAP, and 0. 5 mg/l NAA. Somatic embryos were formed at different stages. They were excised when they appeared to be at the beginning of the cotyledonary stage. Embryos developed to complete plants in 1/2 B5 medium without plant growth regulators. After the elongation of shoots and roots, plantlets were transferred to the soil to prepare them for adaptation to glass-house conditions.

RAPD analysis
Total DNA was isolated from each control and regenerated plant using the CTAB protocol (Doyle and Doyle, 1987). PCR reactions were performed in total volume of 25µl and 6 random Primers (from the OPA set) were used as described in Table 1 (all primers were obtained from Operon Technologies Inc., Alameda, CA, USA). DNA amplification was carried out in a T-gradient thermal cycler (Biometra, Göttingen, FRG), PCR reactions were hot –started at 95ºC for 4 min and subjected to 40 cycles as follows; 1 min at 94ºC; 2 min at 35ºC; and 2 min at 72ºC. The last extension phase was prolonged to 7 min at 72ºC. PCR products were separated by electrophoresis in 1.5 % agarose gel and visualized by Ultraviolet illumination after staining with ethidium bromide, and photographed using gel-documentation system (BIO-RAD, Hercules CA).

Results
Callus tissues were initiated from epicotyls segments and shoots tip of two Egyptian faba bean cvs. Giza 2 and 24 Hyto using two different treatments M1 and M2. All the induction media were supplemented with various concentrations of auxins and cytokinins, segments of shoot tips and epicotyls induced a mixture of greenish, brownish and yellowish compact callus (Fig. 1A-F).

The M1 treatments consisted of CIM1 (callus induction medium) and RM1 (regeneration medium). In CIM1 shoot tips and epicotyls of each cultivar were cultured for two months during which time they were sub-cultured every 3–4 weeks. They had produced a compact yellowish callus; after two months the calluses were transferred to regeneration medium RM1. After 3–7 weeks, structures that looked like an embryo with long structure were obtained, or sometimes a globular smooth structures with white-greenish transparent color, were formed on the surface of callus-derived from shoot tips. Cultivar Giza 2 formed these structures after 3 weeks while cv. 24 Hyto formed the same structures after 7 weeks of culturing in RM1 medium. The embryo-like structures were separated and cultured in the same RM1 medium, but supplemented with 20 mg/l glutamine. Within a week these embryo

Table 1. Nucleotide sequences of six primers used in RAPD analysis.

Sequence (5′-3′)	Primer
AATCGGGCTG	OPA04
AGGGGTCTTG	OPA05
GAAACGGGTG	OPA07
GGGTAACGCC	OPA09
GTGATCGCAG	OPA10
CAGCACCCAC	OPA13

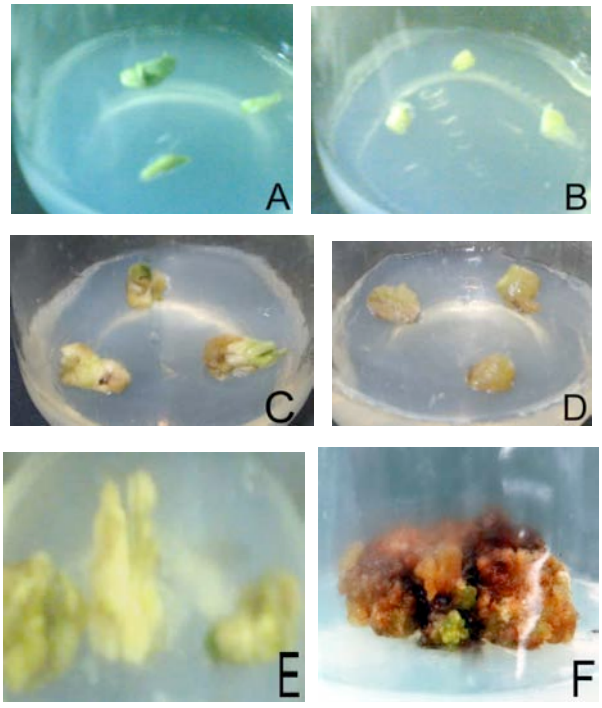

Fig. 1 Examples of embryogenic callus from *V. faba* cvs. Giza 2 and 24 Hyto. Panels **A** and **B** show segments of shoot tips and epicotyls. Panels **C** and **D** show segments of shoot tips and epicotyls on callus induction media after 10–15 days. Panels **E** and **F** show shoot tips and epicotyls respectively after two months on callus induction medium. Panel **E** shows callus derived from shoot tips of cv. 24 Hyto on CIM1 medium. Panel **F** shows callus derived from an epicotyl of cv. 24 Hyto in CIM2 medium which turned brown due to phenolic compounds.

structures grew and developed into globular stage (Fig. 2F, G), 3 weeks later, a lot of somatic embryos with white-greenish color were formed in clusters with embryogenic callus (Fig. 2H). For further development these embryos were transferred to hormone free medium (1/2 B5 medium). It was also noted that, the hormone free medium enhanced the production of new generations of somatic embryos. Each embryo was capable of producing a lot of embryos over a period of 14 months. Therefore there was asynchronous development of embryos to complete plants over that time. The somatic embryos which derived from shoot tips of cv. Giza 2 readily developed to fertile plants, while the cultivar 24 Hyto did not, being arrested at the torpedo stage. Although the embryos of cv. Giza 2 developed slowly, this cultivar produced more embryos than that of cv. 24 Hyto (Table 2).

Epicotyls from cv. Giza 2 which cultured on M1 also formed embryogenic callus with somatic embryos in the globular stage. However, these globular structures did not develop further and eventually became contaminated. The experiment was repeated many times without any success. Epicotyls from cv. 24 Hyto formed callus but not embryos.

The other treatment (M2) induced callus from epicotyls of each cv. but failed to induce callus from shoot tips where, shoots formed directly (Fig. 3A). These shoots were transferred to 1/2 B5 medium supplemented with 3 mg/l IBA for rooting. Epicotyls from cv. Giza 2 induced small calluses with a greenish- yellow color, but all failed to produce any somatic embryos. Only a few adventitious shoots were obtained from buds on the callus surface (Fig. 3B).

Calluses derived from epicotyls of 24 Hyto formed creamy- brownish masses (Fig. 1F), that increased in weight rapidly (Table 5). These calluses were embryogenic after 7 weeks of culturing on CIM2 medium (Fig. 2A). To develop the embryos, calluses were transferred to RM2 medium (Fig. 2B). Calluses developed and produced somatic embryos in clusters at different growth stages including globular and heart stages and the early torpedo stages (Fig. 2C). The embryos were separated at the beginning of the cotyledonary stage (Fig. 2D), and each transferred to hormone free medium (1/2 B5) for their development. The hormone free medium increased the numbers of embryos formed by stimulating many generations of somatic embryos with different stages forming on the first embryo explants. The adventitious embryos developed in the same medium to become young plantlets (Fig. 2E). These plantlets then transferred to the green house for adaptation.

The number of total shoots which developed from somatic embryos varied between each cultivar. In cultivar Giza 2, the calluses that derived from shoot tips were reproducible and produced a lot of somatic embryos. Hence within 8 months after the formation of the first embryo, 400 shoots per callus were developed from somatic embryos. However, in cultivar 24 Hyto, within 8 months, only 127 shoots had developed from somatic embryos derived from the epicotyl calluses.

Therefore cultivar differences and explant differences play important roles determining both callus formation and regeneration percentages; the type of structures that develop callus and the regeneration frequency of those structures (Tables 2 and 3).

A significant response towards callus, and regeneration percentage were observed, due to the hormonal compositions of each treatment (Table 4). Although the M2 medium had the highest value of the

Table 2. Effect of cultivar generally on fresh weight of callus, Callus (%) and regeneration through somatic embryogenesis (%).

Cultivar	Fresh weight of callus	Callus (%)	Calluses producing somatic embryos (%)
Giza 2	2.14[b]	69.38[b]	4.14[a]
24 Hyto	3.69[a]	72.91[a]	3.31[b]

Means followed by the same letter are not significantly different at $p \leq 0.05$ using Duncan's multiple range test.

Table 3. Effect of explants generally on, fresh weight of callus, callus (%) and regeneration through somatic embryogenesis (%) for both cultivars (Giza 2 and 24 Hyto).

Type of explant	Fresh weight of callus	Callus (%)	Calluses producing somatic embryos (%)
Shoot tip	1.64[b]	49.58[b]	6.76[a]
Epicotyl	4.19[a]	92.71[a]	0.70[b]

Means followed by the same letter are not significantly different at $p \leq 0.05$ using Duncan's multiple range test.

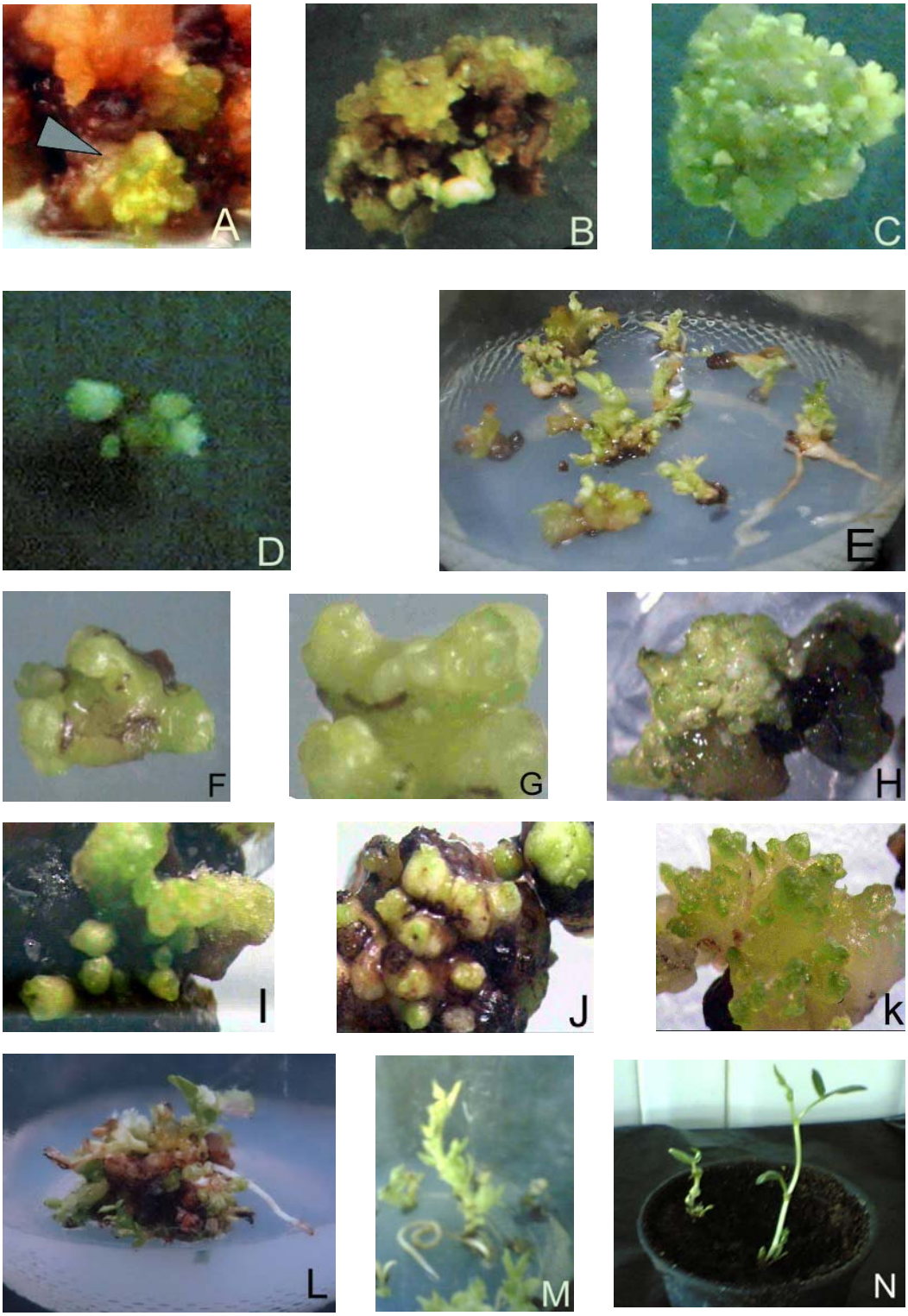

Fig. 2. *In vitro* regeneration through indirect somatic embryogenesis from *V.faba* cvs. Giza 2 and 24Hyto. **A–E** Somatic embryos derived from calluses of epicotyls of cv. 24 Hyto. **A** Embryogenic cells formed on callus derived from epicotyls in CIM2 medium. **B** Differentiation of the embryogenic cells in RM2 medium. **C** Different stages of somatic embryos were formed in RM2 medium including globular, heart and torpedo stages. **D** Maturation of somatic embryos into the cotyledonary stage in hormone free medium with asynchronous development. **E** The development of somatic embryos to young plantlets. **F–N** Different stages of somatic embryos of cv. Giza 2 which formed on RM1 medium from calluses derived from shoot tips. **F–G** The stages of embryos formation including formation of globular stage from a single embryo, this process took place in RM1 medium supplemented with 20 mg/l glutamine. **H** White-green structures identified as the early stages of somatic embryos were obtained on the previous medium including formation of embryogenic callus, these embryos were transferred subsequently to hormone free medium for their development. **I–K** Different stages of somatic embryos were formed in clusters in hormone free medium.**L** A cluster of somatic embryos developed before separating. **M** Development of embryos to young plantlets with shoots and roots in the hormone free medium. **N** Rooted plants that were transferred to the soil for acclimatization.

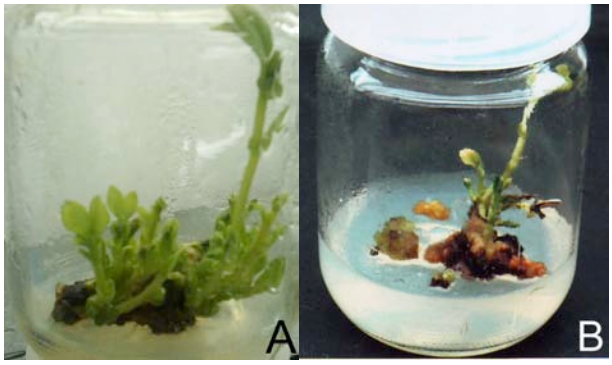

Fig. 3. **A** Direct organogenesis was formed from the shoot tips of cv. Giza 2 on CIM2 medium. **B** Adventitious shoots were formed from callus of epicotyls of cv. Giza 2 on M2 treatment.

callus fresh weight, the M1 treatment was the most effective in each of regeneration and callus percentages, so we detected that there was not any relationship between the weight of callus and the frequency of the regeneration.

Also the interactions between the three elements of this study (cultivars, explants and treatments) in all of the callus and regeneration percentages, showed that the best explant which led to the highest percentage of regeneration was the shoot tip of cv. Giza 2 using M1 treatment (Tables 5–8).

RAPD analysis

RAPD analyses were performed to investigate the genetic stability of the regenerated plants of each cultivar obtained from different treatments and different explants. Therefore six random primers were used (Table 1) to amplify DNA from many genomic locations. The 100 bp DNA molecular marker (with range of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1,000 bp) was used in this experiment. Among the RAPD profiles (Fig. 4) the cultivar Giza 2 showed identity between the control and the earliest regenerated plant in all profiles, except for new fragments detected at 1000 bp (OPA-04), 550 bp (OPA-05), and 500 bp of (OPA-07). Cultivar 24 Hyto showed identity in DNA banding patterns between the control and the earliest regenerated plant only with primer OPA-13. The other 5 primers derived DNA banding patterns showed new fragments at 300 bp (OPA-05) and 400 bp (OPA-09), whereas some fragments were absent at 750 bp (OPA-04), 450 bp (OPA-07) and 800 bp (OPA-10). Therefore, the cultivar Giza 2 exhibited more genetic stability than cultivar 24 Hyto.

Table 4. Effect of treatments generally on fresh weigh of callus, callus (%) and regeneration through somatic embryogenesis(%) for both cultivars (Giza 2 and 24 Hyto).

Treatment	Fresh weight of callus	Callus (%)	Calluses producing somatic embryos (%)
M1	2.66[a]	97.52[a]	6.89[a]
M2	3.17[a]	44.78[b]	0.56[b]

Means followed by the same letter are not significantly different at $p \leq 0.05$ using Duncan's multiple range test.

Table 5. Effect of interaction between treatments and cultivars on fresh weight of callus, callus (%), and regeneration through somatic embryogenesis (%).

Treatment	Cultivar	Fresh weight of callus	Callus (%)	Calluses producing somatic embryos (%)
M1	Giza 2	2.99[a]	96.20[b]	8.28[a]
	24 Hyto	2.33[a]	98.83[a]	5.50[b]
M2	Giza 2	1.29[a]	42.57[d]	0.00[d]
	24 Hyto	5.05[a]	46.98[c]	1.12[c]

Means followed by the same letter are not significantly different at $p \leq 0.05$ using Duncan's multiple range test.

Table 6. Effect of interaction between treatments and explants on fresh weight of callus, callus (%), and regeneration through somatic embryogenesis (%) for both cultivars (Giza 2 and 24 Hyto).

Treatment	Explant	Fresh weight of callus	Callus (%)	Calluses producing somatic embryos (%)
M1	Shoot tip	3.28[b]	99.17[a]	13.51[a]
	Epicotyl	2.04[c]	95.87[b]	0.27[c]
M2	Shoot tip	0.00[d]	0.00[d]	0.00[c]
	Epicotyl	6.35[a]	89.55[c]	1.12[b]

Means followed by the same letter are not significantly different at $p \leq 0.05$ using Duncan's multiple range test.

Table 7. Effect of interaction between cultivars and explants on fresh weight of callus, callus (%), and regeneration through somatic embryogenesis (%).

Cultivar	Explant	Fresh weight of callus	Callus (%)	Calluses producing somatic embryos (%)
Giza 2	Shoot tip	1.71[c]	49.33[c]	8.01[a]
	Epicotyl	2.57[b]	89.43[b]	0.27[d]
24 Hyto	Shoot tip	1.57[c]	49.83[c]	5.50[b]
	Epicotyl	5.82[a]	95.98[a]	1.12[c]

Means followed by the same letter are not significantly different at $p \leq 0.05$ using Duncan's multiple range test.

Discussion

This study describes a new reproducible system for plant regeneration via somatic embryogenesis using callus cultures derived from shoot tips and epicotyls of two Egyptian cultivars of *V. faba*. Many investigators have suggested that the difficulties of indirect regeneration from faba bean were due to the accumulation of phenolic compounds, which led to cell death (Böttinger *et al*., 2001), Here it was shown to be very important to avoid the accumulation of the phenolic compounds within the first two weeks of culturing the explants on the callus induction media. However some calluses that turned to brown or black colors later (Fig. 1 F), presumably due to the phenolic compounds, still showed a good ability to divide and produce several generations of somatic embryos. (Fig. 2 A, B, I, G). The addition of ascorbic and citric acids to the media helped to decrease or prevent the production or oxidation of the phenolic compound, therefore enhancing the production of somatic embryos. The addition of citric acid to media was reported by Anthony *et al*., 2004, to

Table 8. Effect of interactions among treatments, cultivars and explants on fresh weight of callus, callus (%), and regeneration through somatic embryogenesis (%).

Treatment	Cultivar	Explant	Fresh weight of callus	Callus (%)	Calluses producing somatic embryos (%)
M1	Giza 2	Shoot tip	3.42[b]	98.67[a]	16.02[a]
		Epicotyl	2.55[b]	93.73[b]	0.53[d]
	24 Hyto	Shoot tip	3.13[b]	99.67[a]	11.00[b]
		Epicotyl	1.53[c]	98.00[a]	0.00[d]
M2	Giza 2	Shoot tip	0.00[d]	0.00[d]	0.00[d]
		Epicotyl	2.59[b]	85.13[c]	0.00[d]
	24 Hyto	Shoot tip	0.00[d]	0.00[d]	0.00[d]
		Epicotyl	10.11[a]	93.97[b]	2.25[c]

Means followed by the same letter are not significantly different at $p \leq 0.05$ using Duncan's multiple range test.

prevent the browning and to enhance the formation of somatic embryos in *Conostephium pendulum*. Also the addition of ascorbic acid was beneficial for long-term common bean (*Phaseolus vulgaris*) callus maintenance (Mohamed *et al*., 1993).

In this study, callus induction media used enrichment with cytokinin (as BAP) that was higher in concentration than auxins in each treatment. Cytokinin was important for the formation of callus and initiation of somatic embryogenesis. Recently, attempts to regenerate plants via somatic embryogenesis focused on pre-cultivation of the initial plants on cytokinin- enriched medium (Veltcheva *et al*., 2005). High cytokinin to auxin ratios may modify the physiological state of the initial explants and initiate a somatic embryogenesis program.

The hormone free medium enhanced the development of somatic embryos through different stages (Fig. 5) and led to regeneration of complete plants (Pickardet *et al*., 1989; Albrecht and Kohlenbach, 1989). Also the hormone free medium enhanced the formation of secondary embryos.

Here, within 8 months after the formation of the first embryo, 400 shoots per callus were developed from the somatic embryos derived from shoot tips of the cultivar Giza 2 on M1 treatment. However, these calluses maintained embryogenic potential over a period of 14 months. The number of shoots was increased due to the effect of the hormonal composition of the M1 treatment. Further, the type of explant used (shoot tip) with its endogenous hormones may have played an important role in this regeneration system. In the M2 treatment within 8 months only 127 shoots per callus were developed from the somatic embryos that derived from calluses of epicotyl of cultivar 24 Hyto. The difference between each cultivar in the number of shoots was varied due to the type of explants used and the hormonal composition of each treatment. Cultivar genetics seems to have an important effect on the regeneration rate.

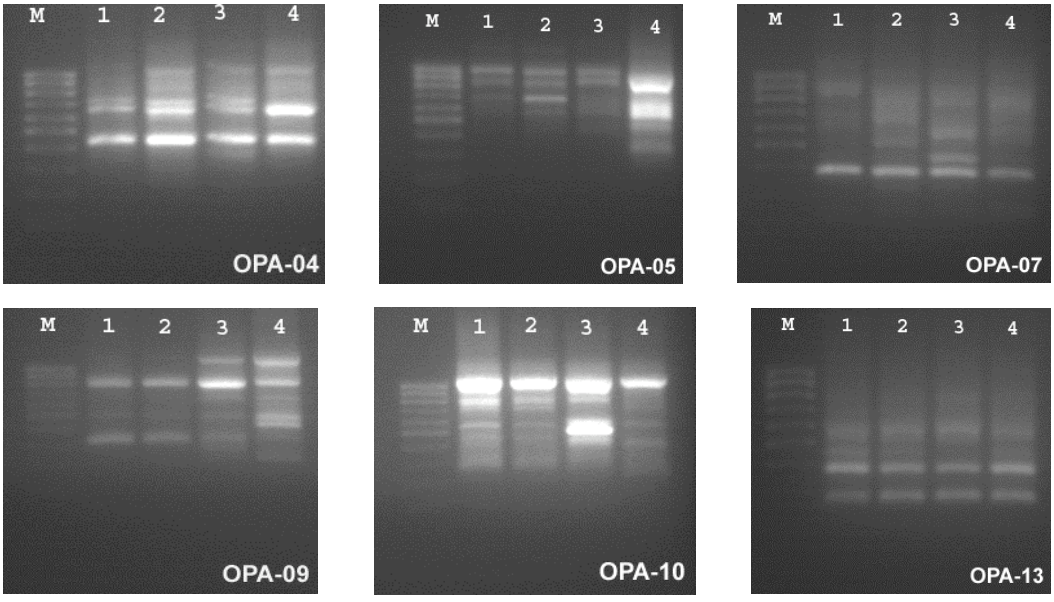

Fig. 4. RAPD profiles of faba bean cvs. were amplified with six RAPD primers. M: DNA molecular marker 100 bp. Lane 1: Control plant of cv. Giza 2 Lane 2: Regenerated plant of cv. Giza 2 via somatic embryogenesis . Lane 3: Control plant of cv. 24 Hyto. Lane 4: Regenerated plant of cv. 24 Hyto via somatic embryogenesis.

Fig. 5. Different stages of somatic embryogenesis (globular stage, heart stage, torpedo stage, cotyledonary stages, and young plantlets) of cv. Giza 2 were formed in the hormone free medium.

During the development of somatic embryos some of the developed plantlets formed roots rapidly in the same hormone free medium. However, sometimes the number of roots was up to 3 per plantlet (Fig. 6). A delay in the formation of roots was observed in some plantlets, in some cases the normal shoots were developed but root formation was suppressed.

Difficulties in the regeneration from the callus tissues of faba bean led to a limited number of publications on cell and tissue culture of this species. A regeneration system of faba bean through indirect somatic embryogenesis was reported by Griga *et al.* (1987). An indirect somatic embryogenesis system for *Vicia narbonensis* was reported by Pickardet *et al.* (1989) and also reported by Albrecht and Kohlenbach (1989). Another kind of regeneration is the organogenesis that was described in faba bean by Taha and Francis, (1990), Tegeder *et al*. (1995) and Böttinger *et al*. (2001).

Fig. 6. Plants developed from somatic embryogenesis of cv. Giza 2 with number of roots ranged from 1–3 roots.

PCR-based RAPD (Random amplified polymorphic DNA) markers have been applied to many types of genetic studies because of their simplicity and ease of use (Gutierrez *et al*., 2006). In this study, RAPD analysis was performed to identify potential somaclonal variations among the regenerated plants from somatic embryogenesis. To investigate the effects of the media and subcultures, on genetic stability, the regenerated plants were obtained after long-term of subcultures (after about 13 months of the first culture, from the later generations).

More new bands were detected in the regenerated plants of cultivar Giza 2 while more bands were missing in regenerated plants of cultivar 24Hyto. Both the deletions underlying the loss of DNA fragments and the modifications underlying the appearance of new fragments among the regenerated plants may be used to help to improve faba bean genetically. Furthermore, RAPD analysis could be used to trace the genetic changes in the regenerated plants at the genome level. RAPD polymorphism in callus-derived plants has been reported in wheat by Brown *et al*., 1993, and turmeric (Salvi *et al*., 2001). However, Isabel *et al.* (1993) detected no polymorphism in regenerated plants of *Picea Mariana*. The source of the explants, mode of regeneration (somatic embryogenesis/ organogenesis/axillary bud multiplication), genotypes used and the plant growth regulators applied are known to play a major role in determining the presence or absence of variations (Razdan, 1994; Damasco *et al*., 1996). Several investigators (Link *et al*., 1995; Torres *et al*., 1993; Vaz Patto *et al*., 1999; Gutierrez *et al*., 2006; and Gutierrez *et al*., 2007) used RAPD molecular markers to study the genetic variability and relationships among accessions, lines and cultivars of faba bean. Therefore, a link between natural and induced genetic variation might be made by further analyses.

Acknowledgments

We thank Dr. Said Abd-ELTawab (Cotton Research institute, Agricultural Research Center, Egypt) for his assistance with statistical analysis.

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