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Genetic Engineering Studies In Some Plants

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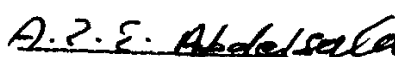
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Abstract

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Faba bean (*Vicia faba* L.) is one of the most important legume crops worldwide particularly in Egypt. The present investigation was designed to establish the regeneration and transformation systems of two cultivars of faba bean (cv. G 461 and G 674). The regeneration system was carried out via organogenesis using shoot apices and cotyledon explants obtained from the two cultivars used. The VSI medium (MS plus B5 vitamins) containing different concentrations of BAP and NAA for callus as well as shoot induction was used and the cotyledon explants appeared to be more effective than shoot apex explants. The VSI medium which was supplemented with 2 mg/l BAP and 1 mg/l NAA and 3 mg/l BAP and 0.05 mg/l NAA were successful for producing calli for the two cultivars, respectively, after 3 weeks from culturing at $28 \pm 2^\circ\text{C}$ and photoperiod 16/8 h light/dark. The VSI medium combination with 5 mg/l BAP and 0.5 mg/l NAA and 3 mg/l BAP and 0.05 mg/l NAA were the best media for shoot induction using the shoot apex explants from the two cultivars (G 461 and G 674), respectively. Six different media were used for root induction and the M4 medium (B5 medium free hormones) was the most successful one. For transformation of faba bean, the same explants of the two cultivars used were transformed via *Agrobacterium*-mediated gene transfer system. The *Agrobacterium* strain was transformed by introducing the pBI121 plasmid carrying NPTII and GUS genes which was produced in a large scale production from *E. coli*. The explants (cotyledon and shoot apices) were then transformed by co-cultivation with the recombinant *Agrobacterium* in VSI liquid medium for 24 and 48 h. Using microprojectile bombardment. The cotyledon explants and calli derived from shoot apex explants were transformed by shooting with different concentrations of DNA plasmid at a distance of 6 cm and 1100 or 1350 psi. The transformants were selected on VSI medium with 100 mg/l kanamycin. The results showed that the concentration of 500 ng/ μl and 1100 psi were the most suitable conditions for explants transformation. The transformants were evaluated for the presence of NPT II by PCR and Southern blot hybridization with a DNA probe labelled with ^{32}P , while the expression of the introduced GUS gene was determined by GUS assay.

Key Words: Faba bean, Regeneration, Organogenesis, *Agrobacterium*-mediated gene transformation, Microprojectile bombardment, GUS assay, NPT-II gene, PCR, Southern blot.

Contents

	Page
1. Introduction	1
2. Review of literature	3
2.1. Regeneration in legumes	3
2.1.1. Regeneration via organogenesis in legumes	3
2.1.1.1. Regeneration of <i>Vicia faba</i> via organogenesis	5
2.1.2. Regeneration via embryogenesis in legumes	6
2.1.2.1. Regeneration of <i>Vicia faba</i> via embryogenesis	8
2.1.3. Rooting stage	8
2.2. Transformation in plants	9
2.3. Kanamycin sensitivity of culture tissues	10
2.4. <i>Agrobacterium</i> -mediated plant transformation	12
2.4.1. Genetic colonization of plant via <i>Agrobacterium</i>	12
2.4.2. <i>Agrobacterium</i> infection and molecular organization of Ti- plasmid	13
2.4.3. Ti- plasmid vectors for introducing genes into plants	14
2.5. Transformation in legumes	16
2.5.1. <i>Agrobacterium</i> -mediated gene transfer in legumes	16
2.5.2. Transformation using biolistic gun	18
2.5.2.1. Transformation of legume(s) by microprojectile bombardment	20
2.6. Detection of transformed plants	20
3. Materials and Methods	23
3.1. Materials	23
3.1.1. Source of seeds	23
3.1.2. Bacterial strains and plasmids	23
3.2. Methods	24
3.2.1. Establishment of regeneration system in <i>V. faba</i> via organogenesis	24
3.2.1.1. Seed sterilization	24
3.2.1.2. Seed germination	25
3.2.1.3. Explants	25
3.2.1.4. Callus and shoot induction	25
3.2.1.4.1. Using shoot apex explants	25
3.2.1.4.2. Using cotyledon explants	25
3.2.2. Rooting stage	26
3.3. Establishment of transformation system in <i>V. faba</i>	26
3.3.1. Kanamycin sensitivity	26
3.3.2. <i>Agrobacterium</i> -mediated gene transfer system	27
3.3.2.1. Isolation and purification of DNA plasmid	27
3.3.2.2. Plasmid digestion	29

3.3.3.	<i>Agrobacterium</i> transformation	29
3.3.3.1.	The 3-Ketolactose test	29
3.3.3.2.	Preparation of <i>Agrobacterium</i> competent cells	29
3.3.3.3.	Transformation procedure	30
3.3.4.	Transformation of <i>V. faba</i>	30
3.3.4.1.	<i>Agrobacterium</i> preparation	30
3.3.4.2.	Transformation procedure	31
3.3.5.	Transformation via microprojectile bombardment	31
3.3.5.1.	Large scale production of DNA plasmid	31
3.3.5.2.	Particles sterilization and coating	31
3.3.5.3.	Preparation of shoot apex and cotyledon explants for bombardment	32
3.3.5.4.	Bombardment of explants	32
3.4.	Selection of transgenic tissues	33
3.5.	Detection of transgenic tissue	33
3.5.1.	Histochemical assay	33
3.5.2.	PCR detection	33
3.5.2.1.	Extraction of total nucleic acids	33
3.5.2.2.	PCR reaction	34
3.5.3.	Southern blotting hybridization	35
3.5.3.1.	DNA blotting	35
3.5.3.2.	Probe preparation	36
3.5.3.2.1.	Purification of NPT-II insert	36
3.5.3.2.2.	Probe labelling system	36
3.5.3.2.3.	Hybridization	37
3.5.3.2.4.	Autoradiography	38
4.	Results and discussion	43
4.1.	Establishment of regeneration system in <i>V. faba</i> via organogenesis	43
4.1.1.	Callus induction stage	44
4.1.2.	Shoot induction stage	52
4.1.3.	Rooting stage	57
4.1.4.	Acclimatization stage	57
4.2.	Establishment of transformation system in <i>V. faba</i>	61
4.2.1.	Kanamycin sensitivity	61
4.2.2.	Confirmation of the plasmid used for transformation	63
4.2.3.	<i>Agrobacterium</i> -mediated transformation	64
4.2.3.1.	3-Ketolactose test	64
4.2.3.2.	Transformation of <i>Agrobacterium</i>	65
4.2.3.3.	Transformation of <i>V. faba</i>	66
4.2.3.3.1.	Via <i>Agrobacterium</i> system	66
4.2.3.3.2.	Via biolistic gun system	68
4.2.4.	Detection of transgenic tissue	70
4.2.4.1.	Histochemical assay	70

4 2 4 2	PCR detection	74
4 2 4 3	Southern blot hybridization	74
5	Summary	78
6	References	80
7	Arabic summary	

List of Tables

Table		Page
1	Survival, callus induction and average weight of calli on MS medium plus B 5 vitamins and different concentrations of NAA and BAP for shoot apex explants of <i>V. faba</i> cv. G 461.	46
2	Survival, callus induction and average weight of callus regenerated on MS medium plus B 5 vitamins and different concentrations of NAA and BAP for shoot apex explants of <i>V. faba</i> cv. G 674.	47
3	Survival, callus induction and average weight of callus regenerated on MS medium plus B 5 vitamins and different concentrations of NAA and BAP for mature cotyledon explants of <i>V. faba</i> cv. G 461.	48
4	Survival, callus induction and average weight of callus regenerated on MS medium plus B5 vitamins and different concentrations of NAA and BAP for cotyledon explants of <i>V. faba</i> cv. G 674.	49
5	Shoot induction using MS medium with B 5 vitamins and containing different concentrations of NAA and BAP with shoot apex explants obtained from <i>V. faba</i> cv. G 461.	53
6	Shoot induction using MS medium with B5 vitamins and containing different concentrations of NAA and BAP with shoot apex explants obtained from <i>V. faba</i> cv. G 674	54
7	Selection of kanamycin resistant explants co-cultivated with <i>A. tumefaciens</i> carrying pBI121 plasmid containing NPT II and GUS genes	68
8	Effect of different DNA concentrations and pressures on transformation of <i>V. faba</i> cotyledon explants and calli from shoot apices using biolistic gun technique	69

List of Figures

Figure		Page
1	The binary Ti based plasmid pBI121 carrying NPT-II as selectable marker for Kanamycin resistance and GUS genes. The plasmid had T-DNA left and right borders.	24
2	Callus induction using cotyledon explants obtained from <i>V. faba</i> after 3 weeks from culturing on VS/ medium.	50
3	Callus induction using shoot apex explants obtained from <i>V. faba</i> after 3 weeks from culturing on VS/ medium.	51
4	Production of plantlets from shoot apex explants of <i>V. faba</i> 4 weeks post culturing on VS/ medium.	55
5	Shoot induction and elongation of cotyledon explants obtained from <i>V. faba</i> cv. G 674 four weeks post culturing on VS/ medium.	56
6	Rooting stage of <i>V. faba</i> cvs. G 461 and G 674 using different media.	58
7	Complete shoot cultured on a rooting medium (B5 major and minor salts without hormone).	59
8	Normal regenerated <i>V. faba</i> plants acclimatized using soil mixture composed of peat-moss:clay (2:1,v/v).	60
9	Effect of Kanamycin concentration on non transformed tissues.	62
10	Electrophoresis separation of the undigestion and digestion products of plasmid pBI121 with different restriction enzyme.	63
11	3-Keto lactose test of <i>A. tumefaciens</i> strain EHA101	65
12	PCR product profiles of <i>A. tumefaciens</i> strain EHA101 transformed by pBI121 plasmid containing NPT-II and GUS genes.	66
13	Histochemical assay of β - glucuronidase (GUS) gene in transformed cotyledon explants in <i>V. faba</i> , via <i>Agrobacterium</i> transformation.	71
14	Histochemical assay of β - glucuronidase (GUS) gene in transformed calli derived from shoot apex explants in <i>V. faba</i> , via <i>Agrobacterium</i> transformation.	72
15	Histochemical assay of β - glucuronidase (GUS) gene in transformed cotyledon explant(s) of <i>V. faba</i> after 48 h from transformation by bombardment.	73
16	PCR products (875 bp) amplified from the total	75

	nucleic acid extracts prepared from some putative transgenic explants or calli.	
17	Southern blot detection of PCR products amplified from the total nucleic acid extracts prepared from some putative transgenic explants or calli.	75

1. Introduction

Faba bean (*Vicia faba* L.) is one of the most important legume worldwide (Haider *et al.*, 1994). It is grown as a winter annual crop in warm temperate and subtropical areas and it is now introduced and cultivated in north and south America, China and Uganda.

Egypt is reported to be the third country in the production of faba bean as produces 444,800 metric tons (Anonymos, 1997). Faba bean is not only important for human nutrition but also for animal feed due to its high protein content as the dry seeds contain 25-30% protein with almost 50-60% soluble carbohydrates, 10-15% fibers and 5% minerals specially phosphorus and potassium (Saleh, 1998).

A dramatic reduction in seed yield/ha and total production of faba bean is mainly due to the infection by different pathogens that decrease the production of the crop. Traditional plant breeding so far has not overcome this problem, the recent development in recombinant DNA technology (Antonio *et al.*, 1988) and plant transformation (Selva *et al.*, 1989) may allow alternative approaches to enhance faba plants resistance to such pathogens. Recent advances in genetic engineering have clearly demonstrated the possibility of incorporating foreign genes for desired agronomic traits while preserving the existing characteristic of improved genotypes (Powell-Abel *et al.*, 1989).

The interest in tissue culture research on legumes has increased considerably, as an important initial step in introducing new genes through recombinant DNA technology (Schroeder *et al.*, 1984 and Thomashow *et al.*, 1984). Through this technique, improving nutritional quality, resistance to environmental stress, resistance to viral diseases and increasing the yield of legume crops can be achieved (Hussey *et al.*, 1989). Plant regeneration from somatic cells was difficult to be

achieved among large seeded leguminous species in contrast to forage legumes and from all large seeded legumes, faba bean has received little attention (Tegeder *et al.*, 1995).

This work was designed to study the following items:

1. Establishment of regeneration system for *V. faba* cultivars Giza 461 and Giza 674 via organogenesis using different explants and different combination of cytokinin(s) and auxin(s).
2. Establishment of transformation system for *V. faba* cvs. Giza 461 and Giza 674 via *Agrobacterium*-mediated gene transfer system and/or microprojectile bombardment using NPT-II and GUS genes.
3. Evaluation of the transformed tissues and/or plants by polymerase chain reaction (PCR), Southern blot hybridization and histochemical assay.

2. Review of Literature

The procedures of plant tissue culture have been developed to such a level that any plant species can be regenerated *in vitro* through three methods, organogenesis, somatic embryogenesis and embryo culture (Murashige, 1974). He also reported that several processes of plant regeneration for each species could be used but usually only one type is most efficient. The rate of plant regeneration in tissue culture varies greatly from one species to another (Evans *et al.*, 1981).

2. 1. Regeneration in legumes

Plant regeneration from somatic cell *in vitro* culture has been difficult to achieve among large seeded leguminous species, in contrast to forage legumes (Morginski and Kartha, 1984). However, successful plant regeneration has been reported in several species of seed legumes such as *Vigna unguiculata* (Kartha *et al.*, 1981), *Cicer arietinum* (Kartha *et al.*, 1981), *Arachis hypogae* (Morginski *et al.*, 1981), *Vicia faba* (Busse, 1986), *Paseolus vulgaris* (Allavena and Rossetti, 1986), *Glycine max* (Barwale *et al.*, 1986 and Wright *et al.*, 1987) and *Lens culmaris* (Saxena and King, 1987 and Malik and Saxena, 1992) and *Pisum sativum* (Malik and Saxena, 1992).

2.1.1. Regeneration via organogenesis in legumes

The production of adventitious shoots *in vitro* is more common and easier to control than the development of somatic embryos from cultured explants. Murashige (1974) reported that regeneration of plants through organogenesis can be achieved through one of the following modes, production of adventitious organs from a callus developed from the explant, emergence of adventitious organs directly from the explant without an intervening callus phase, and production of plantlet from out growth of axillary buds.