GENETIC TRANSFORMATION AND REGENERATION OF COMMON BEAN (*Phaseolus vulgaris* L.) USING *Agrobacterium* SYSTEM

E. A. EISSA

Genetics Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt

Common beans (*Phaseolus vulgaris* L.) are the most important food legume in the developing world, and an important human dietary constituent being a rich source of protein for millions of people (Colpaert *et al*., 2008; Kelly, 2010; Amugune *et al*., 2011; Kwapata *et al*., 2012). Immature pods of common beans are popular as a vegetable for human consumption in Africa, Asia and Latin America (Veltcheva *et al*., 2005; Delgado-Sánchez *et al*., 2006; Varisai Mohamed *et al*., 2006; Gatica Arias *et al*., 2010; Kwapata *et al*., 2010). Therefore, there is considerable interest in the introduction of useful traits into beans. The lack of an efficient regeneration system for common bean is a major bottleneck to genetically transform this species. Plant biotechnology, and conventional breeding methods, could facilitate bean improvement since resistance to biotic and abiotic stress could be increased (Liu *et al*., 2005). Nevertheless, efficient *in vitro* culture system that results in efficient regeneration is an essential requirement for improvement of common bean through genetic transformation (Svetleva *et al*., 2003; Kwapata *et al*., 2010). In addition to genetic improvement, *in vitro* culture is an important tool for the recovery and conservation of germplasm (Delgado-Sánchez *et al*.,

2006). With the development of transformation technology, genetic engineering has emerged as an alternative, potentially important approach to create new varieties.

Difficulties in obtaining plants from somatic cells in common beans have till now hampered the production of transgenic plants by the application of the most common methods available for the introduction of foreign DNA into cells. Regeneration from callus in *P. vulgaris* is hard to attain but it has been reported only by Dillen *et al*. (2000) and Arellano *et al*. (2009). Direct regeneration from explants via shoot organogenesis, is certainly recommendable for gene transfer technology. However, most large seeded legumes have proven to be recalcitrant to regeneration. In case of beans, shoot regeneration can be an important step in plant transformation procedures, and many attempts have been made with results showing that the genotype and the hormones both have great effect (Eissa *et al*., 1999 and 2002; Gatica Arias *et al*., 2010; Kwapata *et al*., 2010). Though scientists have tried extensively, there is still little reproducible and efficient protocol for bean genetic transformation (Kwapata *et al*., 2012). Micropropagation of *Vicia faba* L. culti-

vars has been achieved by somatic embryogenesis and nodal segment explants raised seedlings (Hamdy and Hattori, 2006). Naz *et al*. (2007) reported the efficient plant regeneration from chick pea *via* direct regeneration that can be used in genetic transformation techniques.

Successful plant transformation requires specific knowledge of *Agrobacterium*-host compatibility (Godwin *et al*., 1992). *Agrobacterium*-mediated gene transfer can be one of the methods of choice to introduce gene/genes into common beans. The use of the naturally occurring compound acetosyringone in a preinoculation culture step has been shown to increase the level of virulence in *Agrobacterium* (Vernade *et al*., 1988) and lead to higher transformation rates (Becker *et al*., 1994; Lee *et al*., 2001).

Several protocols have been reported for shoot organogenesis from apical or axillary meristems in *Phaseolus* (McClean and Grafton, 1989; Franklin *et al*., 1991; Malik and Saxena, 1991 and 1992; Mohamed *et al*., 1992; Eissa *et al*., 2002; Veltcheva and Svetleva, 2005; Delgado-Sánchez *et al*., 2006; Varisai Mohamed *et al*., 2006) or through somatic embryogenesis (Zambre *et al*., 1998; Schryer *et al*., 2005). Although several protocols have been described for bean regeneration, an optimal *in vitro* culture system still remains a major challenge since this species from the *Phaseolus* genus are recalcitrant for regeneration (Veltcheva *et al*., 2005). The best characterized and most commonly used trans-

formation system for plants is the disarmed *A. tumefaciens* strains. Since long time, preliminary data regarding the capability of *Phaseolus* to establish a compatible reaction with *Agrobacterium* were reported by Allavena (1985) and McClean *et al*. (1988). Putative transformants have been reported in one common bean cultivar (Mariotti *et al*., 1989). While McClean *et al*. (1991) were able to introduce genes into beans using the *Agrobacterium* system; they were unable to regenerate transgenic plants. Early efforts to produce transgenic bean plants failed due to poor DNA delivery and regeneration systems. Franklin *et al*. (1993) produced transformed *gusA* positive callus from a kidney bean cultivar inoculated with *Agrobacterium* strain EHA101. So far, transformation of *P. vulgaris* has been achieved through direct gene transfer into the apical meristem region of seedlings of one cultivar (Russell *et al*., 1993). Embryonic axes and the apical meristems of *P. vulgaris* exposed using biolistic method, however, the frequency of transgenic plants they obtained was much lower 0.03% (Aragão *et al*., 1996), and Aragão *et al*. (1999) attempted also to introduce a gene coding for methionine in bean via the same method.

To date only a few reports are available on attempts to transform common beans. Therefore, the objective of the present study was to optimize an *in vitro* plant transformation and regeneration systems for two commercial common beans varieties (*P. vulgaris* L.). Two *Agrobacterium* strains were used, one containing the

neomycin phosphotransferase II (*nptII*) and *β*-glucoronidase (*gusA*) marker genes, and the other containing phosphinothricin acetyl transferase (*bar*) and *β*-glucuronidase (*gusA*) marker genes. A marker gene and a selectable gene were used in co-transformation of regenerated common bean plants. According to these experiments engineered strains of *A. tumefaciens* were used to attempt transgenic plant recovery directly from common bean meristematic tissues. Using an optimized regeneration protocol via organogenesis from axillary and adventitious buds derived from nodes explants obtained kanamycin resistant, *gusA*-positive shoots and plantlets, and herbicide resistant, *gusA*-positive shoots and plantlets. The achieved results have been utilized for establishing genetic transformation and regeneration method in order to improvement common beans varieties.

MATERIALS AND METHODS

Plant materials and surface sterilization method of seeds

Two commercial common beans (*Phaseolus vulgaris* L.) varieties "Fönix" and "Maxidor" seeds were used as source of plant materials. The seeds are kindly supplied by the Genetics and Horticultural Plant Breeding, Faculty of Horticultural Sciences, Corvinus University, Budapest, Hungary, to whom my thanks are due. For surface-sterilization method, seeds were selected based on a healthy and similar size. Seeds were soaked for 4 min in 75% ethanol, disinfected with 20% solution of commercial bleach containing (5.25%

sodium hypochlorite) with four drops of tween 20 solution for 20 min and rinsed three times with sterile double-distilled water.

In vitro seeds germination method and explants preparation

Surface sterilized seeds of the common beans were then germinated in test tubes on MS (Murashige and Skoog, 1962) agar medium. The pH of the media was adjusted to 5.7 with KOH and supplemented with 30 g/l sucrose and 7 g/l agar. Media were dispensed into glass tubes or bottle glasses, autoclaved at 120°C for 20 min. The explant cotyledonary nodes containing a portion of the hypocotyl, epicotyl and cotyledons cultures were initiated and prepared from excised three-days-old seedlings. Approximately, 2/3 of each cotyledon was removed from the explant, leaving the cotyledon fragments attached to the nodal region. Full strength MS medium, supplemented with 1 mg/l BA (6-benzyladenine) and 0.1 mg/l NAA (α-naphthalene acetic acid) were used for shoot induction from the explant nodes. Phenolics compound acetosyringone was added after autoclaving when media were cooled to below 60°C. The presence of acetosyringone during co-cultivation generally enhanced the virulence of *Agrobacterium* strains. Axenic seeds explant cultures for transformations and regenerations were incubated on the media at 24-26°C under 16/8 hours photoperiod at white light in growth cabinet. The early events of multiple shoot

formation were examined using scanning electron microscopy.

Agrobacterium strains, binary vectors and cultures

The disarmed *A. tumefaciens* strain EHA101 contains the binary plasmid (pEHA101::pTd33), and strain A281 (pRGG bar H1) were used in the present work for transformation experiments. Strain EHA101 is kindly provided by Dr. Oláh Róbert (Corvinus University, Budapest, Hungary). Strain A281 is kindly provided by Prof. István Nagy (Agricultural Biotechnology Center ABC, Gödöllő, Hungary). The binary vector (pEHA101::pTd33) encodes a plant expressible neomycin phosphotransferase II (*nptII*) gene as selectable marker and *β*-glucuronidase (*gusA*) sequence coding region as a reporter gene. The protein product of *nptII* gene phosphorrylates kanamycin and renders the drug non toxic to plant cells. The binary vector (pRGG bar H1) contains *bar* gene as a selection marker, encodes a modifying enzyme phosphinothricin acetyl transferase (PAT), which confers resistance to herbicide phosphinothricin (PPT), bialaphos as selectable marker and *gusA* as a reporter gene (Table 1). The bacterial cultures of each *Agrobacterium* strain were grown in solid Luria Bertani (LB) medium (10 g/l bacto-tryptone, 5 g/l bacto-yeast extract, 10 g/l NaCL) with appropriate antibiotic kanamycin at concentration of 50 mg/l. The cultures of each bacterial strain were incubated over-night at 28ºC.

Explants inoculation with Agrobacterium strains

The procedure used to obtain common bean plants resistant to kanamycin or phosphinothricin began with the germinating "Fönix" and "Maxidor" seeds on MS medium for three days. Explants (cotyledonary nodes) of common beans were inoculated with *Agrobacterium* strain EHA101 or A281. Hence, for each variety, 500 explants were cultured, cocultivated with each strain. Freshly streaked an overnight cultures of each *Agrobacterium* strain were suspended in MS medium (pH 5.2) or sterile distilled water. 100 µM Acetosyringone were added to the co-cultivation medium plus 1 mg/l BA and 0.1 mg/l NAA to increase the infection rate of *Agrobacterium* on the regenerated buds and shoots including differentiating tissues around the nodal region. Cotyledonary nodes with lateral meristems explant were excised, and the nodal region was punctured numerous times wounded and inoculated below and above the point of excision with *Agrobacterium* strains EHA101 (pEHA101::pTd33) or A281 (pRGG bar H1). Either by immersion in a diluted overnight bacterial suspension for three hours or by repetitive puncturing of meristems with a needle or a surgical blade previously dipped or stabbed into the diluted bacterial culture. The actively dividing cells resulting from wound-induced cell division were conceived to show more transformation. Newly synthesized cell wall was found to be essential for the production attachment of *Agrobacterium* pre-

ceding transformation. The active DNA replication of rapidly dividing cells was also proposed to be very important for the integration of plasmid DNA. The explant nodes were cultured on solidified co-cultivation media for three days. The explant nodes were then washed in sterile water, blotted dry on sterile paper towels, and cultured on MS regeneration media without the use of any chemical selections for 24 hours, to allow expression of the resistance gene. The regeneration media are containing 1 mg/l BA, 0.1 mg/l NAA, 500 mg/l carbenicillin and 800 mg/l cefotaxime, and pH 5.7.

Sensitivity of cotyledonary node explants to kanamycin, and bialaphos (phosphinothricin)

An effective concentration of kanamycin, and bialaphos for the selection of transformants were assessed by preliminary tests. For both bean varieties, cotyledonary node explants were cultured on shoot regeneration MS media, plus various concentrations of kanamycin (0, 50, 75, 100, 150 and 200 mg/l), and bialaphos (0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l). Kanamycin and bialaphos was filter sterilized and added to the media after autoclaving. Kanamycin at a concentration of 100 mg/l, and bialaphos at a concentration of 4 mg/l were used for the selection of transformed shoots and plants for both bean varieties.

Stable genetic transformation, kanamycin, and bialaphos resistance

Co-cultivated nodes with strain EHA101, or A281 were transferred at first to selection media plus only 50 mg/l kanamycin, or 1 mg/l bialaphos, and 500 mg/l carbenicillin and 800 mg/l cefotaxime to eliminate *Agrobacterium* overgrowth for one week. Nodes were then transferred to selection media plus 100 mg/l kanamycin, or 2 mg/l bialaphos. The transformed explant nodes were transferred three times at three day intervals to fresh MS media plus 500 mg/l carbenicillin and 800 mg/l cefotaxime to eliminate *Agrobacterium* overgrowth of the target regions. The explants of both bean varieties co-cultivated with strain EHA101 were then transferred to MS medium plus 200 mg/l kanamycin and 500 mg/l carbenicillin. The kanamycin concentration was increased incrementally over three months period to 300 mg/l. To prevent the escape of non-transformed buds and shoots regenerated from the explants of both two varieties co-cultivated with strain A281, 2 mg/l bialaphos was added to regeneration medium as a selection agent. The selection of stable transgenic shoots for both bean varieties was based on the use of 4 mg/l bialaphos selection for the *bar* herbicide resistance marker gene, and stable transgenic green shoots continued on this medium. Putative transformed green shoots were transferred onto kanamycin, or bialaphos containing media to verify their ability to root.

Histochemical staining of transformed tissues

Segments from putative transgenic green shoot and plant tissues for both bean varieties selected on kanamycin, or

bialaphos transformed with the (pEHA101::pTd33), and (pRGG bar H1) were tested and assessed for expression of the *gusA* reporter gene by the histochemical assay using X-gluc. This substrate works very well, giving a blue precipitate at the site of enzyme activity. All of the procedures using the *gusA* reporter gene system are based on the work of Jefferson (1987). The vials samples were then incubated in a thermostat at 37°C dark incubator for overnight, next morning the samples are checked.

RESULTS AND DISCUSSION

The results obtained in this investigation showed that full strength MS medium supplemented with 1 mg/l BA and 0.1 mg/l NAA were the optimal for shoot induction from meristematic ring of cotyledonary nodes explant in both bean varieties "Fönix" and "Maxidor" (Fig. 1). Early events on the development of highly regenerable tissue culture procedure from the meristematic ring on nodal explants were examined by scanning electron microscope (SEM) in both bean varieties after 48 hours cultured on MS regeneration medium, which showed organogenesis and bud formation from the meristematic cells in the cotyledonary nodes explant (Fig. 2). The results indicated that the shoots were formed from actively dividing cells located at the axillaries bud regions. A method was designed to optimize rapid and high frequency direct shoot regeneration of the commercial important common beans. Therefore, this method for regeneration was suitable for

indirect *Agrobacterium*-mediated transformation protocol and used in transformation and regeneration experiments in both common bean varieties. *Agrobacterium* strain EHA101 (pEHA101::pTd33) and A281 (pRGG bar H1) attachment to cotyledonary nodes explant in both common bean varieties were examined by scanning electron microscope during *Agrobacterium*-mediated transformation. Scanning electron microscope studies demonstrated that cells of *A. tumefaciens* strains attach to cells of cotyledonary nodes in both common bean varieties (Fig. 3). The development of two common beans varieties "Fönix" and "Maxidor" plants that are able to grow in the presence of high concentrations of kanamycin and that express the gene product required, for resistance to the drug, or in the presence of high concentrations of phosphinothricin and that express the gene product required, for resistance to the herbicide were reported.

Transformation and successful regeneration procedures were based on a direct shoot organogenesis protocol previously described by Zhang *et al*. (1997) and Eissa *et al*. (2002). The natural ability of *A. tumefaciens* to introduce DNA into plant cells is being widely exploited for the genetic transformation of plants (van Wordragen and Dons, 1992). This system is, therefore, considered to be suitable for the transformation of common beans. It was tried to improve the *Agrobacterium* infection by the application of acetosyringone which is a phenolic compound produced by the wounding of plant

tissues and which induces the transfer of T-DNA from *Agrobacterium* to the plant genome (Godwin *et al*., 1991; Lee *et al*., 1995). *Agrobacterium* infection can readily deliver DNA in *Phaseolus* (McClean *et al*., 1991). The lack of *in vitro* regeneration capacity in *P. vulgaris* has prompted investigators to devise regeneration independent approaches, and one such approach has met with success (Russell *et al*., 1993). Apical meristems of seedlings were submitted to particle bombardment, and after stimulation of axillary shoot proliferation, transgenes plants were recovered at 0.03% of shoots. Using the same strategy, Aragão *et al*. (1996) obtained transformants of cv. Olathe. Aragão and Rech (1997) hypothesized that a good ability for axillary bud formation, and proper apex morphology the features render a genotype suitable for transformation through meristem bombardment. Success with bean transformation has been very limited; the few successful transgenic bean varieties have been developed by physical methods that circumvent problems with plant regeneration (Kelly, 2010). In the present study, the *nptII* gene coding was tested by selection of kanamycin stable transgenic shoots. The transgenic character of kanamycin-resistant shoots was confirmed by detection of *β*-glucuronidase activity in resistant tissues. Thus, these studies provide strong confirmation for a kanamycin resistant gene transferred to plant cells. Similar results were achieved by Jaiwal *et al*. (2001) who regenerated transformed shoots directly from cotyledonary node explants of mungbean cultured on medium contain-

ing 75 mg/l kanamycin after co-cultivation with LBA4404 (pTOK233). Dillen *et al*. (1995) delivered DNA to intact embryonic axes of common bean through electroporation and expression of the *gusA* reporter gene was observed in tissues.

In the present research, addition of 100 μM acetosyringone to freshly cut cotyledonary nodes tissues could increase the transformation efficiency. The increase in the transformation efficiency of freshly cut tissues in the presence of acetosyringone suggests that the synthesis of *vir* inducers during incubation, and the accumulation of *vir*-inducing compounds in the wounded and preincubated plant tissues are an important factors contributing to the increased competence of the incubated common bean cotyledonary node tissues to *Agrobacterium*-mediated transformation. Stimulation of plant cell division and activation of the DNA replication machinery during the incubation period may play an important role in the integration of plasmid DNA leading to stable transformation (Baron and Zambryski, 1995). The results of the current work showed that the use of cotyledonary node explants is more efficient compared for genetic transformation of a commercial important *P. vulgaris* common bean varieties.

Sensitivity of cotyledonary node explants to kanamycin, and herbicide

A gene coding for the enzyme neomycin phosphotransferase II, or phosphinothricin acetyl transferase is ex-

pressed constitutively in host plant cells to which it is transferred during transform induction and confers kanamycin, or herbicide phosphinothricin (bialaphos) resistance and transcripts on transformed plant cells, and confers on transformed cells the ability to grow in the presence of normally lethal levels of the antibiotic kanamycin, or herbicide (bialaphos). Kanamycin and phosphinothricin a widely used markers for plant transformation that can be phytotoxic and inhibit untransformed tissues. These genes can be used as selectable markers in plants. The development of efficient transformation protocols for common beans will open up the possibility of transferring an enormous range of useful genes into this crop, including gene for herbicide resistance. Kanamycin at a concentration of 50 mg/l, or phosphinothricin at a concentration of 1 mg/l were sufficient to completely kill any morphogenetic event in both bean varieties "Fönix" and "Maxidor". All the cotyledonary node explants of both varieties produced an average of 14.58 and 27.10 buds and shoots, respectively, in 100% of the cultures on kanamycin-free or bialaphos-free MS media. Kanamycin at a concentration of 100 mg/l, or phosphinothricin (bialaphos) at a concentration of 4 mg/l was used for the selection of transformed plants in both bean varieties (Tables 2 and 3). These results showed that kanamycin, and phosphinothricin are effective selection markers for common beans. Influence of carbenicillin and cefotaxime on shoot initiation and on subsequent growth was also checked by culturing explants on shoot regeneration medium containing 500 mg/l carbenicillin and 800 mg/l cefotaxime. This concentration had no effect on shoot initiation and subsequent growth, but effectively controlled the *Agrobacterium* growth.

Kanamycin selection

The aim of the present work was to start the elaboration of a general transformation and regeneration system from *P. vulgaris* L. using *A. tumefaciens* gene transfer procedure. In order to test the efficiency of the *nptII* gene as selection marker for obtaining transformants, cotyledonary nodes explant of two common beans varieties "Fönix" and "Maxidor" were co-cultivated with *Agrobacterium* EHA101 containing *nptII* gene as selectable marker and *gusA* reporter gene. The availability of simple selection assay becomes extremely useful such as selection method may facilitate the identification of the transgenic plants. The co-cultivated explants were incubated for 24 hours without drug selection to allow expression of the resistance gene. Explants were cultured on regeneration media with 50 and 75 mg/l kanamycin as selection agent for regeneration. Stable transformants were then selected by culturing the explants on medium containing 100 mg/l kanamycin (Fig. 4). The percentage of kanamycin resistant explants that was obtained per co-cultivation with *Agrobacterium* EHA101 is listed in Table (2). The percentage of transformed cotyledonary nodes explant by strain EHA101 cultured on selective MS regeneration medium containing 100 mg/l kanamycin

was 15 and 20% in "Fönix" and "Maxidor" varieties respectively (Table 2). As shown in Table (2), co-cultivation with cotyledonary node explants results in high percentage of kanamycin resistant shoots. On the contrary, following several transfers on MS media containing kanamycin, most of the shoots obtained from co-inoculation turned yellow, browned and died (Fig. 4). Transformed shoots was selected on 100 mg/l kanamycin. Selection pressure was applied when excised shoots were rooted on MS medium containing 50 mg/l kanamycin. Subsequent selection on media with kanamycin resulted in the recovery of shoots and plants resistant to the kanamycin. Kanamycin-resistant shoots and plants appeared 2-3 weeks following plating on solid medium. The presence of the DNA insert in the bean genome was shown by its resistance to kanamycin.

The results obtained here in the present work are comparable with the results obtained by other workers in common beans, mungbean, soybean, pea, pigeonpea, chickpea, lentil, strawberry, tobacco, lettuce, eggplant, cabbage, potato, and other plants with little differences due to plant species and genotypes. The transformed state of regenerated *P. vulgaris* L. plantlets after cutting the shoot apexes co-cultivated with *Agrobacterium* was assessed by kanamycin resistance test (Mariotti *et al*., 1989). Becker *et al*. (1994) reported that 50 and 100 mg/l kanamycin, allowed growth of cotyledonary nodes explant of different cultivars of *P. vulgaris* transformed with *Agrobacterium*

A281 for about two and one week. Apical meristems of *P. vulgaris* cv. Jalo bombarded with tungsten microprojectiles and then inoculated with an *A. tumefaciens* wild-type showed a high frequency of tumor formation, 50 to 70% (Brasileiro *et al*., 1996). The hypocotyl and epicotyl of two-week-old plantlets were stabbed using a syringe needle dipped in the *Agrobacterium* strain A281 solution contains oncogenes binary vector or harbors *nptII* and *gusA* marker genes to determine the susceptibility of 16 bean cultivars, four resulted in high frequencies of tumor formation (Karakaya and Özcan, 2001). Amugune *et al*. (2011) evaluated the potential of two common bean varieties to *Agrobacterium*-mediated transformation. Germinated embryos for 1-2 days, were inoculated with *Agrobacterium* LBA4404 (pBI121), EHA105 (pCAMBIA1201) and EHA105 (pCAMBIA1301), harboring *gusA* plasmids, co-cultivated for 3-4 days on MS medium or with 10 μM BAP and cultured on regeneration and selection medium consisting of 10 μM BAP and 50 mg/l kanamycin. Transformed shoots were obtained in 40 weeks old Mwitemania plantlets from explants infected with *Agrobacterium* LBA4404 (pBI121). Soybean cotyledonary nodes inoculated with *Agrobacterium*, 50 mg/l kanamycin used for selection and plant regeneration (Trick *et al*., 1997). Nodus explants seedlings of *P. sativum* were transformed using *Agrobacterium* carrying *nptII* gene as selectable marker, transformed tissue via organogenesis was selected on 50 mg/l kanamycin (De Kathen and Jacobsen, 1990). Cotyledonary node explants of pigeonpea were

transformed by co-cultivation with *Agrobacterium* LBA4404 carrying the *gusA* gene and the *nptII* gene, cultured on shoot regeneration MS medium with 2 mg/l BAP and 50 mg/l kanamycin for selection (Geetha *et al*., 1999). Embryogenic axis from germinated seedlings of chickpea infected with LBA4404 (pASKαAI1) *Agrobacterium*, and cultured on shoot induction MS medium with 1.0 mg/l BAP and 100 mg/l kanamycin for selection (Ignacimuthu and Prakash, 2006).

A shoot regeneration system from leaf disks of strawberry inoculated with *Agrobacterium* contains *nptII* and *gusA* genes cultured on medium containing 50 mg/l kanamycin was described by Nehra *et al*. (1990). In tobacco, leaves were wounded by microprojectile prior to inoculate with *Agrobacterium* strain LBA4404 containing *nptII* gene, and transferred to regeneration medium with 100 mg/l kanamycin for selection (Bidney *et al*., 1992). High frequency of transformation, based on kanamycin resistance in selection medium containing 100 mg/l kanamycin was obtained from lettuce cotyledon explants co-cultivated with *A. tumefaciens* vectors (Torres *et al*., 1993). When leaves explant of eggplant were co-cultivated with *A. tumefaciens* containing *nptII* and *gusA* genes, transgenic buds and shoots were observed on the selection medium with 50 mg/l kanamycin (Billings *et al*., 1997). Petiole segments explants of *Cyclamen persicum* Mill. infected by *Agrobacterium* contains a vector includes the *nptII* gene as selection marker cultured on MS medium with 100 mg/l kanamycin for regeneration were reported by Aida *et al*. (1999). Transgenic plants were obtained from *B. oleracea* L. and *B. rapa* L. by *Agrobacterium* strain EHA101 contains *nptII* and *gusA* genes, green shoots were obtained on medium containing 50 mg/l kanamycin (Kuginuki and Tsukazaki, 2001). Gene transfer by *Agrobacterium* is the established method of choice for the genetic transformation of most plant species. It is perceived to have several advantages over other forms of transformation, including the ability to transfer large segments of DNA and with fewer copies of inserted genes at higher efficiencies (Wu *et al*., 2003). Although plant tissue can be transformed using different direct DNA methods, the foreign genes tend to integrate as multiple copies. Sarker *et al*. (2003) and Hassan *et al*. (2007) reported that decapitated embryo of lentil was more effective in formation of multiple shoots on MS medium with 50 mg/l kanamycin following *Agrobacterium* LBA4404 harboring binary plasmid pBI121, containing the *nptII* and *gusA* genes infection and selection. Two regeneration systems were developed in cowpea, from shoot apices of 3-5-day-old seedlings (Mao *et al*., 2006), and *via* embryonic axes explants (Yusuf *et al*., 2008). Harini and Sathyanarayana (2009) reported the induction of somatic embryos in *Mucuna pruriens* belongs to the family *Fabaceae* from different explants cultured on MS medium with 11.31 μM 2,4-D produced embryogenic callus. Optimizing factors for T-DNA delivery with transformation efficiency more than 20% resulted in kanamycin-resistant transgenic shoots of *Thlaspi caerulescens*

L. using *Agrobacterium* EHA105 harboring pBI121 with the *nptII* and the *gusA* genes were described by Safavi and Asgari (2011). Transformed potato were obtained by co-cultivating leaf explants with *Agrobacterium* contains the *nptII* gene as selectable in MS regeneration medium plus 50 mg/l kanamycin (González *et al*., 2008).

Herbicide phosphinothricin, bialaphos selection

Phosphinothricin is a widely used marker for plant transformation that can be phytotoxic and inhibit untransformed tissues. In order to test the efficiency of the *bar* gene as selection marker for obtaining transformants, nodes explant of both bean varieties were co-cultivated with strain A281 containing *bar* gene as selectable marker and *gusA* reporter gene. The co-cultivated explants were incubated for 24 hours without herbicide selection to allow expression of the resistance gene. Cotyledonary nodes which were cocultivated with *Agrobacterium* strain A281 for three days were grown at first on regeneration media containing 1 and 2 mg/l herbicide bialaphos, phosphinothricin as selection agent and 500 mg/l cefotaxime. Stable transformants were then selected by culturing the explants on medium containing 4 mg/l phosphinothricin (Fig. 5a). The percentage of herbicide resistant explants that was obtained per co-cultivation with *Agrobacterium* A281 is listed in Table (3). The percentage of transformed cotyledonary

nodes explant by strain A281 cultured on selective MS regeneration medium containing 4 mg/l phosphinothricin was 9 and 10% in "Fönix" and "Maxidor" varieties, respectively (Table 3). Transformed tissue was selected on 1 mg/l phosphinothricin. Selection pressure was applied when excised shoots were rooted on MS medium containing 1 mg/l phosphinothricin. Subsequent selection on media with phosphinothricin resulted in the recovery of shoots resistant to the herbicide. Herbicide resistant shoots and plants appeared 2-3 weeks following plating on solid medium. As shown in Table (3), cocultivation with cotyledonary node explants results in high percentage of herbicide resistant shoots. On the contrary, following several transfers on MS media containing herbicide, most of the shoots obtained from co-inoculation turned yellow, browned and died (Fig. 5a). Herbicide at a concentration of 4 mg/l was chosen for selecting transformed tissues, buds, shoots and plants of both common bean varieties. The presence of the DNA insert in the bean genome was shown by its resistance to phosphinothricin. In the present research, the development of two commercial common beans varieties "Fönix" and "Maxidor" plants that are able to grow in the presence of high concentrations of herbicide phosphinothricin (bialaphos) and that express the gene product required, for resistance to the herbicide were reported. Putative plant transformation and successful regeneration procedures were based on a direct shoot organogenesis protocol.

The results obtained in the present investigation are agreement with the results obtained by other workers in common beans, soybean, tobacco, wheat, rice, bentgrass, tomato, and other plants with little differences due to plant species and genotypes. Becker et al. (1994) reported that, cotyledonary nodes explant of different cultivars of *P. vulgaris* transformed with *Agrobacterium* A281 allowed growth on medium with 1 mg/l phosphinotricin, and the explants died within two weeks at 5 and 10 mg/l phosphinotricin. The results of Lee et al. (2001) indicated that to prevent the escape of chimeric or non-transformed buds and shoots, 2 mg/l glufosinate was added to shoot induction medium as a selection agent from cotyledonary node explants of *P. vulgaris* using *Agrobacterium* EHA101 containing *bar* gene. Rech et al. (2008) describes a method for recovery of transgenic common bean plants by combining resistance to the herbicide imazapyr as a selectable marker, multiple shoot induction from embryonic axes of mature seeds and biolistics techniques, and the average frequency of transgenic plants is 2.7. Aragão and Faria (2009) used particle bombardment protocol to insert *bar* gene for herbicide resistance and virus resistance (RNAi) into the pinto bean cv. Olathe. Transgenes included the *bar* herbicide (glufosinate ammonium) resistance selectable marker used for selection of transgenic cultures which confirmed herbicide resistant common bean plants and *gusA* color marker which visually confirmed transgenic events (Kwapata et al., 2012).

Soybean cotyledon explants inoculated with *A. tumefaciens* pTiT37-SE conferring glyphosate tolerance, about 6% shoots were transgenic based on selected glyphosate tolerance (Hinchee et al., 1988). Öktem (1998) used *Agrobacterium* LBA4404 containing the *bar* gene to develop transgenic tobacco plants resistant to herbicide gluphosinate using the leaf disc. The result of Mihály et al. (2002) showed that all the transformed wheat hybrid embryos by selectable *bar* gene germinated onto 5 mg/l bialaphos medium expressed resistance, while the control showed sensitivity. Wu et al. (2003) reported that selection was carried out with 2-4 mg/l phosphinotricin for wheat immature embryos co-cultivated with *Agrobacterium* strain AGL1 harboring the *bar* gene, and transformation efficiencies ranged between 0.3% and 3.3% only. A procedure was developed to produce rice tolerant to the herbicide phosphinothricin by *in vitro* selection (Toldi et al., 2000). Chai et al. (2002) produced transgenic bentgrass plants via biolistic system; 5 mg/l phosphinothricin inhibited the growth of non-transformed plantlets but did not affect the growth of transformed. Plasmid JS101 containing the *bar* herbicide resistance gene was used as a selectable marker, 1 mg/l zeatin and 0.1 mg/l IAA were enhanced shoot regeneration in tomato (Hussain et al., 2008).

GUS analysis

Tissues which developed on non-transformed explants showed no detectable *gusA* activity when stained with X-

gluc, while tissues on transformed explants stained blue (Fig. 5b). This suggests that the *gusA* gene be expressed in common bean tissues, after co-cultivation with EHA101, or A281 100% of kanamycin resistance, or of phosphinothricin resistant of the nodes explant showed *gusA* activity (Tables 2 and 3). This procedure exhibits distinct advantages over these previously reported, and the highly regenerable cotyledonary nodes rapidly produce transgenic shoots without an intermediate callus phase. The results of this study revealed that, in a binary vector system, cotyledonary node explants is effective at delivering DNA into common bean. Ideally, transgenic plant production involves *Agrobacterium*-mediated gene transfer, followed by selection of transformed tissues and regeneration from them. Integration of foreign DNA was confirmed by selective analysis and GUS-test. Shoots formed roots in half strength MS medium with 0.1 mg/l indole-3-butyric acid (IBA) cultured for 15 days prior to transfer to greenhouse.

Conclusion

In the present investigation, transgenic plants with *nptII* gene or *bar* gene in two varieties of the common bean were achieved. In view of the present results, there is a possibility of achieving high levels of kanamycin resistance, or herbicide phosphinothricin resistance in beans by using *Agrobacterium*-mediated strain EHA101 (pEHA101::pTd33), or A281 (pRGG bar H1), and phenolics compound acetosyringone. Traits such as herbicide resistance cannot be introduced from other

species of plants or from distantly related species (soybean) as this would require genetic engineering. The development of efficient transformation protocols for common beans will open up the possibility of transferring an enormous range of useful genes into this crop, including genes for herbicide and pest resistance. This protocol should help achieve *Agrobacterium*-mediated genetic transformation to improve this important food legume.

SUMMARY

Agrobacterium-mediated transformation and regeneration systems has been achieved for two varieties "Fönix" and "Maxidor" of common beans. Transformation ability of cotyledonary nodes explant were tested by *A. tumefaciens* EHA101 (pEHA101::pTd33) with the *nptII* and *gusA* genes, or A281 (pRGG bar H1) with the *bar* and *gusA* genes. Transgenic for both bean varieties with *nptII* gene, or *bar* gene were produced through this approach. Among different selection methods, 100 mg/l kanamycin, or 4 mg/l herbicide phosphinothricin turned to be optimal, resulting in the highest transformation efficiency. Transgenic common bean plants demonstrated enhanced growth ability under antibiotic kanamycin, or herbicide phosphinothricin stress conditions. The increased resistance was also reflected by delayed development of damage symptoms caused by kanamycin, or phosphinothricin stress. Cotyledonary nodes were found to be effective in formation of multiple shoots cultured on se-

lection MS medium supplemented with 1 mg/l BA, 0.1 mg/l NAA and plus 100 mg/l kanamycin, or 4 mg/l phosphinothricin. Stable expression of the *gusA* was observed in various parts of the transformed tissues. Optimal transformation conditions were obtained for both bean varieties by co-cultivating cotyledonary node explants with *Agrobacterium* in MS regeneration medium plus 100 μM acetosyringone for 3 days. The nodal region was punctured, wounded and infected with *Agrobacterium* strain by overnight immersion in bacterial suspension plus 100 μM acetosyringone. This wounding pattern permits *Agrobacterium* to penetrate deeper and more completely throughout the tissue, and increasing the probability of infecting plant cells. After inoculation for 3 hours and co-cultivation for 3 days, the explant nodes were cultured on MS medium supplemented with 1 mg/l BA and 0.1 mg/l NAA, pH 5.7. The explant nodes were transferred three times at three day intervals to fresh MS medium plus 500 mg/l carbenicillin and 800 mg/l cefotaxime to eliminate *Agrobacterium* overgrowth of the target region. After co-cultivation for 24 hours on MS medium, the explants nodes were transferred to a selective medium containing 100 mg/l kanamycin, or 4 mg/l phosphinothricin. Gene expression of kanamycin resistance (*nptII*), or herbicide phosphinothricin resistance (*bar*) and *gusA* in transformed buds and shoots for both bean varieties was demonstrated by selection test and *gusA* histochemical analysis. Shoots and buds of transformed explants continued on selective media. Putative transformed green shoots were transferred onto kanamycin, or phosphinothricin containing medium to verify their ability to root. Expression of the *β*-glucuronidase reporter gene was verified by histochemical staining.

REFERENCES

Aida, R., Y. Hirose, S. Kishimoto and M. Shibata (1999). *Agrobacterium tumefaciens*-mediated transformation of *Cyclamen persicum* Mill. Plant Science, 148: 1-7.

Allavena, A. (1985). Infection of *Phaseolus vulgaris* with strains of *Agrobacterium tumefaciens* and *A. rhizogenes*. Annual Report of the Bean Improvement Cooperative, 28: 90-91.

Amugune, N. O., B. Anyango and T. K. Mukiama (2011). *Agrobacterium*-mediated transformation of common bean. African Crop Science Journal, 19: 137-147.

Arellano, J., S. I. Fuentes, P. Castillo-España and G. Hernández (2009). Regeneration of different cultivars of common bean (*Phaseolus vulgaris* L.) *via* indirect organogenesis. Plant Cell, Tissue and Organ Culture, 96: 11-18.

Aragão, F. J. L., L. M. G. Barros, A. C. M. Brasileiro, S. G. Ribeiro, F. D. Smith, J. C. Sanford, J. C. Faria and E. L. Rech (1996). Inheritance

of foreign genes in transgenic bean (*Phaseolus vulgaris* L.) co-transformed *via* particle bombardment. Theor. Appl. Genet., 93: 142-150.

Aragão, F. J. L. and E. L. Rech (1997). Morphological factors influencing recovery of transgenic bean plants (*Phaseolus vulgaris* L.) of a Carioca cultivar. International Journal of Plant Sciences, 158: 157-163.

Aragão, F. J. L., L. M. G. Barros, M. V. de Sousa, M. F. Grossi de Sá, E. R. P. Almeida, E. S. Gander and E. L. Rech (1999). Expression of a methionine-rich storage albumin from the Brazil nut (*Bertholletia excelsa* H.B.K., lecythidaceae) in transgenic bean plants (*Phaseolus vulgaris* L., *Fabaceae*). Genetics and Molecular Biology, 22: 445-449.

Aragão, F. J. L. and J. C. Faria (2009). "First transgenic geminivirus-resistant plant in the field". Nature Biotechnology, 27: 1086-1088.

Baron, C. and P. C. Zambryski (1995). The Plant response in pathogenesis; symbiosis; and wounding: variations on a common theme?. Annual Review of Plant Genetics, 29: 107-129.

Becker, J., T. Vogel, J. Iqbal and W. Nagl (1994). *Agrobacterium*-mediated transformation of *Phaseolus vulgaris*. Adaptation of some conditions. Annual Report of the Bean Improvement Cooperative, 37: 127-128.

Bidney, D., C. Scelonge, J. Martich, M. Burrus, L. Sims and G. Huffman (1992). Microprojectile bombardment of plant tissues increases transformation frequency by *Agrobacterium tumefaciens*. Plant Molecular Biology, 18: 301-313.

Billings, S., G. Jelenkovic, C. K. Chin and J. Eberhardt (1997). The effect of growth regulators and antibiotics on eggplant transformation. Journal of the American Society for Horticultural Science, 122: 158-162.

Brasileiro, A. C. M., F. J. L. Aragão, S. Rossi, D. M. A. Dusi, L. M. G. Barros and E. L. Rech (1996). Susceptibility of common and tepary beans to *Agrobacterium* spp. strains and improvement of *Agrobacterium*-mediated transformation using microprojectile bombardment. Journal of the American Society for Horticultural Science, 121: 810-815.

Chai, B., S. B. Maqbool, R. K. Hajela, D. Green, J. M. Vargas, Jr, D. Warkentin, R. Sabzikar and M. B. Sticklen (2002). Cloning of a chitinase-like cDNA (*hs2*), its transfer to creeping bentgrass (*Agrostis palustris* Huds.) and development of brown patch (*Rhizoctonia solani*) disease re-

sistant transgenic lines. Plant Science, 163: 183-193.

Colpaert, N., S. Tilleman, M. van Montagu, G. Gheysen and N. Terryn (2008). Composite *Phaseolus vulgaris* plants with transgenic roots as research tool. African Journal of Biotechnology, 7: 404-408.

De Kathen, A. and H. J. Jacobsen (1990). *Agrobacterium tumefaciens*-mediated transformation of *Pisum sativum* L. using binary and cointegrate vectors. Plant Cell Reports, 9: 276-279.

Delgado-Sánchez, P., M. Saucedo-Ruiz, S. H. Guzmán-Maldonado, E. Villordo-Pineda, M. González-Chavira, S. Fraire-VelÁzquez, J. A. Acosta-Gallegos and A. Mora-Avilés (2006). An organogenic plant regeneration system for common bean (*Phaseolus vulgaris* L.). Plant Science, 170: 822-827.

Dillen, W., G. Engler, M. Van Montagu and G. Angenon (1995). Electroporation- mediated DNA delivery to seedling tissues of *Phaseolus vulgaris* L. (common bean). Plant Cell Reports, 15: 119-124.

Dillen, W., M. Zambre, J. De Clercq, A. Goossens, J. Kapila, E. Vranová, M. Van Montagu and G. Angenon (2000). *In vitro* regeneration and *Agrobacterium*-mediated transformation of *Phaseolus vulgaris* L. (common bean) and *P. acutifolius*

A. gray (tepary bean). Acta Horticulturae, 521: 59-65.

Eissa, A. E., Gy. Bisztray and I. Velich (1999). Regeneration in *Phaseolus vulgaris* L.: High-frequency induction of shoot formation in intact seedlings and seedling node explants. Publications of the University of Horticulture and Food Industry, LVIII: 113-118.

Eissa, A. E., Gy. Bisztray and I. Velich (2002). Plant regeneration from seedling explants of common bean (*Phaseolus vulgaris* L.). Acta Biologica Szegediensis, 46: 27-28.

Franklin, C. I., T. N. Trieu, R. A. Gonzales and R. A. Dixon (1991). Plant regeneration from seedling explants of green bean (*Phaseolus vulgaris* L.) *via* organogenesis. Plant Cell, Tissue and Organ Culture, 24: 199-206.

Franklin, C. I., T. N. Trieu, B. G. Cassidy, R. A. Dixon and R. S. Nelson (1993). Genetic transformation of green bean callus *via Agrobacterium*-mediated DNA transfer. Plant Cell Reports, 12: 74-79.

Gatica Arias, A. M, J. M. Valverde, P. R. Fonseca and M. V. Melara (2010). *In vitro* plant regeneration system for common bean (*Phaseolus vulgaris*): effect of N6-benzylaminopurine and adenine sulphate. Electronic Journal of Biotechnology, 13: 1-8.

Geetha, N., P. Venkatachalam and G. Lakshmi Sita (1999). *Agrobacterium*-mediated genetic transformation of pigeonpea (*Cajanus cajan* L.) and development of transgenic plants *via* direct organogenesis. Plant Biotechnology, 16: 213-218.

Godwin, I. D., G. Todd, B. V. Ford-Lioyd and H. J. Newbury (1991). The effects of acetosyringone and pH on *Agrobacterium*-mediated transformation vary according to plant species. Plant Cell Reports, 9: 671-675.

Godwin, I. D., B. V. Ford-Lioyd and H. J. Newbury (1992). *In vitro* approaches to extending the host-range of *Agrobacterium* for plant transformation. Australian Journal of Botany, 40: 751-763.

González, R. G., D. S. Sánchez, Z. Z. Guerra, J. M. Campos, A. L. Quesada, R. M. Valdivia, A. D. Arencibia, K. Q. Bravo and P. D. S. Caligari (2008). Efficient regeneration and *Agrobacterium tumefaciens* mediated transformation of recalcitrant sweet potato (*Ipomoea batatas* L.) cultivars. Asia Pacific Journal of Molecular Biology and Biotechnology, 16: 25-33.

Hamdy, M. A. A. and K. Hattori (2006). *In vitro* micropropagation of (*Vicia faba* L.) cultivars 'Waza Soramame and Cairo 241' by nodal explants

proliferation and somatic embryogenesis. Biotechnology, 5: 32-37.

Harini, S. S. and N. Sathyanarayana (2009). Somatic embryogenesis in *Mucuna pruriens*. African Journal of Biotechnology, 8: 6175-6180.

Hassan, F., M. Imdadul Hoque, H. Kiesecker and H. J. Jacobsen (2007). Transient GUS expression in decapitated lentil embryos. Plant Tissue Culture and Biotechnology, 17: 97-102.

Hinchee, M. A. W., D. V. Connor-Ward, C. A. Newell, R. E. McDonnell, S. J. Sato, C. S. M. Gasser, D. A. Fischhoff, D. B. Re, R. T. Fraley and R. B. Horsch (1988). Production of transgenic soybean plants using *Agrobacterium*-mediated DNA transfer. Bio/Technology, 6: 915-922.

Hussain, A. F., G. H. Anfoka and D. S. Hassawi (2008). Transformation of tomato with TYLCV gene silencing construct using optimized *Agrobacterium*-mediated protocol. Biotechnology, 7: 537-543.

Ignacimuthu, S. and S. Prakash (2006). *Agrobacterium*-mediated transformation of chickpea with α-amylase inhibitor gene for insect resistance. Journal of Biosciences, 31: 1-7.

Jaiwal, P. K., R. Kumari, S. Ignacimuthu, I. Potrykus and C. Sautter (2001).

Agrobacterium tumefaciens-mediated genetic transformation of mungbean (*Vigna radiata* L. Wilczek) - a recalcitrant grain legume. Plant Science, 161: 239-247.

Jefferson, R. A. (1987). Assaying chimeric genes in plants: The GUS gene fusion system. Plant Molecular Biology Reporter, 5: 387-405.

Karakaya, A. and S. Özcan (2001). Susceptibility of different bean (*Phaseolus vulgaris* L.) cultivars to *Agrobacterium tumefaciens*. Turkey Journal of Biology, 25: 447-452.

Kelly, J. D. (2010). The story of bean breeding, white paper prepared for bean CAP & PBG works on the topic of dry bean production and breeding research in the USA. Michigan State University, June 2010: 1-29.

Kuginuki, Y. and H. Tsukazaki (2001). Regeneration ability and *Agrobacterium*-mediated transformation of different cultivars in *Brassica oleracea* L. and *B. rapa* L. (syn. *B. campestris* L.). Journal of Japanese Society for Horticultural Science, 70: 682-690.

Kwapata, K., R. Sabzikar, M. B. Sticklen and J. D. Kelly (2010). *In vitro* regeneration and morphogenesis studies in common bean. Plant Cell, Tissue and Organ Culture, 100: 97-105.

Kwapata, K., T. Nguyen and M. Sticklen (2012). Genetic transformation of common bean (*Phaseolus vulgaris* L.) with the *Gus* color marker, the *Bar* herbicide resistance, and the barley (*Hordeum vulgare*) *HVA1* drought tolerance genes. International Journal of Agronomy, 2012: 1-8.

Lee, Y. M., S. Jin, W. S. Sim and E. W. Nester (1995). Genetic evidence for direct sensing of phenolic compound the *Vir* A protein of *Agrobacterium tumefaciens*. Proceeding of National Academy for Sciences USA, 92: 12245-12249.

Lee, T. J., D. P. Coyne, Z. Zhang, T. E. Clemente and A. Mitra (2001). Approaches to develop an *Agrobacterium*-mediated transformation system *via* direct shoot organogenesis in common bean (*Phaseolus vulgaris* L.). Annual Report of the Bean Improvement Cooperative, 44: 37-38.

Liu, Z., B. J. Park, A. Kanno and T. Kameya (2005). The novel use of a combination of sonication and vacuum infiltration in *Agrobacterium*-mediated transformation of kidney bean (*Phaseolus vulgaris* L.) with *lea* gene. Molecular Breeding, 16: 189-197.

Malik, K. A. and P. K. Saxena (1991). Regeneration in *Phaseolus vulgaris* L. Promotive role of N_6-benzylaminopurine in cultures

from juvenile leaves. Planta, 184: 148-150.

Malik, K. A. and P. K. Saxena (1992). Regeneration in *Phaseolus vulgaris* L.: High-frequency induction of direct shoot formation in intact seedlings by N$_6$-benzylaminopurine and thidiazuron. Planta, 186: 384-389.

Mao, J. Q., M. A. Zaidi, J. T. Arnason and I. Altosaar (2006). *In vitro* regeneration of *Vigna unguiculata* (L.) Walp. cv. Blackeye cowpea *via* shoot organogenesis. Plant Cell, Tissue and Organ Culture, 87: 121-125.

Mariotti, D., G. S. Fontana and L. Santini (1989). Genetic transformation of grain legumes: *Phaseolus vulgaris* L. and *P. coccineus* L. Journal of Genetics and Plant Breeding, 43: 77-82.

McClean, P., J. Simental, P. Chee and J. L. Slightom (1988). Transformation of dry bean tissues *via Agrobacterium* vectors. Annual Report of the Bean Improvement Cooperative, 31: 47-48.

McClean, P. and K. F. Grafton (1989). Regeneration of dry bean (*Phaseolus vulgaris* L.) *via* organogenesis. Plant Science, 60: 117-122.

McClean, P., P. Chee, B. Held, J. Simental, R. F. Drong and J. L. Slightom (1991). Susceptibility of dry bean (*Phaseolus vulgaris* L.) to *Agrobacterium* infection: Transformation of cotyledonary and hypocotyl tissues. Plant Cell, Tissue and Organ Culture, 24: 131-138.

Mihály, R., E. Kótai, O. Kiss and J. Pauk (2002). *In vitro* selection of transformed foreign gene (*bar*) in wheat anther culture. Acta Biologica Szegediensis, 46: 9-10.

Mohamed, M. F., P. E. Read and D. P. Coyne (1992). Plant regeneration from *in vitro* culture of embryonic axis explants in common and tepary beans. Journal of the American Society for Horticultural Science, 117: 332-336.

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

Naz, S., A. Ali, F. A. Siddique and J. Iqbal (2007). Multiple shoot formation from different explants of chick pea (*Cicer arietinum* L.). Pakistan Journal of Botany, 39: 2067-2073.

Nehra, N. S., R. N. Chibbar, K. K. Kartha, R. S. S. Datla, W. L. Crosby and C. Stushnoff (1990). Genetic transformation of strawberry by *Agrobacterium tumefaciens* using a leaf disk regeneration system. Plant Cell Reports, 9: 293-298.

Öktem, H. A. (1998). Development of phosphinothricin resistant fertile transgenic tobacco plants. Acta Agronomica Hungarica, 46: 97-104.

Rech, E. L., G. R. Vianna and F. J. L. Aragão (2008). High-efficiency transformation by biolistics of soybean, common bean and cotton transgenic plants. Nature Protocols, 3: 410-418.

Russell, D. R., K. M. Wallace, J. H. Bathe, B. J. Martinell and D. E. McCabe (1993). Stable transformation of *Phaseolus vulgaris via* electric-discharge mediated particle acceleration. Plant Cell Reports, 12: 165-169.

Safavi, K. and M. J. Asgari (2011). Heavy metal resistance in transgenic plants. International Conference on Food Engineering and Biotechnology-IPCBEE, 9: 286-290. (2011) © (2011) IACSIT Press, Singapore

Sarker, R. H., A. Biswas, B. M. Mustafa, S. Mahbub and M. I. Hoque (2003). *Agrobacterium*-mediated transformation of lentil (*Lens culinaris* Medik). Plant Tissue Culture, 13: 1-12.

Schryer, P. A., Q. Lu, A. Vanderberg and K. E. Bett (2005). Rapid regeneration of *Phaseolus angustissimus* and *P. vulgaris* from very young zygotic embryos. Plant Cell, Tissue and Organ Culture, 83: 67-74.

Svetleva, D., M. Velcheva and G. Bhowmik (2003). Biotechnology as a useful tool in common bean (*Phaseolus vulgaris* L.) improvement. Euphytica, 131: 189-200.

Toldi, O., S. Tóth, A. S. Oreifig, E. Kiss and B. Jenes (2000). Production of phosphinothricin-tolerant rice (*Oryza sativa* L.) through the application of phosphinothricin as growth regulator. Plant Cell Reports, 19: 1226-1231.

Torres, A. C., D. J. Cantliffe, B. Laughner, M. Bieniek, R. Nagata, M. Ashraf and R. J. Ferl (1993). Stable transformation of lettuce cultivar South Bay from cotyledon explants. Plant Cell, Tissue and Organ Culture, 34: 279-285.

Trick, H. N., R. D. Dinkins, E. R. Santarem, R. Di, V. Samoylov, C. A. Meurer, D. R. Walker, W. A. Parrott, J. J. Finer and G. B. Collins (1997). Recent advances in soybean transformation. Plant Tissue Culture and Biotechnology, 3: 9-24.

Van Wordragen, M. F. and H. J. M. Dons (1992). *Agrobacterium tumefaciens*-mediated transformation of recalcitrant crops. Plant Molecular Biology Reporter, 10: 12-36.

Varisai Mohamed, S., J. M. Sung, T. L. Jeng and C. S. Wang (2006). Organogenesis of *Phaseolus*

angularis L.: high efficiency of adventitious shoot regeneration from etiolated seedlings in the presence of N6-benzylaminopurine and thidiazuron. Plant Cell, Tissue and Organ Culture, 86: 187-199.

Veltcheva, M. and D. Svetleva (2005). *In vitro* regeneration of *Phaseolus vulgaris* L. *via* organogenesis from petiole explants. Journal Central European Agriculture, 6: 53-58.

Veltcheva, M., D. Svetleva, Sp. Petkova and A. Perl (2005). *In vitro* regeneration and genetic transformation of common bean (*Phaseolus vulgaris* L.) Problems and progress. Scientia Horticulturae, 107: 2-10.

Vernade, D., A. Herrera-Estrella, K. Wang and M. Van Montagu (1988). Glycine betaine allows enhanced induction of the *Agrobacterium tumefaciens vir* genes by acetosyringone at low pH. Journal of Bacteriology, 170: 5822-5829.

Wu, H., C. Sparks, B. Amoah and H. D. Jones (2003). Factors influencing successful *Agrobacterium*-mediated genetic transformation of wheat. Plant Cell Reports, 21: 659-668.

Yusuf, M., A. A. Raji, I. Ingelbrecht and M. D. Katung (2008). Regeneration efficiency of cowpea [*Vigna unguiculata* (L.) Walp.] *via* embryonic axes explants. African Journal of Plant Science, 2: 105-108.

Zambre, M. A., J. De Clercq, E. Vranová, M. Van Montagu, G. Angenone and W. Dillen (1998). Plant regeneration from embryo-derived callus in *Phaseolus vulgaris* L. (common bean) and *P. acutifolius* A. gray (tepary bean). Plant Cell Reports, 17: 626-630.

Zhang, Z., D. P. Coyne and A. Mitra (1997). Factors affecting *Agrobacterium*-mediated transformation of common bean. Journal of the American Society for Horticultural Science, 122: 300-305.

Table (1): *Agrobacterium tumefaciens* strains and binary plasmid used in the transformation and regeneration experiments.

Strain	Binary plasmid	Genes	Encode genes
EHA101	pEHA101::pTd33	*nptII* and *gusA*	kanamycin resistance and *β*-glucuronidase expression
A281	pRGG bar H1	*bar* and *gusA*	herbicide phosphinothricin, bialaphos resistance and *β*-glucuronidase expression

nptII = neomycin phosphotransferase II;   *gusA* = *β*-glucuronidase;
bar = phosphinothricin acetyl transferase.

Table (2): Transformation efficiency from cotyledonary node explants of two common bean varieties transformed by strain EHA101 contains the binary vector (pEHA::pTd33) cultured on regeneration MS medium supplemented with 1 mg/l BA and 0.1 mg/l NAA.

Variety	Kanamycin for selection mg/l	Number of cotyledonary nodes explant	Number of kanamycin resistant explant/Total	Percentage of transformed	Co-transformation (GUS positive)
Fönix	100	500	75	15	15
Maxidor	100	500	100	20	20

Percentage of transformed explants (explants giving rise to one or more kanamycin resistant shoot compared to the total number of explants.

Table (3): Transformation efficiency from cotyledonary node explants of two common bean varieties transformed by strain A281 contains the binary vector (pRGG bar H1) cultured on regeneration MS medium supplemented with 1 mg/l BA and 0.1 mg/l NAA.

Variety	Phosphinothricin for selection mg/l	Number of cotyledonary nodes explant	Number of phosphinothricin resistant explant/Total	Percentage of transformed	Co-transformation (GUS positive)
Fönix	4	500	45	9	9
Maxidor	4	500	50	10	10

Percentage of transformed explants (explants giving rise to one or more phosphinothricin resistant shoot compared to the total number of explants.
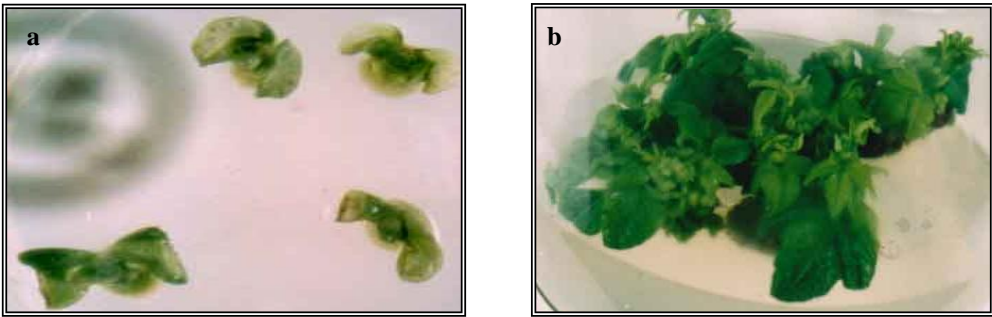
Fig. (1): (a and b) Buds and shoots regeneration from cotyledonary nodes meristematic ring of bean cultured on MS medium supplemented with 1 mg/l BA and 0.1 mg/l NAA.

Fig. (2): (a and b) Scanning electron photomicrographs of organogenesis from cotyledonary nodes of bean cultured on MS medium supplemented with 1 mg/l BA and 0.1 mg/l NAA. (c and d) Bud formation from the meristematic cells in the axillary region of cotyledonary node explants of bean cultured on MS medium supplemented with 1 mg/l BA and 0.1 mg/l NAA after 48-h of culture.
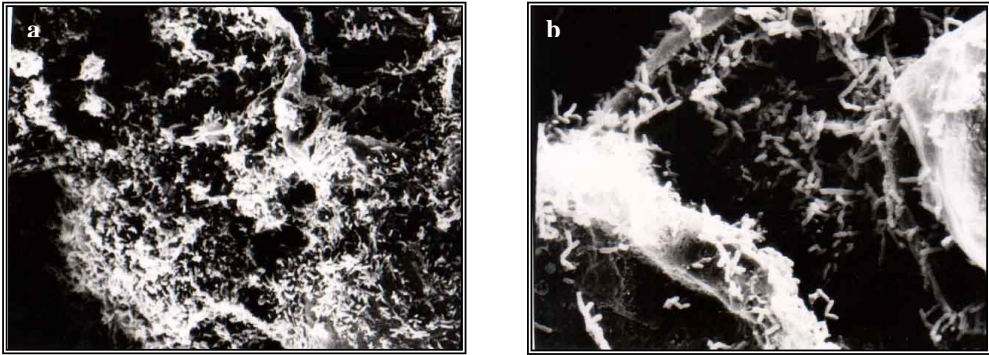
Fig. (3): (a and b) Scanning electron photomicrographs of cotyledonary node inoculated with *A. tumefaciens* strain A281 showing bacterial attachment cells to common bean plant cells
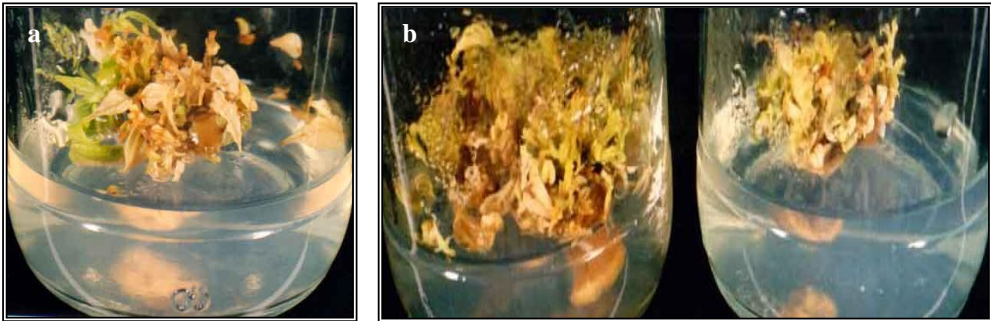

Fig. (4): (a) Selection of stable kanamycin resistance transgenic green shoots of bean growing on selective MS regeneration medium with 100 mg/l kanamycin, while (b) nontransgenic bean shoots (control) cultured on selective MS regeneration medium with 100 mg/l kanamycin turned yellow, brown and died.
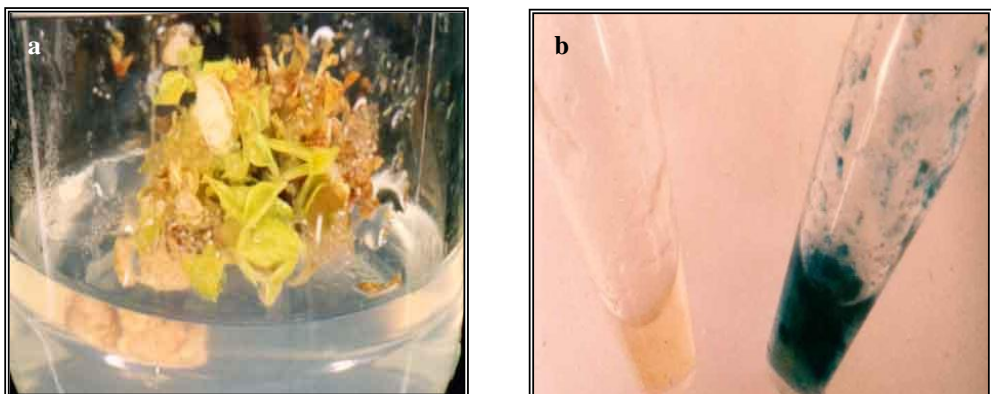

Fig. (5): (a) Selection of stable herbicide phosphinothricin resistance transgenic bean green shoots growing on selective MS regeneration medium with 4 mg/l herbicide, (b) transgenic bean tissues showed *gusA* positive activity and stained blue with X-gluc, while non-transgenic showed *gusA* negative and no *gusA* activity.