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Original Research Article

Effect of N6-benzylaminopurine and adenine sulphate in *In-vitro* plant regeneration of *Phaseolus vulgaris* L.

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Common bean, *in vitro* culture, morphogenesis, *Phaseolus vulgaris* L., plant growth regulators.

A B S T R A C T

A method for regeneration of the commercially important common bean (*Phaseolus vulgaris*) using N^6-benzylaminopurine(BAP) and adenine sulphate (AS) was established. Embryogenic axes of the five Indian common bean cultivars viz. string bean, field bean, flageolet bean, French bean and garden bean were cultured on Murashige and Skoog medium supplemented with 100 mgl^{-1} myo-inositol, 1 mgl^{-1} thiamine, 30 gl^{-1} sucrose, BAP (0, 5 and 10 mgl^{-1}), AS (0, 20 and 40 mgl^{-1}) and 8 gl^{-1} agar. Regardless of the concentration of BAP and AS in the induction medium, the number of shoots and leaves differed significantly among the common bean cultivars evaluated. The higher average of shoots was obtained for V2 > V5 > V1 > V4 > V3. Moreover, independently of the cultivar, the induction medium supplemented with 5 mgl^{-1} BAP and 20 or 40 mgl^{-1} AS resulted in the higher average of shoots formation. Culture of five different varieties axes on induction medium supplemented with different BAP and AS resulted in a differential response. Successful acclimatization of common bean *in vitro* plants were achieved in the greenhouse, and plants appeared morphologically normal. The regeneration system developed in this investigation for this important crop could be a useful tool for the genetic modification through mutagenesis or genetic transformation.

Introduction

Common bean (*Phaseolus vulgaris*) is an economic important crop and one of the major grain legumes for human consumption in Latin America, Africa and Asia (Varisai Mohamed et al. 2006). Despite its importance, production growth rates are limited by viral, fungal and bacterial pathogens, insects, lack of drought tolerances and nutritional deficiencies (Aragao et al. 1996). Therefore, there is considerable interest in the development of new bean cultivars with useful agronomical traits. Plant biotechnology, together with conventional breeding methods, could facilitate bean improvement since resistance or tolerance to biotic and abiotic stress could be increased and seed quality, plant architecture and reproduction modes could be altered (Veltcheva et al. 2005). Nevertheless, a reliable and efficient *in vitro*

culture system that results in efficient differentiation, shoot development and whole plant regeneration is an essential requirement for improvement of common bean through genetic transformation or mutagenesis (Svetleva et al. 2003). In addition to genetic improvement, *in vitro* culture is an important tool for the recovery, conservation of germplasm and embryo rescue.

In vitro plant regeneration of *Phaseolus* has been reported by organogenesis (Malik and Saxena, 1992; Ahmed et al. 2002) or through somatic embryogenesis (Zambre et al. 1998; Schryer et al. 2005). Although several protocols have been described in the literature for bean regeneration, development of an optimal *in vitro* culture system still remains a major challenge since this and other species from the *Phaseolus* genus are recalcitrant for *in vitro* regeneration .

Therefore, the objective of the present study was to optimize an *in vitro* plant regeneration system for five commercial Indian *Phaseolus vulgaris* cultivars by studying the influence of N^6-benzylaminopurine and adenine sulphate, as a prerequisite for improvement of common bean through genetic transformation or mutagenesis.

Materials and Methods

Plant material and ex-plant preparation

Indian common bean (*Phaseolus vulgaris*) was obtained from market. Seeds were washed with running tap water and soaked for 4 min in 70% ethanol, disinfected with 20% (v/v) sodium hypochlorite with two drops of Tween 20 solution for 20 min and rinsed three times with sterile distilled water. Then, the seeds were immersed in 0.01% Benomyl solution for 4 min and rinsed three times with sterile distilled water. The seeds were soaked in sterile distilled water overnight at 26 ± 2ºC to soften the seed coat. The embryogenic axes were excised from the seeds and cotyledons and root meristems were removed.

Induction and plant regeneration medium: The embryogenic axes were cultured on Petri dishes containing 20 ml of induction medium which consisted of MS mineral salts (Murashige and Skoog, 1962) supplemented with 100 mgl^{-1} myo-inositol, 1 mgl^{-1} thiamine, 30 gl^{-1} sucrose and 8 gl^{-1} agar. The induction medium was supplemented with a combination of BAP (0, 5 and 10 mgl^{-1}) and AS (0, 20 and 40 mgl^{-1}) to comprise 9 treatments (Delgado-Sanchez et al. 2006). Experimental treatments consisted of three replicates of three Petri dishes with 10 explants in each one. All medium pH was adjusted to 5.6 before autoclaving for 21 min at 121ºC and 1.07 kg cm^{-2}.

The shoots regenerated from bud clusters were excised from the original ex-plant and transferred to baby food jars, closed with polyethylene food wrap , containing 20 ml of induction medium to promote elongation and root formation. The *in vitro* cultures were maintained at 26 ± 2ºC under a 16 hrs light photoperiod (30 µmol m^{-2} s^{-1}). The average number (mean ± SE) of shoots, leaves and roots per ex-plant were estimated after 30 days of culture on each induction medium. The efficiency of the treatments [(Number of embryogenic axes with de *novo* shoots/total of embryogenic axes) x 100] were calculated.

Acclimatization and field transfer

Regenerated shoots (approximately 3 cm long) with well developed leaves and roots were transferred to pots covered with plastic bags and maintained in the greenhouse

under 12 hrs light photoperiod at 26 ± 2ºC. After one week the plastic bags were removed. The plantlets were watered twice a week.

Results and Discussion

Multiple shoot formation began with the swelling of embryogenic axes (Figure 1a), and apical bud clusters formed after 30 days of culture (Figure 1b). The apical bud clusters were multiplied by culturing them in the induction medium (Figure 1c) until shoots were developed (Figure 1d).

Data were recorded after 30 days of culture on induction medium supplemented with different BAP and AS concentrations.

The Table 1 shows the effect of genotype on the average number of shoots, leaves and roots induced from embryogenic axes of common bean (*Phaseolus vulgaris*) regardless of the BAP and AS concentration. The average number of shoots and leaves differed significantly among the common bean cultivars evaluated. The higher multiple shoot formation was obtained using Field bean > Garden bean > String bean > French bean > Flageolet bean. Moreover, the higher average of leaves was obtained using Field bean > Flageolet bean > French bean > String bean > Garden bean. On the other hand, no significant differences were observed in the number of roots formed from embryogenic axes cultured on induction medium supplemented with different BAP and AS concentrations

The shoots regenerated from the apical bud clusters and developed into plants with leaves and roots. The *in vitro* plants were successfully acclimated with 100% survival. The entire procedure starting from apical bud cluster induction to establishing a plant under greenhouse conditions took approximately 3 months.

Regeneration protocols using different explants of *Phaseolus* spp. which has been histologically demonstrated by Malik and Saxena (1992) and Arellano et al. (2009). In the present study an efficient and reproducible method for regeneration of the commercially important Indian common bean using BAP and AS was established.

Many factors induce development of new structures via organogenesis, but it has been demonstrated that regenerative capacity and response to growth conditions is species and genotype dependent as reported in *Helianthus annuus* (Deglene et al. 1997) and *Solanum melongena*. (Sharma and Rajam, 1995). Our results confirmed earlier observations that the genotype influences the regeneration process via organogenesis in the bean (Santalla et al. 1998). The genotype effects suggest that genetic factors are important in the response to *in vitro* tissue culture. In this sense, due to the reduced genetic variability in modern bean cultivars, the screening of a large number of cultivars may be useful in the attempt to achieve plant regeneration (Svetleva et al. 2003; Veltcheva et al. 2005).

The composition of the shoot induction medium is important in the regeneration process through organogenesis since in this medium a higher number of buds and shoots are formed (Santalla et al. 1998). In this study, BAP and AS played an important role in the induction of organogenic structures in the Indian bean cultivars evaluated. It is well known that cytokinins stimulate plant cell division and participate in the release of lateral bud dormancy, in the induction of adventitious bud formation, in the growth of lateral buds and in the cell cycle control (Gaspar et al 2003). Malik and Saxena (1991) reported that 5 µM BAP had a favorable effect on shoot formation from cultured leaf explants of *P. vulgaris*

compared to the explants cultured on MS medium depleted of BAP. Moreover, Varisai Mohamed et al. (2006) indicated that BAP and thidiazuron at 5 µM was the best concentration for shoot formation in *P. angularis* (binomial name *Vigna angularis*) cvs. (azuki bean). In the absence of BAP, no shoot bud development was found in *P. angularis*. (Varisai Mohamed et al. 2006). Delgado-Sanchez et al. (2006) observed that 22.2 or 44 µM BAP induced the formation of buds in two Mexican *P. vulgaris* cultivars. BAP has also been reported to be effective for shoot induction in *P. vulgaris* and *P. coccineus* (Malik and Saxena 1992; Santalla et al. 1998). The beneficial effects of BAP on adventitious shoot induction were also observed in other legumes such as *Vigna angularis* (Avenido and Hattori, 2000) and *V. mungo* (Saini and Jaiwal, 2002).

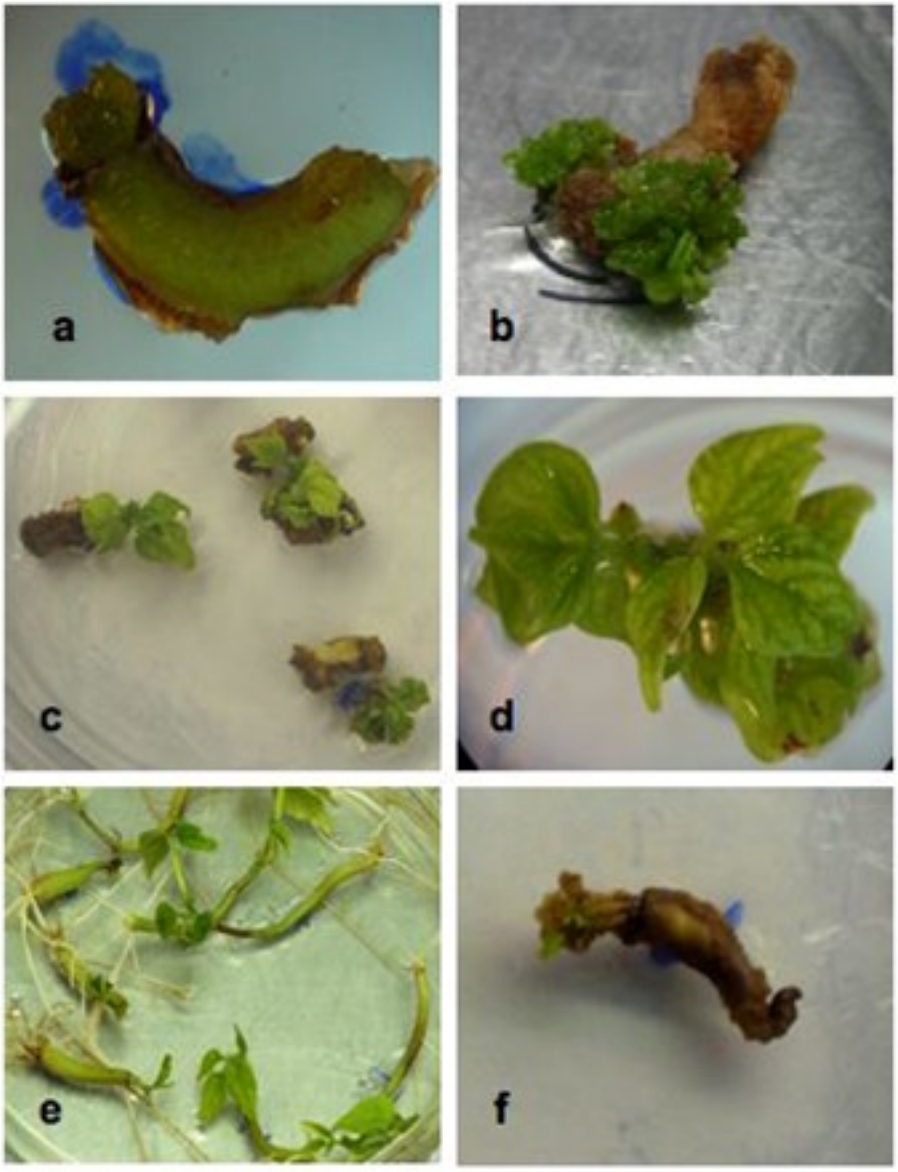
The conclusion of this study is culture of five different varieties axes on induction medium supplemented with different BAP and AS resulted in a differential response. Successful acclimatization of common bean *in vitro* plants were achieved in the greenhouse, and plants appeared morphologically normal. The regeneration system developed in this investigation for this important crop could be a useful tool for the genetic modification through mutagenesis or genetic transformation. The average number of shoots and leaves differed significantly among the common bean cultivars evaluated. The higher multiple shoot formation was obtained using Field bean > Garden bean > String bean > French bean > Flageolet bean. Moreover, the higher average of leaves was obtained using Field bean > Flageolet bean > French bean > String bean > Garden bean.

The shoots regenerated from the apical bud clusters and developed into plants with leaves and roots. The *in vitro* plants were successfully acclimated with 100% survival. The entire procedure starting from apical bud cluster induction to establishing a plant under greenhouse conditions took approximately 3 months and the future strategies are the *in vitro* protocol reported in this study could be used for clonal propagation of Indian bean and to obtain competent target tissue for genetic modification through in order to generate plants resistant to diseases and pests.

Table.1 Effect of genotype on the average number of shoots and leaves induced from embryogenic axes of common bean *(Phaseolus vulgaris* L.) regardless of the BAP and AS concentration

Genotype	Shoots[1]	Leaves[1]
V1- String bean	0.26 ± 0.04[2] b	0.27 ± 0.03 b c
V2 -Field bean	0.54 ± 0.06 a	0.41 ± 0.05 b
V3- Flageolet bean	0.10 ± 0.03 c	0.36 ± 0.04 b c
V4 -French bean	0.18 ± 0.02 c	0.23 ± 0.03 c
V5- Garden bean	0.42 ± 0.04 b	0.16 ± 0.08 a

Figure.1 Apical bud cluster induction from embryogenic axes of common bean (Phaseolus vulgaris L.)



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