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Short Communication

Agrobacterium tumefaciens mediated genetic transformation of moth bean [*Vigna aconitifolia* (Jacq) Marechal]

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Moth bean is an important grain legume of tropics, subtropics and warm temperate areas of different countries. It is rated as one of the most adapted arid legume in hot region due to its tolerance to drought and heat [1]. The transfer of foreign genes into the genome of moth bean has previously been achieved by co-cultivation of protoplast with *Agrobacterium* [2]. Moreover, an alternative approach utilizing the particle bombardment technology is also available using protoplast [3] and mature embryos [4]. The two techniques are more difficult than the present report where transgenics were obtained from leaf expants using *Agrobacterium.* The stable transfer of transgene and its expression has also been shown by histochemical GUS assay.

Seeds of two Moth bean varieties Jadia and Jawala were surface sterilized and inoculated in sterilized test tubes. Primary leaves and Cotyledonary node from 7-8 days old seedlings were used as explants for co-cultivation. Prior to attempting transformation experiments, the concentration of kanamycin appropriate for the selection of transformed cells was determined. Both types of explants were inoculated on MS medium supplemented with BAP 3 mg 1^{-1} and IAA 1 mg 1^{-1} along with different levels of Kanamycin (25, 50, 100, 200, 300, 400, 500, 600, and 700 mg 1^{-1}). A concentration of 100 mg l"^{1} kanamycin in regeneration medium was found appropriate for selecting kanamycin resistant transformed callus or shoots. At 100 mg 1^{-1} kanamycin there was no callusing and explants showed brown spots indicating toxic effect of kanamycin. Whereas, higher concentration (200-700 mg 1^{-1}) showed more chlorosis, browning and death in both varieties. Similar results were obtained by Mathews in *V. radiata* [5] and Karthikeyan in *V. mungo* [6].

Similarly, the concentration of cefotaxime (300-900 mg 1^{-1}) that can be tolerated by explants and at the same time kill the bacterium was selected. In both the explants growth and callusing was not affected by cefotaxime upto 900 mg 1^{-1}. However, presence of 500 mg 1^{-1} of cefotaxime effectively eliminated the excess of *Agrobacterium* and prevented its further growth.

Agrobacterium tumefaciens strain EHA 105 harboring the binary vector p35SGUSINT [7] was used for transformation experiments. The plasmid contained the *GUS* reporter gene driven by the CaMV35S promoter and *npt* II gene under the control of nopaline synthase promoter for kanamycin resistance as selectable marker. The *Agrobacterium* cultures were grown overnight at 27°C in YEP liquid medium (pH 5.2) containing 50 mg 1^{-1} kanamycin to log phase (A_{600}, 0.6-0.8). Bacteria were collected by centrifugation and resuspended in fresh MS liquid (20:1 v/v).

Leaf discs of 0.5 cm diameter and ctoyledonary node from *in vitro* raised seedling were co-cultivated with *Agrobacterium* solution for 10 minutes. Co-cultivated explants were blotted dry and incubated on hormone free MS medium containing 500 mg 1^{-1} cefotaxime for 2 days at 27 ± 0.5° C. Explains were washed thoroughly with liquid MS medium containing cefotaxime 500 mg 1^{-1}. Explants showed callus induction after 10-15 days

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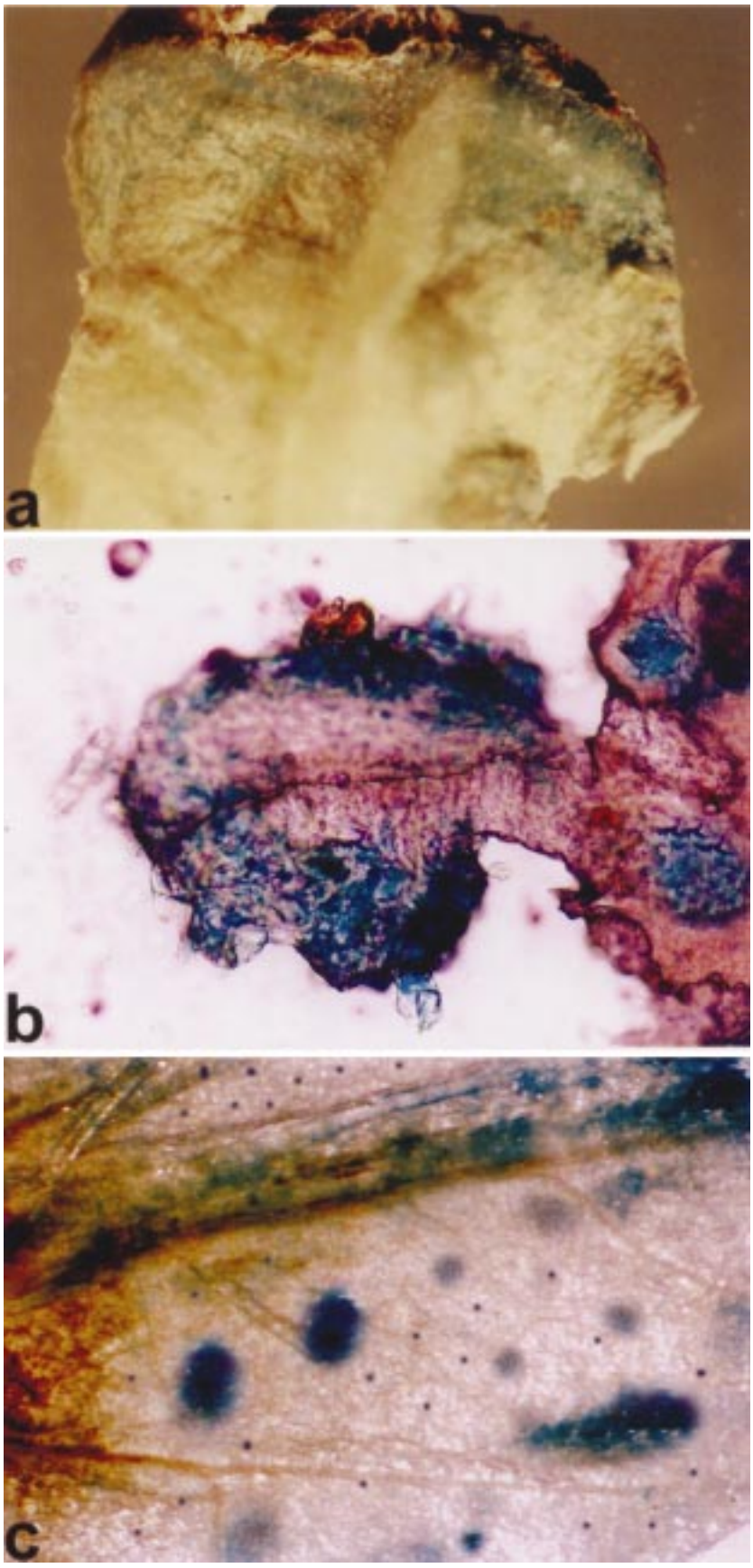

Fig. 1. a. Moth bean leaf explant showing GUS expression along the cut ends after 3 days of co-cultivation with A.t strain EHA105 (pSSSGUSINT); b. Smeared moth bean callus regeneratad from leaf explant co-cultivated with *A.t.* strain EHA105 containing plasmid p35SGUSINT (Photographed at 200X resolution) and c. Leaf taken from transformed plantlet showing GUS expression/the plantlet was obtained from callus derived from leaf explant incubated on kanamycin (100mg/l) co-cultivated with A.t. strain EHA105 (p35SGUSINT)

of inoculation. Regenerating kanamycin resistant callus and shoots were used for detection of GUS activity as described by Jefferson *et al.* [8]. The staining was carried out for 4-5 hrs at 37°C with x-gluc (Banglore, genei). Chlorophyll was removed by soaking the tissues in absolute alcohol after staining.

GUS expression was detected in cut ends of leaf explants (Fig. 1a) of both the varieties after three days of co-cultivation. This GUS activity could be because of transient expression of the gene. However, the presence of GUS activity in the callus and leaf taken from regenerated shoot (Fig. 1b&c) and its absence in control confirmed the stable transformation. The transformation frequency of tested callus was 100 per cent for both the varieties i.e. the entire callus mass taken showed GUS activity. Almost no genotypic effect was noticed as far as transformation frequency is concerned. Hence the developed protocol for leaf explants can be used to produce transgenics using economically important genes in diverse genotypes. However, transformation could not be achieved in Cotyledonary node explants.

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