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Agrobacterium-mediated genetic transformation and production of semilooper resistant transgenic castor (*Ricinus communis* L.)

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Summary

Semilooper resistant transgenic castor plants were produced through *Agrobacterium*-mediated genetic transformation method. Two castor cultivars, Jyothi and VP1 were transformed using the super-binary vector pTOK233 carrying *gus A* and *hpt* genes. Putative transformants were regenerated following selection on the hygromycin containing medium. GUS positive primary transformants, when subjected to Southern analysis, revealed stable integration of *gus A* into their genomes. In the T_1 generation, a monogenic segregation ratio of 3 GUS positive: 1 GUS negative plants was observed. Furthermore, transformation experiments were carried out with the *Agrobacterium* pSB111 super-binary vector carrying a synthetic delta endotoxin gene *cryIAb* and the herbicide resistance gene *bar* both driven by cauliflower mosaic virus 35S promoter. Putative transformants were regenerated through selection on the phosphinothricin containing medium and Basta tolerant transformants were subjected to molecular analysis. PCR analysis revealed the presence of both *bar* and *cryIAb* genes in the Basta tolerant primary transformants. Southern analysis of PCR positive plants with *cryIAb* probe showed a 3 Kb band upon *Hind*III digestion and a >6 Kb band with *Bam*HI digestion, thus suggesting stable integration of *cryIAb* intact expression cassette and independent nature of the transformants. The primary transformants subjected to ELISA disclosed varied levels of Cry protein. These transgenics expressing *cryIAb* — when bioassayed against freshly hatched semilooper larvae — induced substantial (>88%) insect mortality. Southern analysis of $2T_1$ plants revealed the presence of *cryIAb* gene, indicating stable inheritance of the transgene into the next generation. In T_1, all the Southern-positive plants for *cryIAb* invariably exhibited tolerance to Basta, denoting co-segregation of both *bar* and *cryIAb* genes. Transgenics, expressing *cryIAb* exhibited ample resistance against the castor semilooper.

Introduction

Castor (*Ricinus communis* L.), belonging to Euphorbiaceae, is an industrially important non-edible oil seed crop, and is widely cultivated in tropical and subtropical countries of the world. A major constraint to profitable castor production is the seed yield loss caused by various biotic stresses. More than 100 insects were found to feed on castor and cause heavy damage to the crop (Barteneva, 1986; Kolte, 1995). The loss in seed yield due to insect pests was estimated to be in the range of 35–50% (Kolte, 1995).

Among the lepidopteran pests, castor semilooper (*Achoea janata* L.) is a voracious feeder causing extensive defoliation and yield losses in the semi-arid tropics of India and other countries (Narayanan, 1959). Despite the availability of vast germplasm with wide variability for several economic characters, there are no known reliable gene sources of insect resistance in castor that may be readily introduced through traditional methods into elite castor cultivars. Intergeneric hybridizations between castor and *Jatropha* species proved unsuccessful owing to strong barriers to crossability existing between them (Sathaiah

& Reddy, 1985; Reddy et al., 1987; Sujatha, 1996 - unpublished).

Current strategies aimed at reducing crop losses mainly rely on chemical pesticides. However, these strategies are limited by high cost of pesticides, their intensive applications and persistence in the environment. Furthermore, the major insect pests were found to develop resistance to most of the chemical insecticides (Barton et al., 1987). Microbial formulations of *Bt* are being used safely to control various insect pests for more than 30 years (Aronson et al., 1986). However, poor persistence, photosensitivity and prohibitive costs of multiple sprays have restricted their applications (Sneh & Gross, 1983). The high specificity of the *Bt* toxins makes them an attractive alternative to chemical pesticides (Barton et al., 1987) provided the efficiency of application is improved. *Bacillus thuringiensis* (*Bt*) serves as a promising source of *cry* genes encoding insect specific δ-endotoxins. *Bt*-proteins are highly specific toxins that act by disrupting the midgut cells of different insect pests (Adang et al., 1987; Vaeck et al., 1987; Stewart Jr et al., 1996).

A prerequisite for introducing beneficial alien genes into crop plants entails the establishment of suitable regeneration and transformation systems. In castor, an efficient method of *in vitro* propagation, using meristematic explants, was developed to serve as a prelude to meristem-based genetic transformation (Sujatha & Reddy, 1998). *Agrobacterium*-mediated transformation and microprojectile bombardment are the two most widely used methods for achieving genetic transformation. However, *Agrobacterium*-mediated approach proved to be one of the most effective methods of genetic engineering in diverse plant species (Hood et al., 1986; Gasser & Fraley, 1989; Smith & Hood, 1995).

In this investigation, we have attempted to develop a stable genetic transformation system for introducing desired alien gene(s) into castor using embryo axes through *Agrobacterium*-mediated approach. We report, first time, the successful production of transgenic castor with *cryIAb* gene affording resistance against castor semilooper.

Materials and methods

Plant materials

Genetic transformation studies were initially carried out using two castor cultivars, "Jyothi", and "VP1", procured from the Directorate of Oil Seeds Research, Hyderabad, A. P., India.

Transformation vectors

Preliminary transformation experiments were carried out using the *Agrobacterium* super-binary vector pTOK233 harboring the reporter gene *gus A* containing an intron along with the selectable marker gene hygromycin phosphotransferase (Hiei et al., 1994) (Figure 1A).

Construction of pSB111cryIAbbar vector

The pSB111*cryIAbbar* vector was constructed as per the method described earlier (Ramesh et al., 2004). The synthetic delta endotoxin gene *cryIAb,* under the control of CaMV35S promoter and nos terminator (2.845 kb), was cloned into the *Hind*III site of the vector pSB11*bar* containing herbicide resistance gene *bar*, and the resulting vector was designated as pSB11 *cryIAbbar*. This construct was mobilized, into the *Agrobacterium* strain LBA4404 harboring the acceptor vector pSB1, through triparental mating and the resultant co-integrate vector was named as pSB111*cryIAbbar* (Figure 1B).

Castor transformation protocol

Mature seeds of castor were decoated and, were sterilized with 0.1% (w/v) of aqueous mercuric chloride for 10 min followed by three washes (ten minutes each) in sterile distilled water. Embryo axes were excised from seed and planted on the co-cultivation medium containing MS salts (Murashige & Skoogs, 1962) with 0.5 mg/l Benzyl amino purine (BAP) and 0.8% agar, a day prior to infection with the bacterium. All the culture plates were sealed with the parafilm. A total of fifty explants per petri plate were inoculated. *Agrobacterium* cultures (pTOK233 or pSB111*cryIAbbar*) were initiated by inoculating a single colony into 5 ml of ABG liquid medium (Lichtenstein & Draper, 1985) containing 50 mg/l hygromycin or spectinomycin at 29 °C for 24 h. The bacterial cultures were pelletted at 2500 rpm, resuspended in 10 ml of PIM II medium (Aldemita & Hodges, 1996) and incubated for 16 h at 29 °C. Each explant was co-cultivated with 20 μl of the *Agrobacterium* suspension and incubated for three days in dark at 29 °C. After 72 h of co-cultivation, the explants were thoroughly washed with 100 mg/l

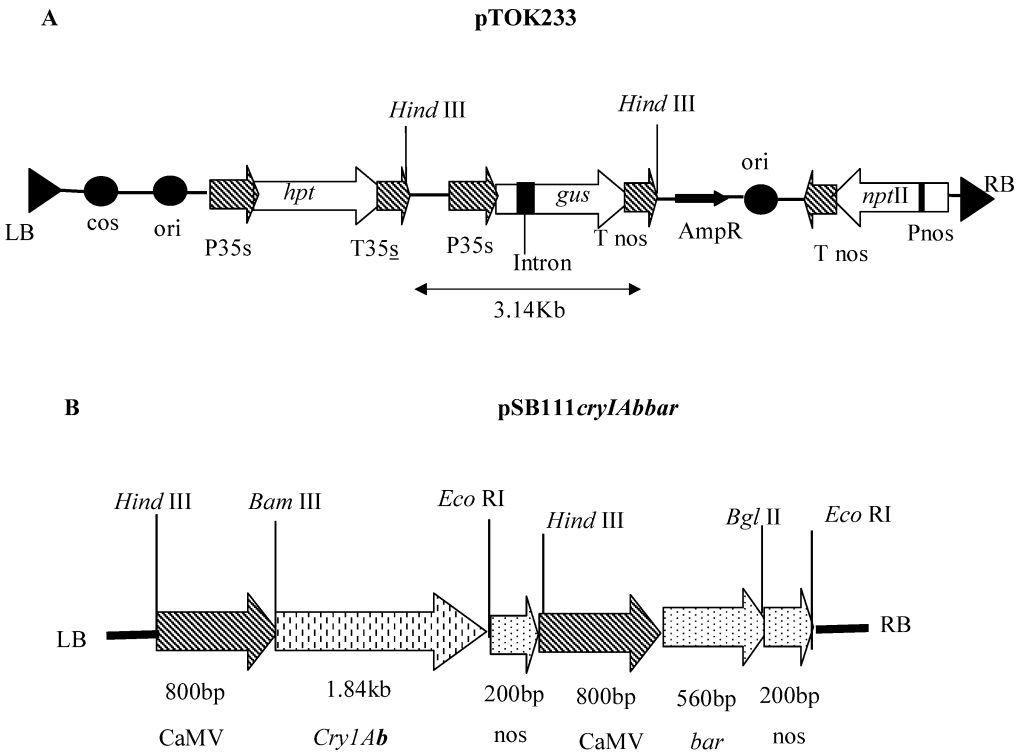

Figure 1. (A) T-DNA region of pTOK233 depicting restriction sites. (B) T-DNA region of pSB111*cryIAbbar* depicting restriction sites.

cefotaxime and 250 mg/l carbenicillin in sterile water for a period of 90 min, and incubated for two weeks on the MS medium containing 0.5 mg/l BAP, 0.5 mg/l Kinetin (KN), 100 mg/l cefotaxime, 250 mg/l carbenicillin and 30 mg/l hygromycin or 6 mg/l phosphinothricin and 0.8% agar (selection I medium). After 15 days of culture, the proliferating regions of the explants were excised and transferred to the MS medium containing 0.5 mg/l BAP, 0.5 mg/l KN and 50 mg/l hygromycin or 8 mg/l phosphinothricin and 0.8% agar (Selection II medium), and were cultured for two weeks. The surviving green or creamy white regions were transferred to the regeneration medium containing MS + 0.2 mg/l BAP + 0.5 mg/l KN and 0.8% agar without any antibiotic, for proliferation of shoots. Regeneration of shoots was observed after 10–15 days; the regenerated shoots were transferred to the MS-medium containing 0.2 mg/l BAP, 0.5 mg/l KN and 0.2 mg/l Gibberellic acid$_3$ (GA_3) for shoot elongation. Elongated shoots were rooted on the half-strength MS medium containing 0.5 mg/l Naphthalene acetic acid (NAA) and 0.3% gelrite. The rooted plants were initially transferred to soilrite + vermiculite mixture (3:1) supplemented with Hoagland's nutrient solution in the glass house, and were later established in pots filled with a mixture of black soil + sand + compost (3:3:1).

Analysis of transformants

GUS assay

Histochemical GUS assay was performed according to Jefferson et al. (1987). For transient GUS assay, the explants, after two weeks of culture on the selection I medium containing 30 mg/l hygromycin, were incubated overnight at 37 °C in a solution containing 50 mM sodium phosphate buffer, (pH-7.0), 0.1% TritonX 100, 10 mM EDTA and 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc). Likewise, leaves collected from two-month-old putative transformants, grown in the glass house, were assayed for GUS activity.

Basta leaf dip assay

Leaves sampled from 45 to 50-day-old putative transformants and control plants were subjected to Basta leaf dip assay to check for the expression of *bar* gene.

These leaves were dipped in 0.125% Basta solution and observations were recorded after 72 h.

PCR and Southern blot analyses

Genomic DNA was isolated from the putative transformants, obtained after co-cultivation with pTOK233 or pSB111*cryIAbbar*, and untransformed control plants by CTAB method (Saghai-Maroof et al., 1984). PCR analyses were carried out on plants obtained from co-cultivation with pSB111*cryIAbbar* using primers, 5′-CTA CCA TGA GCC CAG AAC G-3′; 5′- TCA GAT CTC GGT GAC GGG- 3′ for detection of *bar* coding sequence and primers 5′-GTT CGC AGT CCA GAA CTA CCA AG-3′; 5′-TGG GTG ATT TGA GAG GAA GGA 3′, for detection of *cryIAb* coding sequence, using the PCR amplification kit of Amersham Pharmacia Biotech in MJ Research Inc., Thermal cycler. The DNA extracted from untransformed plants was used as a negative control while the pSB11*cryIAbbar* vector was used as a positive control.

Southern blot analysis was carried out using fifteen micrograms of genomic DNA according to Sambrook and Russel (2001). Genomic DNA was digested with *Hind*III in pTOK233 transformants and with *Hind*III and *Bam*HI independently in *Bt* transformants. The digested DNA was resolved on 0.8% agarose gel and transferred to positively charged nylon membranes as per the manufacturer's instructions (Amersham). The coding sequences of *gus A* and *cryIAb* were radiolabelled with α-^{32}P dCTP using random primer labeling beads (Amersham), and were used as probes.

ELISA

Enzyme Linked Immuno Sorbent Assay (ELISA) was carried out for detection and quantification of CRYIAb protein in the leaf tissue samples of primary transformants. The ENVIROLOGIX CRYIAb plate kit designed for the quantitative laboratory assay was used. Samples of 500 mg of leaf tissue from 2-month-old primary transformants and untransformed control plants were washed, blotted and ground to a fine powder in liquid nitrogen. The ground tissue was resuspended in 0.5 ml of 1X CRYIAb extraction/dilution buffer in a microcentrifuge tube and processed as per the instructions. The plate was read within 30 min of the addition of stop solution. Observations were recorded on an ELISA plate reader set to read the plate at 450 nm wavelength.

Insect bioassays

Insect bioassays were carried out on the Southern positive primary transformants and control plants. Detached leaf bioassays were carried out using freshly hatched neonate larvae of the castor semilooper (*Achoea janata* L.) under controlled conditions in a no-choice environment and the experiments were replicated thrice. The larval weight was measured before releasing them onto leaves in petriplates containing moist filter paper. Twelve larvae were infested on three 4 cm-wide leaf discs at the rate of 4 larvae/leaf disc. The plates were incubated at 25 $\pm$ 2 °C in the dark at 70–80% relative humidity for three days. Data on larval weight and mortality were recorded after 3 days of feeding.

T_1 generation studies

Selfed seeds from the six Southern-positive plants obtained with pTOK233 vector were collected and germinated in the glasshouse. Leaves sampled from two-week-old T_1 plants were assayed histochemically for stable GUS expression as described earlier.

The putative transformants obtained with pSB111*cryIAbbar* vector were selfed and mature seeds were collected plantwise. T_1 seeds of $2T_0$ primary transformant were germinated and the resulting T_1 plants were used for Basta leaf dip assay as described earlier. The DNA isolated from five of the T_1 plants was subjected to PCR and Southern blot analyses as described earlier.

Results and discussion

The present study describes results dealing with the establishment of a reproducible transformation protocol for the introduction of beneficial alien genes into the castor genome using super-binary Ti vectors, viz., pTOK233 and pSB111*cryIAbbar*.

Genetic transformation using pTOK233

The mature embryos, after co-cultivation – when assayed for transient GUS expression – showed characteristic blue coloration of varied intensities. Out of 240 embryos infected, cv. Jyothi showed significantly higher frequency of GUS expressing embryos (84.12%) compared to cv VP1 (67.32%). Based on these observations, cv. Jyothi was used for stable transformation experiments.

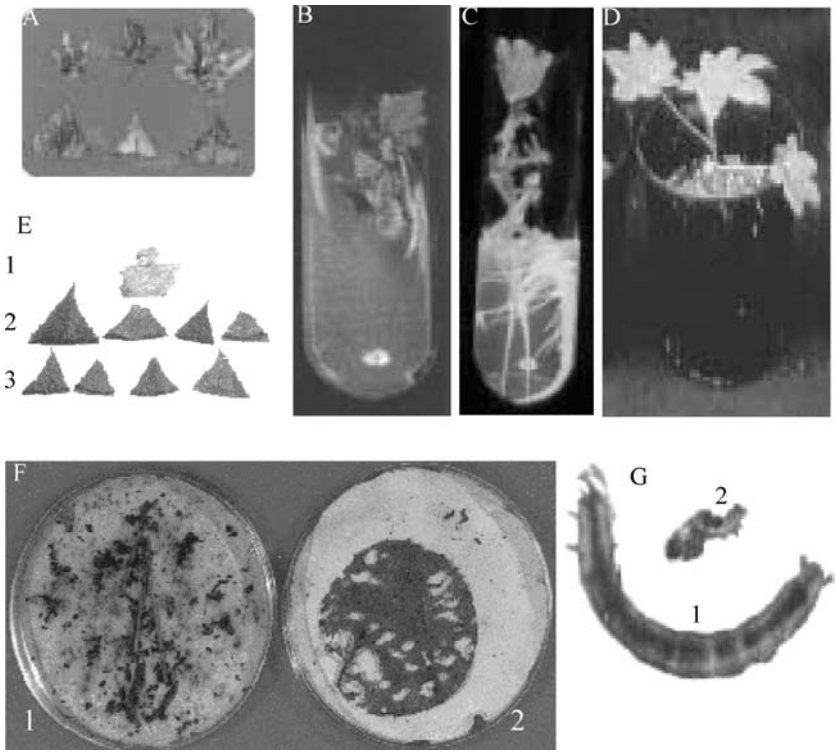

Figure 2. (A)GUS expression in the leaves of primary transformants of castor. (B) Regeneration of putatively transformed regions of cv. jyothi after selection on 8 mg/l phosphinothricin. (C) Profuse rooting observed in the putative transformant. (D) Acclimatization of the transformants in the glass house (pot height 14 cm and diameter 15 cm). (E) Basta leaf dip assay of primary transformants of cv. jyothi. Row 1: Control leaf. Rows 2 and 3 : Transgenic leaves. (F) Insect bioassays on leaves of primary transformant and untransformed control plants of cv. jyothi with castor semilooper larvae after three days of infestation. 1. Extensive feeding damage caused by semilooper on the untransformed control leaf. 2. Partial feeding damage caused by semilooper on the transgenic leaf. (G) Effect of CRY protein on semilooper larva fed on the transgenic castor. 1. Larva fed on untransformed control leaf. 2. Larva fed on transgenic leaf.

More than five thousand mature embryos of cv. Jyothi were co-cultivated with pTOK233, out of which 30% embryos disclosed surviving regions after two stages of selection. The transformed regions were found healthy with green or creamy white appearance, while untransformed regions turned black on the selection medium. The surviving regions were observed randomly among the infected embryos. The healthy surviving tissues, when transferred onto the regeneration medium, gave rise to *de novo* shoot development. The regenerated shoots developed profuse roots in the rooting medium and were subsequently transferred to the glasshouse. Two-month-old regenerated plants (To), when assayed histochemically, showed GUS expression in the leaves (Figure 2A), thereby indicating the stable integration of *gus A* gene. A total of 21 independent putative transformants of cv. jyothi were established from 5000 infected embryos, revealing 0.42% transformation frequency.

Southern analysis of GUS positive primary transformants

Southern blots of GUS positive transgenic plants, when probed with radiolabelled 1.8 kb *gus A* coding sequence, showed a clear hybridization signal in all the six transformants at ~3 kb region corresponding to the expression cassette of *gus A*, affirming the stable integration of reporter gene *into* the genomes of castor plants. However, no such hybridization signal was observed in the untransformed control (Figure 3A).

Inheritance of gusA gene in T_1 generation

Selfed seeds were collected from the six Southern-positive transformants and germinated in pots in the glass house, to analyse the inheritance pattern of transgene. Histochemical GUS assays, using leaves of two-week-old T_1 plants, revealed a monogenic segregation

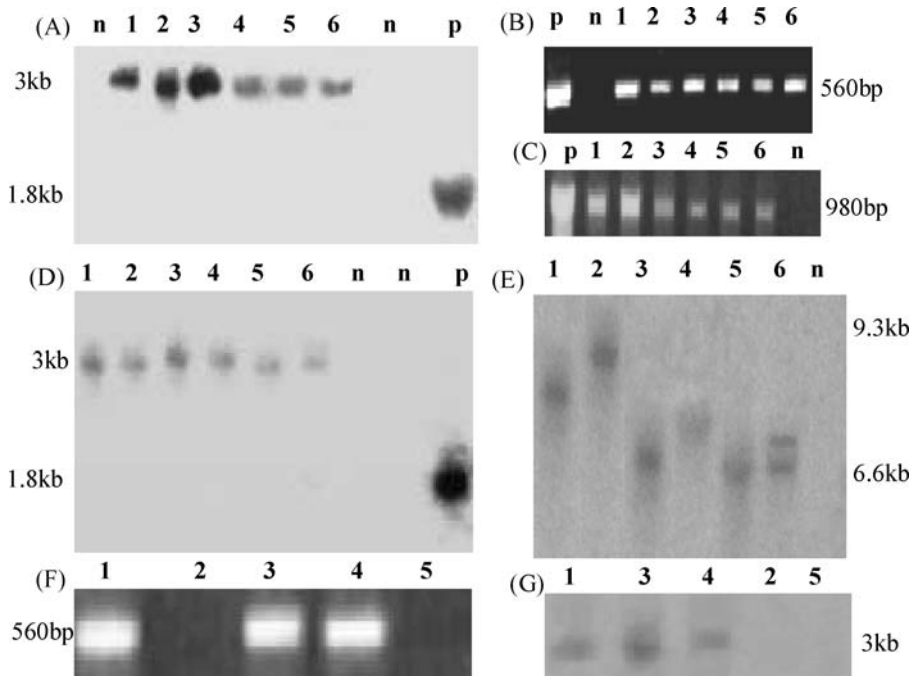

Figure 3. (A) Southern blot analysis for detection of *gus A* in the primary transformants of cv. jyothi obtained after cocultivation with pTOK233, n: *Hind*III digested genomic DNA of untransformed control, 1–6: *Hind*III digested genomic DNA of primary transformants, p : 1.8 kb *gusA* sequence (positive control). (B) PCR analysis of primary transformants of cv. jyothi for the presence of *bar* gene, p: pSB11*cryIAbbar* plasmid (positive control), n: untransformed plant (negative control), 1-6: primary transformants. (C) PCR analysis of primary transformants of cv. jyothi for the presence of *cryIAb* gene, p: pSB11*cryIAbbar* plasmid (positive control), 1–6: primary transformants n: untransformed plant (negative control) (D) Southern blot analysis of primary transformants for the presence of *cryIAb*, 1–6: *Hind*III digested genomic DNA of primary transformants n: *Hind*III digested genomic DNA of untransformed plant (negative control), p: 1.8 Kb *cryI*Ab coding sequence (positive control). (E) Southern blot analysis of primary transformants for the presence of *cryIAb* gene. 1–6: B*am*HI digested genomic DNA of transformants. n: B*am*HI digested genomic DNA of untransformed control. (F) PCR analysis for the presence of *bar* in T_1 generation. 1,3 and 4 : T_1 plants showing the presence of *bar* gene. 2 and 5 : T_1 plants lacking *bar* gene. (G) Southern blot analysis for the presence of *cry*IA*b* in T_1 generation. 1,3 and 4 : T_1 plants showing the presence of *cry*IAb gene. 2 and 5 : T_1 plants lacking *cry*IAb gene.

ratio of 3 GUS positive: 1 GUS negative plants (Table 1), amply indicating that the *gus A* gene is stably integrated into the castor genome and is transmitted to the next generation in a Mendelian fashion.

Development of co-integrate vector pSB111 cryIAb bar

Molecular analysis of constructed Ti plasmid confirmed the successful recombination between pSB11*cryIAbbar* vector and the acceptor vector pSB1 during triparental mating, resulting in a recombinant Ti vector pSB111*cryIAbbar*. PCR analyses of the recombinant Ti vector, using gene specific primers of *bar* and *cryIAb,* revealed amplified bands of 560 bp and 980 bp, respectively. Similarly, *Hind*III digested DNA, probed with *cry* coding sequence, showed a clear hybridization signal at ~3 Kb, connoting the stable formation of the cointegrate (data not shown).

Genetic transformation using pSB111cryIAbbar

In stable transformation studies using pSB111*cryI-Abbar*, it was observed that untransformed embryonic regions became necrotic within ten days on selection I medium, while transformed regions were found healthy and were creamy white or green in colour. The actively growing regions were selected and transferred to the selection II medium. Actively growing tissues that survived two stages of selection were regenerated into shoots (Figure 2B); the regenerated shoots were rooted on half-strength MS medium containing 0.3% gelrite (Figure 2C). The Rooted plantlets were hardened in Hoagland's nutrient solution and transferred to pots in

Table 1. Segregation pattern of the *gus* A gene in T_1 generation of castor plants

Transformant	No. of seeds germinated	No. of plants showing GUS expression	No. of plants without GUS expression	Segregation ratio	χ^2 value*
1	9	7	2	3:1	0.036
2	14	11	3	3:1	0.094
3	15	11	4	3:1	0.021
4	8	6	2	3:1	0
5	7	5	2	3:1	0.046
6	13	9	4	3:1	0.230

Calculated χ^2 values are $<$ tabular χ^2 value (3.841).
*Significant at 5% level.

glass house (Figure 2D). A total of 25 independent putative transformants of cv. Jyothi have been established from 6,185 infected mature embryos approximating to 0.4% transformation frequency.

The leaves from control plants subjected to Basta showed complete damage with dried, dark-brown appearance. Whereas the leaves of transformants exhibited either minimal or no damage and remained green after Basta application (Figure 2E). Basta leaf dip assays revealed that 16 of the 25 transformants exhibited tolerance to the herbicide, indicating the expression of *bar* gene in them.

Molecular characterization of primary transformants

PCR amplification of the genomic DNA from putative transformants with *bar* and *cryIAb* primers, gave 560 bp and 980 bp amplification products of *bar* and *cryIAb*, respectively (Figure 3B & C), which were similar to that of the positive control pSB11*cryIAbbar* vector. Whereas, no such bands were observed in the untransformed controls under similar conditions. This observation clearly indicates the presence of both the transgenes in the genomes of T_0 transformants.

Southern blot analysis of *Hind*III digested genomic DNA of PCR positive plants, probed with radiolabelled *cryIAb* coding sequence disclosed a clear hybridization signal at ~3 kb region indicating the integration of intact *cryIAb* expression cassette into their genomes (Figure 3D). However, no such hybridization signal was seen in the untransformed control. Whereas, *Bam*HI digested DNA, probed with *cryIAb*, exhibited hybridization signals of different sizes >6 kb (Figure 3E), suggesting the independent nature of various transformants. Most of the transformants showed the presence of a single band, implying single copy integration of the transgene in the castor genome. It was reported that single copy integrations were essential for achieving predictable inheritance pattern and for avoiding problems of gene silencing in transgenic plants (Finnegan & McElroy, 1994).

ELISA

ELISA was carried out using microtitre plates coated with CRYIAb antibody to estimate the levels of CRYIAb expression in the primary transformants. In different transformants, a characteristic yellow color was observed at different intensities. The concentration of CRYIAb protein among transformants ranged from 0.23 to 0.47 ng/mg tissue (Table 2). The differences observed in CRYIAb expression levels may be attributed to the random integration of the transgene at different sites in these transformants. Similar results were reported in various transformants of tobacco, tomato, cotton, maize and rice transformed with

Table 2. Insect bioassay on primary transformants

Plants	CRY Protein ng/mg tissue	Mean larval Mortality (%) ± S.D	Mean larval weight (mg) ± S.D
$1T_0$	0.24	88.91 ± 1.19	33.23 ± 1.15
$2T_0$	0.47	97.25 ± 1.78	25.92 ± 2.44
$3T_0$	0.42	94.41 ± 1.39	26.67 ± 3.46
$4T_0$	0.43	94.41 ± 1.34	26.57 ± 3.43
$5T_0$	0.23	88.92 ± 1.25	33.63 ± 1.23
$6T_0$	0.38	94.41 ± 1.53	30.10 ± 1.40
Untransformed control	0	13.92 ± 0.51	142.69 ± 0.26

modified *cryIAb* gene (Ghareyazie et al., 1997; Ramesh et al., 2004).

Insect bioassays

Insect bioassays with castor semilooper on primary transformants confirmed the toxicity of CRYIAb protein expressed in the transgenic castor plants. After three days of infestation the larvae fed on the leaves of different primary transformants disclosed high levels of insect mortality ranging from 88.91% to 97.25% (Table 2). Whereas larvae fed on untransformed control leaves showed least (13.92%) insect mortality. These differences in larval mortality are attributable to the varied levels of CRYIAb protein expressed in different transformants. The larvae fed on the leaves of primary transformants showed marked feeding inhibition and reduced larval growth compared to the voracious feeding and normal larval growth observed on the leaves of susceptible untransformed plants (Table 2; Figure 2F and G). It was reported that *Bt* toxins bind to the cell – membrane – lining of the insect midgut and cause pores that disturb the osmotic balance, thereby inducing swelling and lysis of midgut cells; as a consequence, the insect larvae stop feeding and eventually die (Feitelson et al., 1992).

A high concordance was noted between the observations recorded from the ELISA and that of insect bioassays (Table 2). Transformants expressing higher levels of CRYIAb protein invariably induced higher larval mortality, resulting in substantial resistance against the castor semilooper. The various primary transformants were phenotypically normal and produced fertile seed upon selfing.

Molecular analysis of T1 progeny

The T_1 progeny of the primary transformant ($2T_0$) were subjected to PCR and Southern analyses to determine the mode of inheritance of transgenes. A clear amplification of the 560 bp product (Figure 3F) representing the *bar* gene implicates its stable integration into the castor genome. *Hind*III digested genomic DNA from *bar*-positive plants, probed with radiolabelled *cryIAb* coding sequence, disclosed a clear hybridizable signal of ~3 kb representing the expression unit of *cryIAb* (Figure 3G). These observations amply indicate that the transgenes *cry* and *bar* are stably integrated and are co-segregating. Furthermore, the co-segregation of transgenes also signify the transfer of entire T-DNA region along with the two expression cassettes and their unequivocal integration at a single site in the castor genome.

To our knowledge, no published report(s) dealing with genetic transformation of castor plants, deploying agriculturally useful gene(s), exist. However, a method of transformation of castor through vacuum infiltration of wounded flower buds was patented by McKeon et al. (2003; US patent No. 6, 620,986). Also, an attempt has been made to develop a transformation system for castor using meristem proliferation system and hygromycin phosphotransfarase (*hpt)* as a marker gene (Sujatha & Sailaja, 2005). It was reported that recalcitrant plant species could be made amenable to transformation by the *Agrobacterium*-mediated method using super-binary vectors (Komari, 1990; Li et al., 1996; Saito et al., 1992). In the present investigation, we report the successful genetic transformation of castor using the super-binary Ti vectors. The established transformation protocol proved effective for both the selectable marker genes, viz., *hpt* and *bar*, using mature embryo axes as explants. These transgenics expressing CRYIAb afforded ample resistance against the castor semilooper. Furthermore, the various transformants, endowed with the exotic gene, hold promise as a novel genetic resource in conventional breeding aimed at developing insect resistance.

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