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Comparison between *Corchorus olitorius* and *Corchorus capsularis* at GUS histochemical assay performance for tissue culture independent transformation

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ABSTRACT

Tissue culture independent transformation technique in crops is relatively new and of popular interest due to its faster approach and efficiency. The prospect of this technique in the production of transgenic Jute plants with new genetic properties is promising. In the present study, two varieties of each of *Corchorus olitorius* (var. O-72 and var. OM-1) and *C. capsularis* (var. CVL-1 and var. BJC-83) were used to observe their transformation ability. *Agrobacterium tumefaciens* strain LBA4404 was used for transformation, which harbors a binary vector pBI121 containing selectable marker gene nptII, *gus* (β-glucuronidase) reporter gene and a stress tolerance gene GLY-1. The young shoot tip of the seedlings (20-22 cm) was infected with the bacterial culture. The young leaves were collected after 20-21 days of bacterial culture transformation and thereafter histochemical GUS assay was performed. In the putatively transformed regions of the plants, *gus* reporter gene was expressed showing blue color in the tissues. Non-transformed plants did not show any color. Among the varieties, the percentage of matured plants showing GUS activity was higher in *C. olitorious* var. O-72 (80.66%) and OM-1 (73.33%) compared to *C. capsularis* var. BJC-83 (32.50%) and CVL-1 (40.00%). The result of the study provides an indication that efficiency of transformation by using tissue culture independent direct genetic transformation for the two species of Jute may differ significantly.

Key words: *Corchorus olitorius*, *Corchorus capsularis*, GUS assay, tissue culture independent transformation

Introduction

Jute is the world's front mostbast (bark) fibre and second most important textile fibre after cotton. Bangladesh is the second largest jute fibre producing country after India with its production of about 9.9 lakh tons. Bastfibre is produced from two cultivated species of jute (*Corchorus olitorius* and *C. capsularis*) (Huda et al., 2007). These self-pollinating species of jute are characterized by limited genetic variability with respect to quality and quantity of fibre production as well as agronomic adaptability and disease susceptibility (Basu et al., 2004). However, the production, quality and yield of this crop are affected by various biotic and abiotic stresses. In particular, flood and drought are among the common problems in crop cultivation of Bangladesh. *C. capsularisis* is comparatively more resistant to flood and drought but slightly more susceptible to diseases and pests. On the other hand *C. olitorius*, which is relatively tolerant to diseases and pests, produces the stronger commercial fibre (Roy et al., 2006). So, a desirable combination of useful characters of these two species is of recent interest. There are a number of wild germplasms of jute available in the Gene Bank of Bangladesh Jute Research Institute (BJRI), which can be utilized for the variety improvement program. But interspecific cross-incompatibility stands in the way of employing these wild jute germplasms for transfer of

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significant agronomical characters.

Different techniques of plant tissue culture have been developed as a powerful tool for crop improvement (Carlson PS., 1975; Razdan & Cocking, 1981). However, this technique is a lengthy procedure, which involves the steps such as *in vitro* seed germination, callus induction, maintenance of callus, shoot regeneration. It has been found that *in vitro* regeneration is hard to achieve with *C. capsularis* and almost impossible with *C. olitorius*. Success with whole plant regeneration using tissue culture and transformation was negligible (Sarker et al., 2007). Advances in tissue culture and recombinant DNA technology in recent times have exposed new approaches in transformation of higher plants, which therefore produced many transgenic plants with new genetic properties.

Establishment of an efficient system from explants to matured fertile plants was the foremost requirement of genetic transformation of jute. Transformations of higher plants have been accomplished by different methods (Gardner, 1993; Gasser & Frley, 1989; Paszkowski et al., 1988). The most common and efficient one utilizes non-ontogeny *Agrobacteium* strain as a gene vector (Lindsey, 1992). The most recent successful transformation technique was the tissue culture independent transformation (Sajib et al., 2008). The direct method of transformation is less time consuming and more efficient than the tissue culture dependent methods.

Preliminary studies on *Agrobacterium*-mediated genetic transformation have shown that the *gus* gene can be efficiently transferred to infected explants of jute varieties (Hossain et al., 1988), but there are limited numbers of reports on the recovery of *Agrobacterium*-mediated transformed (transgenic) jute plants and regeneration abnormality as well as failure was often seen (Haseena et al., 2000; Khatun et al., 1990; Ahmed et al., 1999). However, there is one established transformation protocol for jute, which had been developed by using a biolistic particle delivery system for the generation of stable genetic transformation in jute (*C. capsularis* var. JRC321) (Ghosh et al., 2002). But, that study included the use of a costly particle bombardment technique and lengthy tissue culture procedure. The simple tissue culture independent protocol for transformation of Jute demonstrated by Sajib et al. (2008) involved only var. O-72 of *C. olitorius*. The transformation efficiency in that study was shown to be sufficiently high for practical purposes (Sajib et al., 2008). However, no previous study was undertaken to see the efficiency of the other important cultivated species- *C. capsularis* using this methodology. The aim of this study was simply to screen the possibility of *Agrobacterium*-mediated direct gene transformation event through GUS assessment of the matured leaves of both of the *C. olitorius* and *C. capsularis*, the plantlet of which were infected in the meristematic region. From this comparative study, the potentiality of the broader application of *Agrobacterium* mediated direct gene transformation in Jute can be assumed.

Materials and Methods

Plant source

The present transformation experiment was conducted with four varieties from two species of jute (*C. olitorius* L. var. O-72 and OM-1 and *C. capsularis* L. var. CVL-1 and BJC-83). The seeds of four varieties of jute, obtained from the Gene Bank Department of Bangladesh Jute Research Institute, were sown in pots filled with appropriately prepared soil. The germinated seeds were maintained so scientifically that each of the germinated seed grew as a plantlet. No fertilizer and pesticide used during the total experiment. The plantlets were allowed to grow 18-22 cm in height. The apical meristematic regions of young jute plants were infected with *A. tumefaciens* strain LBA4404 harboring pBI121. The experiment was conducted for primary screening and comparison of GUS activity among directly infected *C. olitorius* L. and *C. capsularis* L..

Culture of the A. tumefaciens Strain LBA4404

Genetically engineered *A. tumefaciens* strain LBA4404 was used for infection in the transformation experiment. This strain contains plasmid pBI121 of 14 KDa (binary vector) containing udiA gene encoding *gus* (β-glucuronidase) (Jefferson et al., 1987), the nptII gene and a stress tolerance gene (GLY-1). The bacterium also contains plasmid pAL4404, which is a disarmed Ti plasmid (132 KDa) containing the virulence genes. YMB (10 g/L D-Mannitol, 0.4 g/L Yeast extract, 0.1 g/L NaCl, 0.2 g/L $MgSO_4.7H_2O$, 0.5 g/L K_2HPO_4) medium was used for suspension culture whereas LB (10 g/L Triptone, 10 g/L NaCl, 5 g/L Yeast extract) was used as *Agrobacterium* maintenance medium; both of the media were supplemented with kanamycin monosulphate as antibiotic.

Genetic transformation and GUS assay

The shoot tips of young jute plants (20-22 cm in height) were injured with a fine needle. After an hour, the injured

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regions were infected with few drops of *A. tumefaciens* suspension in YMB medium with an O.D. of 0.8, followed by a second infection at the same regions an hour later. After being placed in the dark for three days, the infected plants were allowed to grow under normal conditions in light.

For preliminary assessment of transformation, GUS activity was assayed with newly formed leaves of infected plants above the infected regions after 20-21 days of infection. The young leaves were immersed in the GUS solution. The tube containing the leaves immersed in the GUS solution was incubated for 24 hours at 37°C in dark. GUS activity was detected as described by Jefferson (Jefferson et al., 1986). The preferred substrate for GUS detection is 5-bromo-4-chloro-3-indolyl-β-D-glucuronide or X-gluc. This colorless substrate has high extinction coefficient (making it readily detectable at low concentrations) and aqueous insolubility of the final cleavage product, dichloro-dibromoindigo (CIBr-indigo). The transverse and longitudinal sections of leaves segments and part of shoot tips showed blue color under compound microscope and the photographs were taken with the help of digital camera (Samsung PL210). The percentage of GUS assay was calculated by the following formula.

$$\text{Percent GUS positive plants} = \frac{\text{Number of GUS positive plants}}{\text{Number of plants assayed for GUS}} \times 100$$

Results

Morphology of Agrobacterium infected plants

Almost all the wounded plants survived and showed normal growth upon infection with *A. tumefaciens*. In the region of the infection the stem of the jute plant has become widen in diameter as compared to surrounding regions shown in Figure 1. Stems of control plants were slimmer than transformed plant's stems.

GUS histochemical assay

All the plants survived following *Agrobacterium* infection were subjected to GUS histochemical assay. Conspicuous GUS positive (blue colour) regions were detected in the transformed leaf surface. The detailed results of the investigation are presented in the Table 1. Following the GUS assay, it was found that all the four varieties showed positive responses unequally towards infection by *A. tumefaciens*. The control leaves did not show any response to the assay. The microscopic views of the blue zone in the transformed tissues are assured in Figure 2.

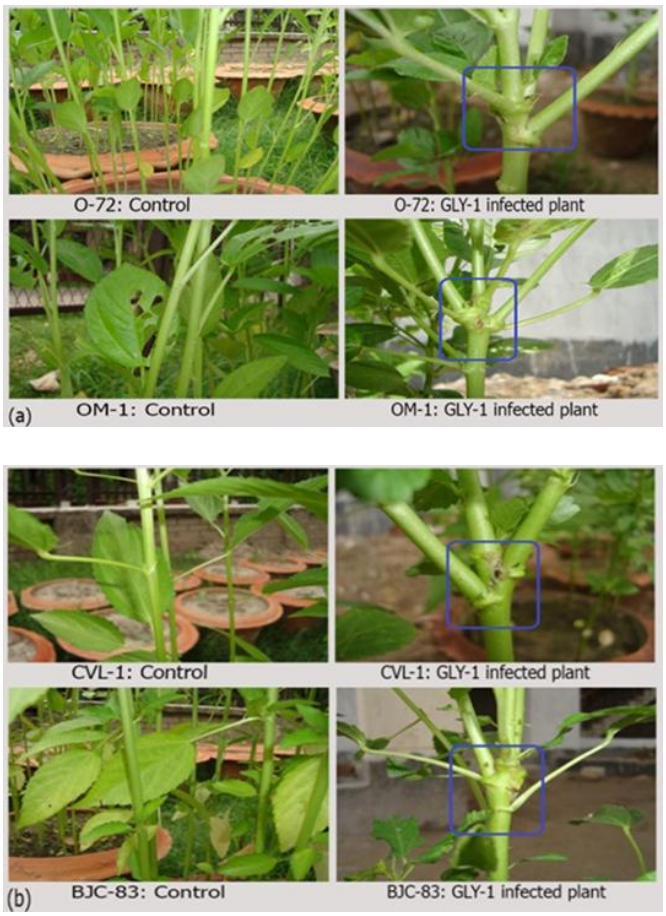

Figure 1. *Phenotypic expression of infected variants with the gene GLY-1 against control plants (a) variants O-72 and OM-1 of C. olitorius (b) variants CVL-1 and BJC-83 of C. capsularis.*

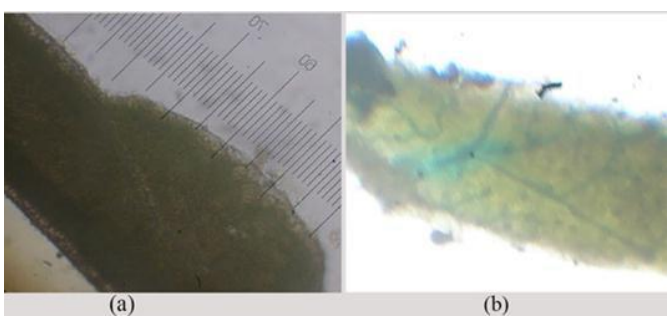

Figure 2. *GUS assay of one of variants (**a**) C. olitorius var. O-72 Control jute leaf at 40x, (**b**) C. olitorius var. O-72 Transformed leaf showing blue zone at 100x.*

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Table 1. *Effect on varieties of transformation analyzed by GUS histochemical assay for GLY-1 gene.*

Jute species	Varieties	Replication	Number of plants assayed for GUS	Number of plants positive for GUS	% of GUS positive plants	Mean (%)
Corchorus olitorius	O-72	R1	30	21	70.00	80.66
		R2	30	25	83.33	
		R3	29	23	79.31	
		R4	30	27	90.00	
	OM-1	R1	30	24	80.00	73.33
		R2	30	19	63.33	
		R3	30	21	70.00	
		R4	30	24	80.00	
Corchorus capsularis	CVL-1	R1	29	12	41.37	38.25
		R2	30	9	30.00	
		R3	30	10	33.33	
		R4	29	14	48.28	
	BJC-83	R1	30	12	40.00	40.00
		R2	30	10	33.33	
		R3	30	12	40.00	
		R4	30	14	46.67	

Discussion

Results of GUS histochemical assay (Table 1) showed that *C. olitorius* variants transformed in greater extent as compared to *C. capsularis* variants. The average percentage of transformed tissues assayed by GUS histochemical assay in this study was found to be maximum 80.66% in *C. olitorius* var. O-72 and minimal 38.25% in *C. capsularis* var. JBC-83.

The study carried out by Sajib et al. (2008) showed similar morphology and GUS activity of infected plants (T_0) of O-72 and after normal growth of the seeds, an average of 76.23% GUS activity of the leaves from T_2 plants. That study showed that, in leaves of T_0 plants some but not all cells were positive for GUS expression, but whole plants were positive in further (T_1 and T_2) generations. Efficiency obtained in the study was shown to be sufficiently high for practical purposes (Sajib et al., 2008). In the present study, the average GUS activity in O-72 and OM-1 was found as 80.66% and 73.33% respectively. Transformation in dividing cells of meristematic regions results in the transfer of transgene(s) to the progeny cells and later differentiation of these progeny cells to floral buds which are followed by seed generation, results in inheritance of the transgene(s) in seeds (Sajib et al., 2008). Thus, it can be assumed that the *C. olitorius* varieties used in this study would also have efficient transformation in T_1 and T_2 plants. On the other hand, the GUS activities in the *C. capsularis* (38.25% for CVL-1 and 40.00% for BJC-83) varieties were much lower in comparison to *C. olitorius* and this may entail a putative transformation not as efficient as with *C. olitorius* of *C. capsularis* in further generations.

The present study was the first of its kind to be undertaken regarding *C. capsularis* with a view to indicate the possibilities of tissue culture independent direct genetic transformation in the same. Further studies involving molecular techniques like *gus* gene specific PCR, RT-PCR, and selection in subsequent generations are needed to affirm whether or not the gene has been stably transformed.

Conclusion

The efficient tissue culture independent protocol in Jute transformation is economically feasible and less time consuming. The direct genetic transformation is considered as efficient for *C. olitorius* which is also an indicative of our study results. On the other hand, *C. capsularis* variants based on primary GUS histochemical assay, may not be as responsive as the *C. olitorius* variants towards tissue culture independent direct genetic transformation.

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