Transient expression of β-glucuronidase reporter gene in embryogenic callus cultures of an elite indica basmati rice (*Oryza sativa* L.)

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Various parameters for the introduction of β-glucuronidase (GUS) reporter gene driven by actin-1 promoter into embryogenic callus cultures of an elite indica basmati rice cultivar (Basmati 370) through biolistic delivery method were studied. Helium gas pressure of 900 psi, arrangement of the callus at the centre of the plate or 1100 psi helium pressure and arrangement of the callus at the innermost concentric ring of the plate along with 6 cm distance from the microcarrier produced best results in terms of transient GUS expression, but with moderate callus survival. However, better survival of the callus with moderate GUS expression was observed when the callus was placed at the centre or the innermost concentric ring of plate with a distance of 9 and 6 cm using 900 and 1100 psi, respectively; and these parameters may be useful in recovering transgenics. These parameters will now be employed for the development of fertile transgenic basmati rice harbouring agronomically useful genes.

SIGNIFICANT progress has been made in the last two decades in the improvement of important cereals, such as rice, through conventional breeding methods.

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However, application of genetic manipulation techniques is becoming increasingly important for rice improvement to cope with its growing need. Although, genetic transformation through *Agrobacterium* is very effective and a widely used method of obtaining transgenic plants in dicots, it is still a difficult proposition in monocots, including rice, though some success has been achieved in this respect, of late[1,2]. Therefore, other methods of gene transfer such as particle gun, electroporation of polyethylene glycol-mediated transformation have been used for the achievement of fertile transgenic rice[3-5] and other cereals[6,7]. However, all of these reports have come from laboratories abroad. Certain elite indica rice cultivars, which are widely grown in our country, have not been used for these studies. Regeneration protocol from callus cultures in Basmati 370 which is an aromatic, fine-grained and highly-priced rice cultivar has been standardized in our laboratory[8] with the aim of establishing transformation protocol in basmati rice. In this communication, we report, for the first time, the transient expression of β-glucuronidase (GUS) reporter gene through biolistic delivery in mature embryo-derived embyrogenic callus cultures of rice (*Oryza sativa* L. cv. Basmati 370).

Mature seeds of rice were de-husked, surface-sterilized with 0.1% $HgCl_2$ for 20 min and then rinsed with several changes of sterile distilled water. For callus induction, the seeds were placed on Murashige and Skoog (MS)[9] medium supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and cultured in dark at 26 ± 1°C. Following three weeks of culture, the callus induced contained both embryogenic as well as non-embryogenic regions. Embryogenic regions were excised and cultured on the same callus medium for another 2 weeks as described earlier[10].

The PDS-1000 He biolistic particle delivery system (Bio-rad, USA) was employed in the present experiments and the microprojectiles were driven by helium pressure. The gene construct used was actin-1 promoter-actin 1 intron-gus-tnos (obtained from D. McElroy, CSIRO, Australia). Other details of this plasmid construct are published elsewhere[11].

The microcarriers (DNA-coated gold particles) were prepared by sterilizing 60 mg gold particles (Bio-rad, USA) with 1 ml of absolute ethanol in an eppendorf tube and kept at room temperature for 20 min. Following incubation, the particles were spun down and washed thrice with sterile distilled water and resuspended in 1 ml 50% glycerol. For DNA/particle precipitation, 12 µg of plasmid DNA (DNA concentration 1 mg/ml) was added to 50 µl of these particles, along with 50 µl of 2.5 M $CaCl_2$ and 20 µl of 5 M spermidine. Following vortexing and 20 min of precipitation at room temperature, the particles were spun down and resuspended in 140 µl 70% ethanol. This procedure was repeated once with 40 µl ethanol. Finally, 6 µl of the suspension was used per shot.

Table 1. Bombardment conditions employed in the present investigation on basmati indica rice callus

Distance between rupture disc and macrocarrier	0.5 inch + 15 mm
Size of gold particles	1 µm
Distance between microcarrier and target	6 and 9 cm
Density of particles/shot	9 mg/shot
Bombardment medium	MS medium supplemented with 2 mg/l, 2,4-D (rice callus medium)
Callus placement in the petri plate	In the centre and in various concentric rings in 90 mm diameter petri plate
DNA concentration	1 mg/ml
Physical parameters:	
Helium pressure	450–1300 psi
Vacuum	26 mm Hg
Post-bombardment culture	On callus medium till GUS assay (48 h)

Table 2. Transient expression of GUS at various arrangements of rice callus at the time of bombardment with variable helium pressure

Helium pressure (psi)	Arrangement of callus in the petri plate	Transient expression of GUS*	Callus survival†
450	At the centre (distance between microcarrier and the target = 6 cm)	–	Good
650	At the centre (distance = 6 cm)	+	Good
900	At the centre (distance = 6 cm)	+++	Moderate
	At the centre (distance = 9 cm)	++	Good
	At the innermost concentric ring of the petri plate (distance = 6 cm)	++	Good
	At the innermost concentric ring of the petri plate (distance = 9 cm)	+	Good
1100	At the centre (distance = 6 cm)	++	Died
	At the centre (distance = 9 cm)	++	Moderate
	At the innermost concentric ring of the petri plate (distance = 6 cm)	++	Good
	At the innermost concentric ring of the petri plate (distance = 9 cm)	+	Good
1300	At the centre (distance = 9 cm)	–	Died#

*Based on the distribution of blue spots on the callus; +, poor expression; ++, moderate expression; and +++, good expression of GUS; †Callus survival was observed in 15-day-old cultures; #Callus died within 48 h of culture.

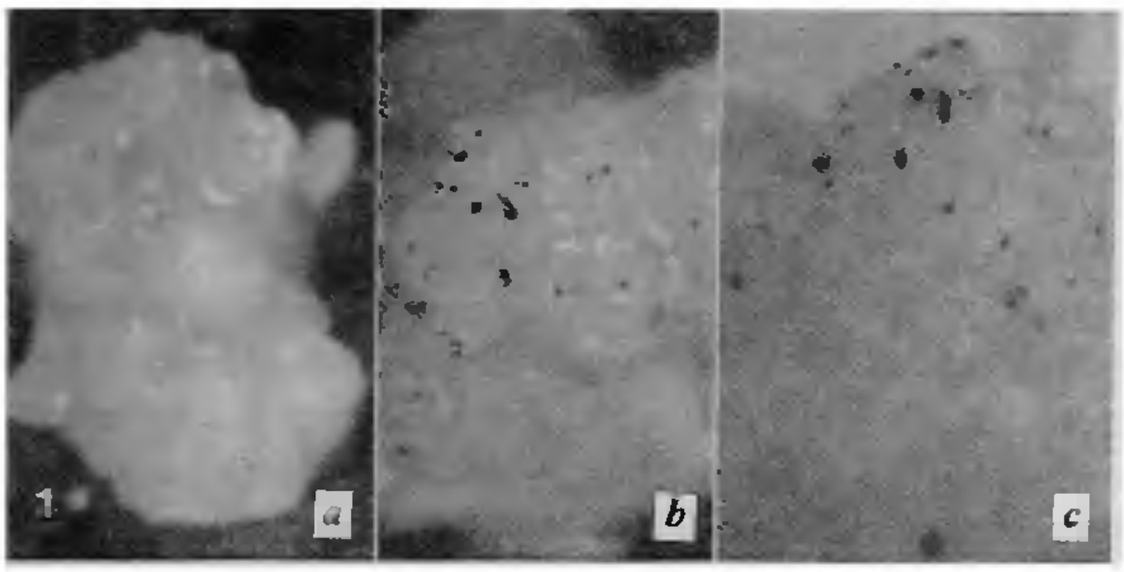

Figure 1. Transient expression of GUS in bombarded callus of rice arranged in the centre of the plate with a distance of 6 cm and 900 psi helium pressure. Non-bombarded callus (*a*) and GUS expression in bombarded callus (*b* and *c*).

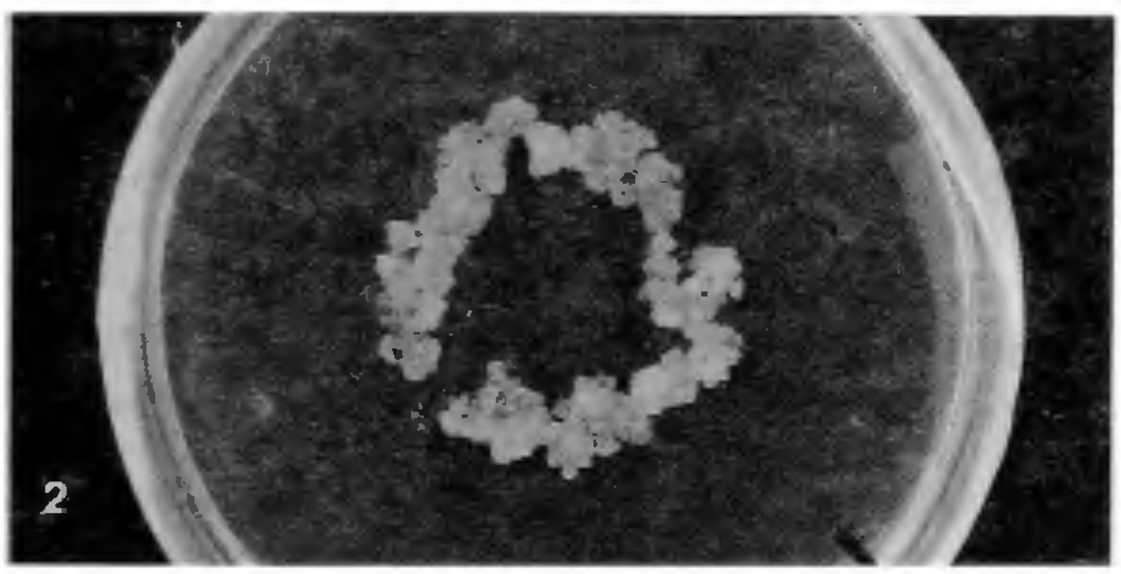

Figure 2. Arrangement of the callus at the innermost concentric ring of the plate on the platform for bombardment.

The target tissue (embryogenic callus) was arranged in concentric rings in sterile petridishes (90 mm diameter). The other physical parameters employed for the use of particle gun are summarized in Table 1. After the bombardment, the calli were cultured in dark for 48 h after which GUS staining was performed at 37°C as described by Jefferson[12].

Various parameters were employed for optimizing conditions for bombardment of rice callus to get transient GUS expression (Table 1). The lower helium pressures (450–600 psi) proved to be suboptimal while higher pressures (900 and 1100 psi) produced optimum results as evident in the form of blue spots on the callus following GUS histochemical assay (Figure 1; Table 2); GUS expression was not detected in control, non-bombarded callus (Figure 1 *a*). However, the highest helium pressure (1300 psi) led to the complete browning of the callus (Table 2). Therefore, further optimizations were carried out using 900 and 1100 psi helium pressures.

Subsequently, the arrangement of the callus on the concentric rings of the plate was standardized (Table 2). First, the callus was placed at the centre followed by placing of the callus in the two innermost concentric rings. The maximum expression of GUS, concomitant with moderate survival of callus, was observed when the callus was placed at the centre or the innermost concentric ring of plate with a distance of 6 cm using 900 and 1100 psi helium pressure, respectively (Figure 1 *b* and *c*; Figure 2). However, better survival of the callus with moderate GUS expression was observed when the callus was placed at the centre or the innermost concentric ring of the petri plate on the platform at a distance of 9 and 6 cm using 900 and 1100 psi, respectively (Table 2). The latter parameters may be useful in recovering transgenics. These standardized bombardment conditions will be utilized for the development of fertile transgenic basmati rice harbouring agronomically-useful genes.

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