(which only points to the extent and depth of their knowledge on environment). From detailed description of the preferred habitat, using overlay analysis of slope, aspect, elevation and vegetation classes, we obtained the preferred habitats of Takin (Figure 8). The preferred habitat constitutes just 0.11% of the sanctuary. In real terms the extent is a mere 30 ha. This is possibly the major reason for the observed low encounter rates. It also points to the need of including available habitat outside of Mehao in a bigger viable conservation unit.

Visualization in biodiversity research entails a whole spectrum of potential benefits at the levels of species, ecosystem and at landscape. An explicit spatial dimension—in terms of patterns of distribution of the above three components of biodiversity, can be provided. This is especially useful in underexplored and relatively unknown field locations using simulated environmental images. The Rapid Biodiversity Assessment (RBA) procedures stand to gain immensely by using visualizing tools. As a sequel to RBA one could effectively use the visualization for evolving comprehensive and appropriate sampling designs for biodiversity description, assessment and monitoring.

For the routine natural history surveys of endangered rare species, one can use available information, howsoever meagre, along with visualization tools to focus the surveys in well-defined geographical locations. Such focused surveys of course, are not only cost effective but could contribute to better formulation of habitat suitability models for a number of species.

We thought it would be better to elicit reaction to these visualizations and perceived impressions on utility for field personnel. We have shown these and the two-dimensional photos to a wide group of people in the sanctuary. It was amazing to find the ease with which the people identified ground features draped on DTM. These persons could identify vegetational and terrain features much more easily and preferred the three-dimensional pictures.

The results on visualization can be altered/improved based on availability of further field data or models of habitat utilization of species of interest. Again, in the area of EIA studies, these visualization tools hold great promise in communicating the data in the form of environmental images to a wide group of users.

In conclusion, we hold the opinion that this methodology and its applications will find wider use in the coming years. This will undoubtedly go a long way in baseline information collection, assessing and monitoring biodiversity in ever-changing environment.


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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 (Basmatic 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. 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 and other cereals. 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 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 embryogenic callus cultures of rice (Oryza sativa L. cv. Basmati 370).

Mature seeds of rice were de-husked, surface-sterilized with 0.1% HgCl₂ 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) 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.

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-tinos (obtained from D. McElroy, CSIRO, Australia). Other details of this plasmid construct are published elsewhere.

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 appendorf 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 CaCl₂ 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.

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<tr>
<th>Table 1. Bombardment conditions employed in the present investigation on basmati indica rice callus</th>
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<tr>
<td>Distance between rupture disc and macrocarrier</td>
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<tr>
<td>Size of gold particles</td>
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<td>Distance between microcarrier and target</td>
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<tr>
<td>Density of particles/shot</td>
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<td>Bombardment medium</td>
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<td>Callus placement in the petri plate</td>
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<td>DNA concentration</td>
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<td>Physical parameters:</td>
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<td>Helium pressure</td>
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<td>Vacuum</td>
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<td>Post-bombardment culture</td>
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<th>Table 2. Transient expression of GUS at various arrangements of rice callus at the time of bombardment with variable helium pressure</th>
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<td>Helium pressure (psi)</td>
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<td>450</td>
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*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.
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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