patch of $1.5 \times 0.5$ km, occurring as a river bench, it is covered by thick sequence of Cretaceous–Tertiary sediments in the south. This channel-filled sandstone is flanked on the west and east by Lum Kuttraw and Nongmalong flood plain sediments, respectively.

2. A north–south patch exposed between Wah Podhra and Um Sophew rivers and south of Phlangdiloin village: The width of this unit is nearly 1 km in the north, which broadens to about 4 km in the south. The Domiasiat uranium deposit is situated on this patch only. This patch of channel-filled sandstone is flanked on the west and east by sediments of Nongmalong and Sindumudum floodplain, respectively.

3. A small patch of $1 \times 1.5$ km exposed north of Phlangmahprow and west of Um Rilang valley: Most part of this sedimentary unit appears to have been eroded away, giving way for the formation of Um Rilang valley.

Sandstone-type uranium deposits are epigenetic concentrations of uranium in unmetamorphosed sandstones of fluvial, lacustrine or marginal marine origin. The minimum requirements for the formation of this type uranium deposit are: (1) a provenance rock containing labile uranium, e.g. granite; (2) a transporting medium to carry the oxidized uranium, e.g. ground water; (3) a favourable host rock having the capacity to reduce and retain uranium.

By virtue of its higher porosity and permeability, incorporation of disseminated reductants like carbonaceous matter and pyrite, presence of permeability barriers such as siltstones and a score of other factors, the channel-filled sandstone of Mahadek formation is considered to be a good host for uranium mineralization. This is evidenced by a number of uranium occurrences like Domiasiat, Phlangdiloin, Tynrai, etc. The aim of the present study was to reduce target areas for survey and exploration, which has been successfully achieved by utilizing effectively the geomorphological characteristics to discriminate the channel-filled facies from the others. By the study of aerial photographs and subsequent field checks, a photogeological map has been prepared delineating the three aforementioned channel-filled sedimentary units. Of these, Domiasiat has already been proved to be having a low-grade medium tonnage deposit. In the Phlangmahprow domain only a small patch of channel-filled sediments are left uneroded after giving way for the Um Rilang valley. Radiometric surveys conducted by scientists of Atomic Minerals Division during the year 1992 have revealed interesting uranium occurrence in this area.

The third patch exposed on the eastern bank of Kynshiang river, north of its confluence with Wah Blei river, which is continuing further south of the confluence had so far remained radiometrically unchecked. During the course of ground truth collection on this channel-filled sedimentary domain in the early months of 1992, the authors have located significant uranium occurrence south of the confluence of Wah Blei and Kynshiang rivers ($91^\circ06'34"^E-25^\circ18'38"^N$). These uranium anomalies range from 0.022% to 0.704% of uranium associated with carbonaceous shale, felspathic sandstone and coal band. This newly discovered uranium occurrence in the channel-filled sedimentary domain of Mahadek formation has geared up the uranium exploration activities in this part of the sedimentary basin.


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Monitoring functional property of the transgene through rapid amplification of cDNA ends in indica rice transformants

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In plant transformation studies, it is realized that we do not have a handy method to test the functional property of a transgene. In that context, it was considered that utility of the RACE (rapid amplification of cDNA ends) technique is worth testing. The test was conducted through an experiment carried out in indica rice cell lines of stable transformants for monitoring the presence of the transcripts of the transferred target genes, viz. gusA and bar. Based on our success, it was realized that the RACE technique may find its utility in many areas of plant molecular biology studies, besides the one tested.

GENE transfer through transformation has become achievable in most of the plant systems. The methods that are adopted could either be Agrobacterium-mediated transformation or any of the direct gene transfer tech-
In all transformation studies, markers are used to ascertain the effectiveness of the introduction of the transforming DNA. In stable transformants, marker gene expression can be influenced by the site of insertion, template copy number, rearrangements and methylation. Thus, the pattern of integration of the transforming DNA in each transformant can influence the function of the transgene. In fact, we do not have a handy tool to follow directly the functional property of the transferred gene. In this context, it was considered that application of the principles of the RACE (rapid amplification of cDNA ends) technique may find its utility.

Two bacterial genes, viz. *gusA* and *bar* were cloned in plant expression vectors pBSS102 and pBSS202 (Figure 1). The DNA to be transferred was delivered to the embryogenic calli of *indica* rice cv. Heera and IR64 through the direct gene transfer method. The embryogenic calli were initiated from the scutellum of matured seeds in MS medium supplemented with 2 mg/l 2,4-D. After one month of initiation, the calli were bombarded with DNA-coated 1.0 μm gold particles using a BioRad PDS 1000/He particle gun. The bombarded calli were cultured in the selection medium containing 3 mg/l bialaphos (Meiji Seika Kaisa Ltd., Japan) in order to recover the bialaphos-resistant cell lines which were utilized for the experiment. The cell lines originating from the bialaphos-resistant cells were then individually tested for the presence of the transgenes following the RACE technique (Figure 2). This consisted of reverse transcription of the 3' end of the polyA mRNA using a hybrid primer consisting of oligo dT (17 residues) linked to a 18-base oligonucleotide primer, used as an adapter, containing the restriction sites (Table 1). Amplification was carried out through PCR using the adapter primer which binds to cDNA containing the hybrid primer at the 3' end and a primer specific towards 5' end of each of the two genes, *gusA* and *bar* (Table 1). By following this course, it was expected that the 3' end of the mRNA (polyA) of only the target gene should be amplified. In cases where amplification could be achieved, they would provide proof for the presence as well as functioning of the transgenes at the level of transcription. Furthermore, since most eukaryotic mRNA have polyA tails and the target gene contained nos polyA termination signal sequences, the target transcripts were expected to contain the polyA tails. Any proof based on RACE technique is expected to distinguish the true transformants from those that are generated from endophytes or from any bacterial contamination. Total RNA of the bialaphos-resistant individual rice cell lines was extracted using the hot phenol method. The polyA mRNA was then isolated by passing the total RNA twice through oligo dT cellulose column. The first cDNA strand (Figure 2) of every sample was synthesized by using 2 μg of polyA mRNA with the hybrid primer (0.5 μg) in the first-strand reaction mixture of the 'youprime' cDNA synthesis kit (Pharmacia). PCR reaction was then performed after purifying the first strand using phenol/chloroform extraction, followed by alcohol precipitation. The PCR mixture contained the following: synthesized first-strand cDNA in 10 μl of H2O, 5 μl of 20 μM of each primer, 10 μl of 10 PCR buffer (without MgCl2), 10 μl of 1 mM dNTPs mix, 10 μl of 20 mM MgCl2, di H2O up to 99 μl. Denaturation was carried out at 95°C for 5 min and 2.5 units of Taq polymerase (Promega) was added and overlaid with two drops of mineral oil. Temperature profiles of each cycle were 95°C for 1 min, 50°C for 1 min, 72°C for 1 min for 40

![Figure 1. Structural details of the chimeric gene used in the plant expression vector a, pBSS102; b, pBSS202.](image)

<table>
<thead>
<tr>
<th>No and types of primer</th>
<th>Nucleotide sequences of primer</th>
<th>Position of the primers</th>
<th>Expected size of the fragment to be amplified (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hybrid 3'</td>
<td>5GACTCGAGTCGACATCGATTTCGGGGG (containing XhoI, SalI and CiaI sites)</td>
<td>+1482</td>
<td>~ 400</td>
</tr>
<tr>
<td>2. Adapter</td>
<td>5GACTCGAGTCGACATCGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. 3' amp gusA</td>
<td>5CTGCCCCGCAAGGCAAACACTGCATGAGG</td>
<td>end of the gene</td>
<td></td>
</tr>
<tr>
<td>4. 3' gusA</td>
<td>5TCATTGGTTTGGGCTCCCTCTGCTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. 3' amp bar</td>
<td>5ATGAGCGGACAGACGACAGCAGCAG</td>
<td>+1</td>
<td>~ 550</td>
</tr>
<tr>
<td>6. 3' bar</td>
<td>5TCAGCATCGGCGGAGGCAGAGGAGAG</td>
<td>end of the gene</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Details of the primers used for RACE
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**Figure 2.** Strategy for rapid amplification of cDNA ends.

**Figure 3.** Agarose gel electrophoresis of DNA after using the RACE technique. a, Rapid amplification of cDNA ends of gusA gene from sample 1 (lane 2) and sample 2 (lane 3) using primers 2 and 3 (Table 1), lane 4 shows PCR amplification of pBSS202 DNA using primers 3 and 4 (Table 1) of the gusA gene; lanes 5 and 6 show PCR amplification of total mRNA of samples 1 and 2, respectively; lane 7 shows PCR amplification of total DNA of sample 1 when the adapter primer 2 and gusA gene 5' primer 3 (Table 1) were used. Lane 1 shows φ X174 DNA cleaved with HaellIII endonuclease as molecular weight marker. b, Rapid amplification of the cDNA ends of the bar gene from sample 1 (lane 2) and sample 2 (lane 3) using primers 2 and 5 (Table 1); lanes 4 and 6 show PCR amplification of total mRNA of samples 1 and 2, respectively, lane 5 represents PCR amplification of total DNA of sample 1. In case of total RNA and DNA, the adapter primer 2 and bar gene 5' primer 5 were used. Lane 7 represents amplification of pBSS102 DNA when 3' primer 5 and primer 6 of the 3' end of bar gene (Table 1) were used. Lane 1 shows φ X174 DNA cleaved with HaellIII endonuclease as molecular weight marker.
cycles. The last cycle was extended for 7 min 30 s at 72°C.

Figure 3a documents evidence for rapid amplification of cDNA ends of gusA gene. It was evident that amplification in lanes 2 and 3 of ~400 bp took place through RACE. No amplification with total RNA and DNA (lanes 5–7) was possible. Similarly, Figure 3b showed evidence for rapid amplification of cDNA ends of the bar gene. As in the case of gusA gene, amplification of ~550 bp of bar gene also materialized, as documented in lanes 2 and 3 in Figure 3b. No amplification could be seen in lanes 4–6, indicating that nonspecific amplification had indeed not taken place. Thus, it was realized that the method worked satisfactorily.

To further confirm the presence of the translated gene product, a bialaphos-resistant cell line (sample 1 of Figure 3b) which provided positive signal for the RACE technique was subjected to phosphinothricin acetyltransferase (PAT) assay following the method of De Block et al.\(^6\). The results presented in Figure 4 show that the bialaphos-resistant cell line expressed the transferred gene product during PAT assay. Additionally, the expression of gusA gene was histochemically tested (as evident in Figure 5) following the method of Jefferson\(^7\) in sample 2 of Figure 3a. The gusA gene is used extensively as a reporter gene in plant and agricultural molecular biology. The enzyme β-glucuronidase catalyses the hydrolysis of a wide variety of glucuronides. Advantages of GUS over other systems are the robustness of the enzyme\(^8\), the simplicity of the assays and the varieties of the substrates available, including the sensitive histochemical substrate 5-bromo-4-chloro-3-indolyl β-d-glucuronide (X-Gluc), as used in the present study.

Methods for detection and analysis of RNA molecules are always an important aspect of biological studies. Based on the present finding, the application of the RACE technique may find its utility in many molecular/cell biology studies. Although in situ hybridization method is considered to be the most sensitive one, its technical complexity, especially in processing a large number of samples, makes its application difficult. On the other hand, the RACE technique could additionally be useful in (i) monitoring the expression of a gene in various plant parts at differential stages of growth, (ii)

**Figure 4.** Detection of PAT activity in rice tissue extract. Lane 1: leaf tissue of untransformed rice plant, lane 2: untransformed indica rice callus, lane 3: bialaphos-resistant callus. Arrow indicates the presence of \(^14\)C-labelled acetylated phosphonothricine (PPT). In lanes 1 and 2 tissue extracts spotted were ten times more concentrated than the extract used for lane 3.

**Figure 5.** Histochemically assayed transformed rice callus showing gusA gene expression.
sequence analysis of individual forms of genes belonging to a multigene family by designing alternative primers, and (iii) situations where the outcome of the genetic end product is dependent upon more than one gene and the expression of each gene involved needs to be monitored. However, the applicability of this technique is limited to only situations where the nucleotide sequence of the gene to be searched is known.


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Changes in nitrate reductase and glutamine synthetase activities in Ziziphus mauritiana by different VAM fungi

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Efficacy of different vesicular arbuscular mycorrhizae (VAM) species towards increasing nitrate reductase (NR) and glutamine synthetase (GS) activities in Ziziphus mauritiana was evaluated under glasshouse conditions. After 45 days of seedling growth, plant samples were analysed. In general GS activity was higher in all the treatments as compared to NR activity. Addition of VAM increased the activities of both these enzymes. However, different VAM species varied in their efficacy to increase these enzymatic activities. Among the five VAM species used during the present investigation, Glomus fasciculatum was found to be the most efficient VAM species for Z. mauritiana as it increased most effectively the activities of GS and NR in this multipurpose fruit tree of the Indian Thar desert. This VAM fungus also increased protein contents more than twofold in Z. mauritiana, which can be of great significance in producing highly proteinaceous leaf fodder of this desert plant.

NITROGEN is the most important mineral nutrient for plant growth. Indirect increased nitrogen uptake by vesicular arbuscular mycorrhizae (VAM) has been well recognized. Due to its beneficial effects, VAM are receiving considerable attention in agriculture and forestry. Ziziphus mauritiana is an important multipurpose fruit tree of arid and semiarid regions. It is a source of fuel, fodder and timber. Cultivation of the plant is done mostly in P-deficient sandy soils of drought-prone areas. The primary stresses imposed on vegetation by environment are lack of water and nutrients. The VAM fungi may be of particular significance in coping with P-deficiency stress in natural ecosystems. There has been less work on nitrogen metabolism in plants colonized by VAM. Nitrate reductase (NR) and glutamine synthetase (GS) are two major enzymes of nitrogen metabolism. Increased NR and GS activities by VAM fungi have been reported by few workers. Efficiency of VAM symbiosis is affected by a variety of factors, including environmental conditions and the host plant; hence, response of different host–fungus combinations and effects of different environmental conditions must be analysed. Keeping all these facts in mind, efficiency of different VAM species were studied towards increasing NR and GS activities in Z. mauritiana in order to select an efficient VAM strain for this neglected multipurpose fruit tree of the Indian Thar desert.

Five VAM species, namely Glomus constrictum Trappe, G. fasciculatum (Thaxter sensu Gerd.) Gerd. and Trappe, G. mosseae (Nicol. and Gerd.) Gerd. and Trappe, Gigaspora margarita Becker and Hall and Scutellospora calospora (Nicol. and Gerd.) Walker and Sanders collected from rhizosphere soils of Z. mauritiana were maintained on Calnherus ciliaris as pot cultures. The soil from these pot cultures along with the roots of C. ciliaris was used as source of inoculum. Ten g of inoculum was used in each pot of 18 cm diameter, containing sterilized soil, by the layering method. The surface-sterilized seeds of Z. mauritiana were sown in these pots and the pots were kept in a glasshouse having 60% humidity and 22–25°C temperature. After 15 days of germination, seedlings were thinned to one per pot. For all studies except that on nitrate reductase, 45-day-old plants were harvested, and the roots and shoots were separated, weighed, immersed in liquid nitrogen, stored at 80°C, and analysed within 48 h. There were 20 replications in each treatment.

Plant tissue, 0.5 g fresh weight of each organ, was