was a difference in the pattern of derepression of *hup* genes, suggesting a role for the genetic make-up of the host strain.

On acquisition of plasmid pIJ1008, the symbiotic energy efficiency of *Cicer*-rhizobial strains was improved, as suggested by RE estimates (Table 2). Energy losses due to H_2 evolution from nodules were reduced by 14 and 18% respectively in Hup^+ transconjugants compared to their respective parental strains. However, in transconjugant G 36–84 (pIJ1008) specific nodule nitrogenase activity was significantly reduced. This may be due to antagonistic effects of pIJ1008, which also carries genetic determinants for nodulation capability as well as for symbiotic N_2 fixation[21].

This study thus opens up the possibility of improving symbiotic efficiency of *Cicer-Rhizobium* by incorporating H_2-recycling ability, which the *Cicer*-rhizobia, as a whole, normally lack.

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Transient expression of GUS and CAT genes in electroporated rice protoplasts

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Bacterial β-glucuronidase (GUS) and chloramphenicol acetyltransferase (CAT) genes were introduced into rice protoplasts by electroporation. Detection of significant levels of GUS and CAT enzyme activity indicated that both genes are expressed in transformed rice protoplasts. GUS and CAT genes can therefore be used as reporter genes in transformation experiments in rice.

DIRECT introduction of DNA as a means of transformation of plant protoplasts has provided a system for monitoring gene expression within hours of transformation and for evaluation of the recombinant-DNA constructs, promoters and marker genes used[1]. In rice, direct gene-transfer methods have also been used to obtain transgenic plants from protoplasts[2–4]. Generally a strong promoter is fused to a gene whose product is required in increased amount to enhance expression of the gene *in vivo*. In studying regulation of gene expression, the experimental approach of fusing the promoter and various segments of upstream regulatory sequences of the gene of interest to the coding region of a 'reporter' gene whose protein product can be easily detected, introducing the constructs into a recipient plant or plant protoplasts, and monitoring expression of the reporter gene is a well-established one. The present communication shows that bacterial β-glucuronidase (GUS) and chloramphenicol acetyltransferase (CAT) genes introduced by electroporation into rice protoplasts are expressed.

The transforming DNA was in the form of plasmids. The plasmid constructs used were: (i) pBI121, carrying the GUS gene fused with the cauliflower mosaic virus (CaMV) 35S promoter; (ii) pDW2, carrying the CAT gene fused with the CaMV 35S promoter; (iii) pNOSCAT, carrying the CAT gene fused with the *Agrobacterium tumefaciens* nopaline synthetase (NOS)

promoter; and (iv) pVE1001, carrying the CAT gene fused with the SV40 promoter (pDW2 and pNOSCAT were kindly provided by J. Paszkowski and I. Potrykus, Zurich; pVE1001 by Y. Nagami, Basel; and pBI121 by R. A. Jefferson, Cambridge).

DNA was introduced into freshly isolated protoplasts from rice callus by electroporation. Ten micrograms of plasmid DNA was used, along with 50 μg of calf thymus DNA as carrier, for 1 ml of protoplasts at 10^6 protoplasts per ml. The procedures for electroporation were essentially as described[5], with the discharging of a 200-μF capacitor from 200 volts providing three pulses with 10-sec intervals. Controls were treated under identical conditions except that no plasmid DNA was added. GUS[6] and CAT[7] assays were performed after culturing the protoplasts for 48 hours.

Figure 1 shows the results of the GUS assays in protoplast lysates from the rice cultivars 'Praerie' (indica) and 'Iwaimochi' (japonica). In both cases, significantly increased GUS activity was observed in the protoplasts that received the plasmid DNA, indicating their genetic transformation and expression of the introduced GUS gene. The background GUS activity in the controls is attributed to either the mutability of 4-methylumbelliferone into other compounds and then cleavage to produce a fluorescent signal, or to contaminating β-glucuronidase activity from the cell wall-digesting enzymes used in protoplast preparation, as suggested by Jefferson[8].

Figure 2 shows the results of the CAT assays. Comparison of the positive control (*E. coli* with pBR328) and the negative control (protoplasts transformed without plasmid DNA) shows that the latter also gave some background activity. However, the protoplasts transformed with the three different CAT-gene constructs gave a significantly higher level of chloramphenicol acetylation, confirming expression of the introduced CAT gene in these protoplasts. The low endogenous CAT activity observed is not unusual, since very high levels of endogenous CAT activity have been reported for other plant systems[9]. Another interesting feature observed here is that the SV40 promoter drives transcription in a plant system. In an earlier report[10], the SV40 promoter was used to drive the expression of the neomycin phosphotransferase II gene in *Brassica napus*.

The results presented here show that both GUS and CAT genes are suitable reporter genes for rice. GUS would be a more suitable reporter gene since lower background activity can be expected in cleaner protoplast preparations; it would not be possible to get

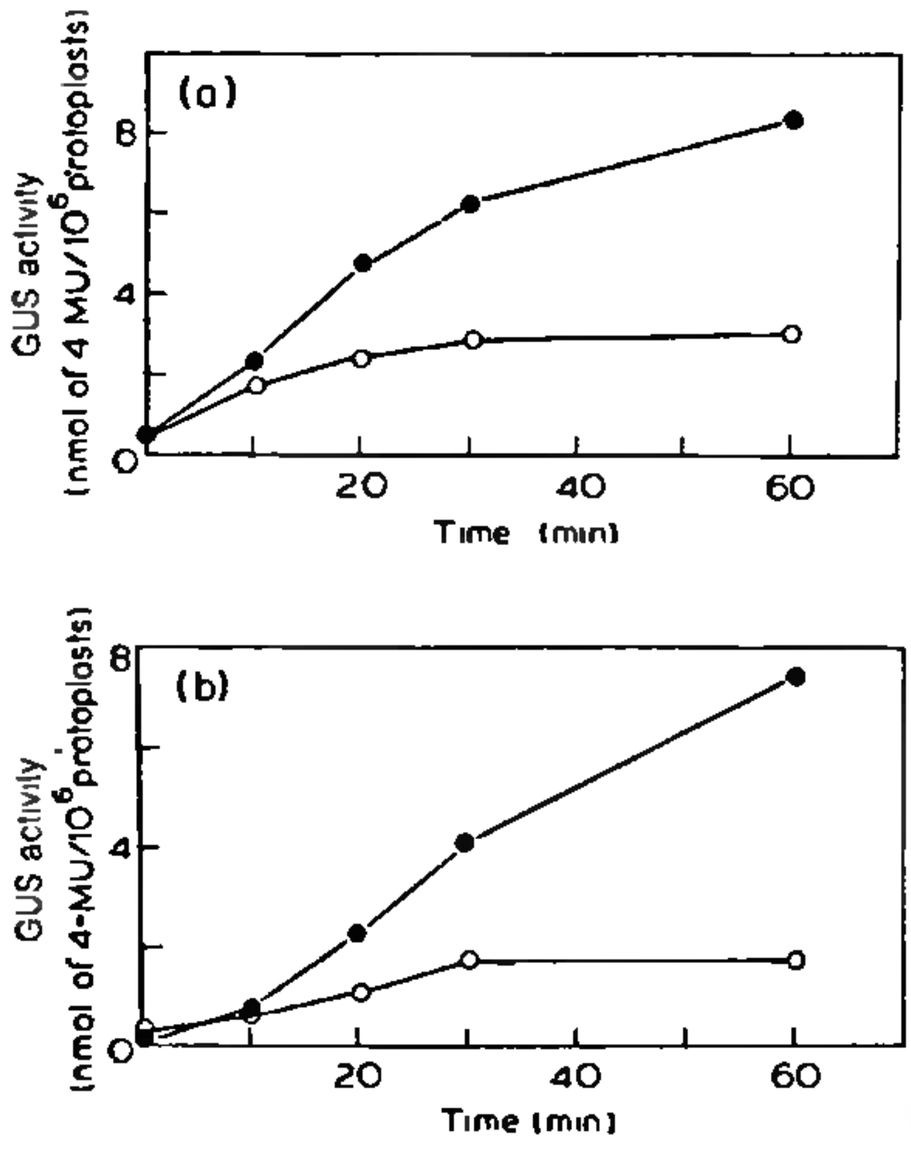

Figure 1. β-Glucuronidase activity[6] in lysates of protoplasts of rice cultivars (*a*) 'Praerie' and (*b*) 'Iwaimochi' 48 hours after transformation with pBI121. The reaction mixture containing the protoplast lysate and 4-methylumbelliferyl glucuronide (4-MUG) was incubated at 37 C. The formation of 4-methylumbelliferone (4-MU) from 4-MUG, catalysed by β-glucuronidase, was monitored using a spectrofluorimeter calibrated with 4-MU standards, with excitation at 365 nm and emission at 445 nm —○—, Protoplasts transformed without plasmid DNA; —●—, protoplasts transformed with pBI121.

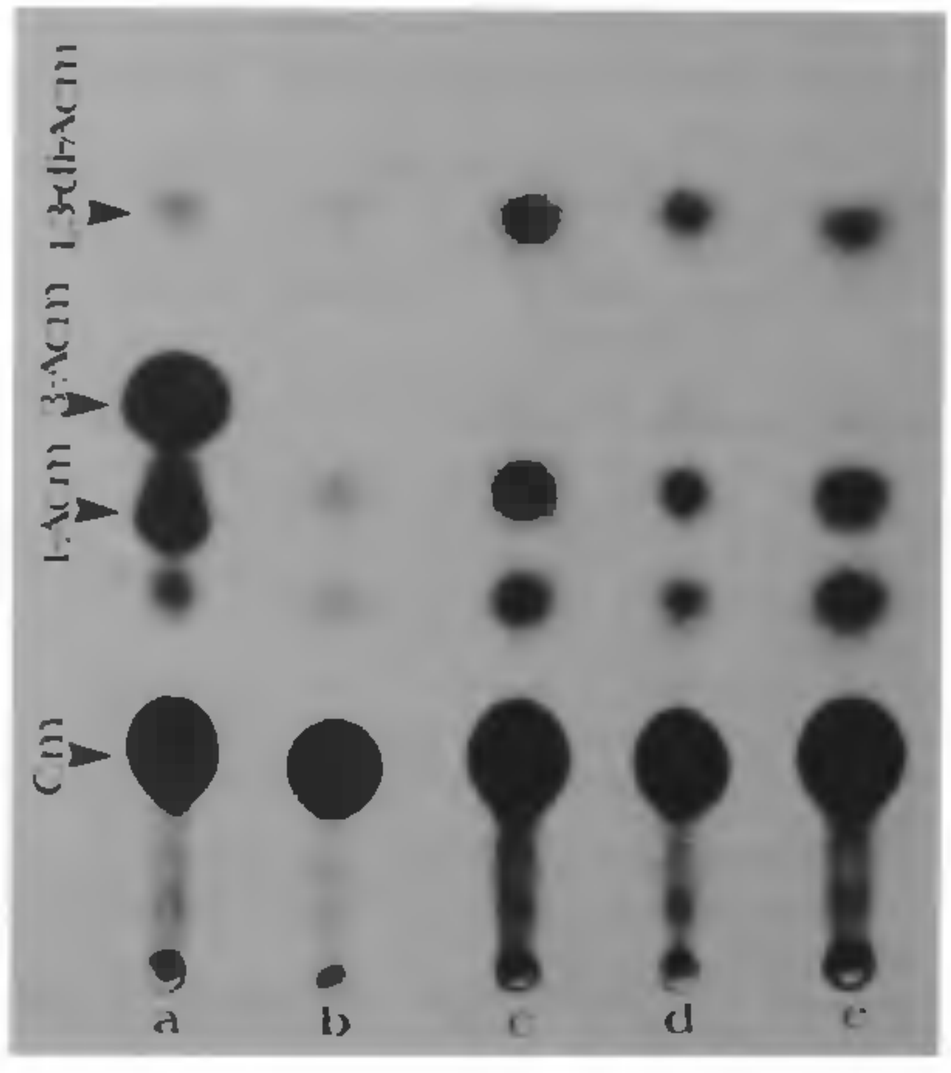

Figure 2. Chloramphenicol acetyltransferase activity[7] in lysates of protoplasts of rice cultivar 'Praerie' 48 hours after transformation with the various CAT-gene constructs. The reaction mixture containing the lysate and [^{14}C]chloramphenicol (Cm) was incubated at 37 C for 60 min. Chloramphenicol and its acetylated derivatives (Acm) were extracted with ethyl acetate and separated on a polyester silica gel TLC plate, using a 95:5 mixture of chloroform and methanol. After air drying, the plate was autoradiographed. Lane a, *E. coli* with pBR328, lane b, rice transformed without plasmid DNA, lane c, rice transformed with pDW2, lane d, rice transformed with pNOSCAT, lane e, rice transformed with pVE1001.

rid of the endogenous CAT activity. These results will be used for standardizing a system for the genetic transformation of rice.

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