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GUS Expression by CaMV 35S and Rice *Act1* Promoters in Transgenic Rice

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To determine the patterns and the levels of expression of the cauliflower mosaic virus (CaMV 35S) promoter and the rice actin 1 (*Act1*) promoter in rice, transgenic rice plants containing CaMV 35S-β-glucuronidase (GUS) and *Act1*-GUS constructs were generated and examined by fluorometric and histochemical analyses. The fluorometric analysis of stably transformed calluses showed that the activity of the rice *Act1* promoter was stronger than that of the CaMV 35S promoter in rice cells. In a histochemical study of the transgenic rices, it was shown that the GUS activity directed by the CaMV 35S promoter was localized mainly in parenchymal cells of vascular tissues of leaves and roots and mesophyll cells of leaves. These results are similar to those of potato, a dicot plant. In contrast, rice plant transformed with *Act1*-GUS fusion construct revealed strong GUS activity in parenchymal cells of vascular tissue, mesophyll cells, epidermal cells, bulliform cells, guard and subsidiary cells of leaves and most cells of the root, suggesting that the rice *Act1* promoter is more constitutive than the CaMV 35S promoter. It was also confirmed that in both types of transgenic rice little or no staining was localized in metaxylem tracheary elements of vascular tissue from leaves or roots. These results indicate that the rice *Act1* promoter can be utilized more successfully for expression of a variety of foreign genes in rice than the CaMV 35S promoter.

Keywords: GUS, CaMV 35S promoter, rice *Act1* promoter, transgenic rice

With respect to the gene transfer technology, the development of a particle bombardment method based on accelerated particles has been utilized for obtaining transgenic plants in agronomically important cereals of which transformation has been hindered by the limited host range of *Agrobacterium*. Recently, in some laboratories, it has been reported that transgenic plants have been produced successfully in monocots such as maize (Fromm *et al.*, 1990), wheat (Vasil *et al.*, 1993) and rice (Christou *et al.*, 1991) using particle bombardment techology.

In addition to the gene transfer technology, the lack of an efficient and strong promoter for the expression of foreign genes is still one of the major limitations for obtaining transgenic monocot plants. In general, CaMV 35S and maize alcohol dehydrogenase 1 (*Adh1*) promoters were often used to express foreign genes in gene transfer studies on monocots (Zhang and Wu, 1988; Fromm *et al.*, 1990; Terada and Shimamoto, 1990; Kyozuka *et al.*, 1991). However, both promoters have been reported to show relatively low activity in the transformed cells of monocots (McElroy *et al.*, 1990; Peterhans *et al.*, 1990; Chibbar *et al.*, 1993). Recently it has been reported that the 5′ region of the rice *Act1* gene (*Act1* promoter) directed high levels of GUS gene expression in transformed rice protoplasts (McElroy *et al.*, 1990) and barley cells (Chibbar *et al.*, 1993). We have previously reported that the *Act1* promoter was more highly active in transient GUS gene expression of transformed rice cells than the CaMV 35S promoter (Jeon *et al.*, 1994). In this report, we examined the activity of both promoters in stably transformed calluses of two rice cultivars, and compared the results with those previously published.

By histochemical analysis of transgenic plants trans-

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formed by fusion of the regulatory region of promoter to the GUS gene, it has become clear that expression of certain plant genes is not only tissue specific but cell type specific in a particular organ. In transgenic rice plants, it has been reported that the CaMV 35S promoter was not active in all cell types (Battraw and Hall, 1990; Terada and Shimamoto, 1990), and that the maize *Adh1* promoter was more highly active in roots than in leaves (Zhang and Wu, 1988; Kyozuka *et al.*, 1991). It has been also reported that the rice *Act1* promoter showed a more constitutive pattern of activity in transgenic rice than either the CaMV 35S or the maize *Adh1* promoter (Zhang *et al.*, 1991). We have previously reported that the activity of the CaMV 35S promoter was high in the phloem cells in and around vascular tissue of the stems and roots, and in the apical meristem of shoots, roots and adventitious shoot buds of transgenic potatoes (Lee and Sung, 1992; Sung *et al.*, 1994).

We here examined the expression patterns of CaMV 35S and rice *Act1* promoters in transgenic rice of an identical cultivar for the first time by microscopic analysis. We compared these results with those previously published including the results of transgenic potato, a dicot plant.

MATERIALS AND METHODS

Plant transformation

Two cell lines of rice embryogenic cell suspensions, which were derived from anther of cv. Seomjinbyeo and seed of cv. Dobongbyeo, were used in obtaining transgenic rice plants (Jeon and Lee, 1992; Jeon *et al.*, 1994). All plasmids used in this experiment, except for pGA643 (An *et al.*, 1988), and all transformation procedures using a particle inflow gun including DNA isolation, DNA coating and osmotic treatment of target cells were described in Jeon *et al.* (1994). pLS201 (Lee and Sung, 1992) containing the combination of CaMV 35S-GUS and neomycin phosphotransferase II (NPTII) gene was used for obtaining calluses containing CaMV 35S-GUS fusion construct. In contrast, to obtain stably transformed calluses containing rice *Act1*-GUS fusion construct (pAct1D, Zhang *et al.*, 1991), pGA643 containing NPTII gene as a selectable marker was cotransformed with pAct1D at a 1 : 1 ratio.

The N6 medium (Chu *et al.*, 1975) supplemented with 2 mg/L 2,4-D and 100 mg/L G418 was used to select transformed calluses. For cotransformed calluses, GUS-expressing calluses were secondarily selected out of G418-resistant calluses by *in situ* GUS assay (Jeon *et al.*, 1994).

Transformed calluses were cultured in N6 liquid medium containing 2 mg/L 2,4-D and 25 μM $AgNO_3$ for 2 weeks and then subcultured on the MS medium (Murashige and Skoog, 1962) containing 0.1 mg/L NAA, 2 mg/L kinetin, 2% sorbitol, 5% sucrose and 1.6% agar for plant regeneration. For active growth of roots, regenerated plants were transferred onto the MS medium containing 1 mg/L NAA.

GUS spectrofluorometric analysis

GUS activity was quantified fluorometrically using 4-methylumbelliferyl-β-D-glucuronide (MUG) as described by Jefferson (1987). GUS activity was expressed as pmol 4-methylumbelliferone (MU) produced per mg protein/min of incubation at 37℃. The GUS extraction buffer consisted of 50 mM sodium phosphate (pH 7.0), 10 mM β-mercaptoethanol, 10 mM EDTA, 0.1% sarcosyl and 0.1% triton X-100. GUS assays were performed 3-5 months after transformation using G418-resistant calluses. Protein concentration of callus extracts was determined by the method of Bradford (1976) using BSA as a standard.

Histochemical analysis

Leaf blades, leaf sheaths and roots, cut by hand with a scalpel to about 3-5 mm, were incubated at 37℃ for 12 h in a solution containing 10 mM sodium phosphate (pH 7.0), 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 10 mM EDTA, 0.1% Triton X-100 and 0.1% 5-bromo-4-chloro-3-indoyl-β-D-glucuronide (X-gluc). Tissue samples were then fixed in a solution of 4% paraformaldehyde, 17 mM sodium phosphate (pH 7.0) and 0.5 M sucrose for 4 h and finally washed with a solution of 0.5 M sodium phosphate (pH 7.0) and 0.5 M sucrose (Battraw and Hall, 1990). Tissues were then frozen to −20℃ and cut into 30 μm thick sections using a Cryo-Cut II microtome (AO).

PCR analysis and Southern hybridization

Polymerase chain reaction (PCR) assay was per-

formed according to Jeon *et al.* (1994). Genomic DNA was isolated from transformed calluses and plants using the procedure of Gordon-Kamm *et al.* (1990). For detecting the presence of the transferred gene, 5′-CTACACCACGCCGAACACCT-3′ (+527 from ATG) and 5′-CAGGCACAGCACATCAAAGA-3′ (+1391 from ATG) for the GUS gene (Omirulleh *et al.*, 1993), and 5′-GAGGCTATTCGGCTATGACTG-3′ (+201 from ATG) and 5′-ATCGGGAGCGGCGATACCGTA-3′ (+900 from ATG) for NPTII gene (Hamill *et al.*, 1991) were used as primers. PCR products were separated by electrophoresis in 0.8% agarose, and then transferred to Hybond N+ membrane (Amersham) according to the method of Southern (1975). Detection of PCR products was performed according to protocol of the manufacturer (Boehringer Mannheim) using the non-radioactive digoxigenin (DIG) method. The filter was hybridized with a DIG-labelled probe, which consisted of the GUS coding region isolated as about a 1.9 kb *Pst*I fragment from pLS201. The hybridized filter was detected by enzyme-linked immunoassay using an antidigoxigenin-alkaline phosphatase conjugate and a subsequent enzyme-catalyzed color reaction with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

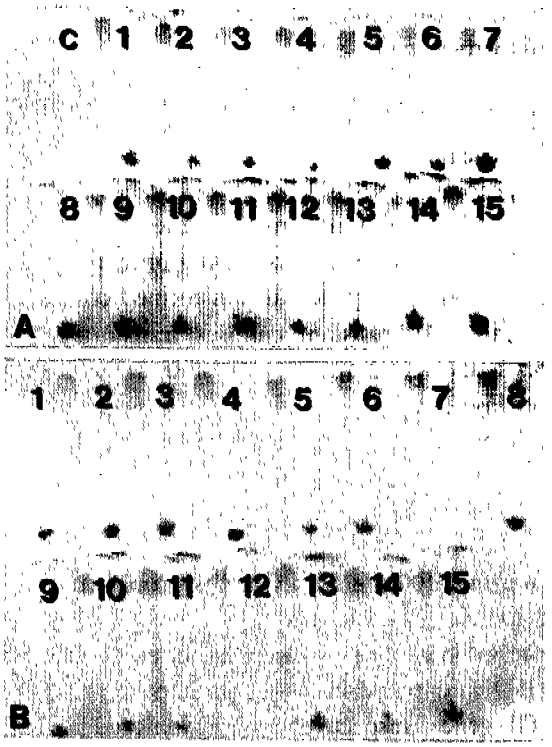


Fig. 1. A, GUS activity in calluses of cv. Seomjinbyeo stably transformed with pLS201. The left tube (C) of the upper part is a result of assay of untransformed callus, and the others (1-15) is a result of assay of putative transformed calluses. B, GUS activity in calluses of cv. Dobongbyeo cotransformed with pAct1D and pGA643. All the tubes contained about 20 μL X-gluc solution and a each section of putative transformed calluses (1-15).

RESULTS AND DISCUSSION

Selection of transformed calluses containing CaMV 35S-GUS or *Act1*-GUS

To select GUS-expressing calluses, the selected G418-resistant calluses were assayed for GUS activity by staining them with X-gluc. A section of each transformed callus was added for GUS assay in a microfuge tube containing X-gluc solution (Fig. 1). Untransformed control calluses showed no blue coloration, whereas almost all of the G418-resistant calluses transformed with pLS201 showed blue coloration (Fig. 1A). In contrast, among calluses cotransformed with pAct1D and pGA643, some tested showed intense blue coloration (Fig. 1B). This result indicates that only some of G418-resistant calluses were cotransformed with both plasmids.

In the G418-resistant calluses transformed with pLS201 containing CaMV 35S-GUS and NPTII genes, 46 out of 66 calluses showed blue coloration 12 h after staining. This means that about 70% of G418-resistant calluses expressed the GUS gene actively. However, it was confirmed in PCR analysis that the other G418-resistant calluses which didn't express GUS gene as well as GUS-expressing calluses contained GUS coding region. It is suggested that partial delection or methylation of inserted GUS gene or position of inserted GUS gene on the genome affected negatively on the GUS gene expression in some G418-resistant calluses.

In the case of calluses cotransformed with pAct1D containing *Act1*-GUS fusion construct and pGA643 containing NPTII gene, 27 calluses out of 60 showed blue coloration after staining, suggesting that about 45% of the selected calluses were cotransformed by pAct1D and pGA643. This result suggests that the cotransformation method can be utilized to deliver unlinked useful genes into plant cells.

PCR analysis and Southern hybridization of transformed calluses

To confirm the presence of foreign genes in the selected calluses, PCR analyses were performed using genomic DNA isolated in each callus and primers for the coding regions in either the GUS or NPTII gene. In PCR analysis using primers for GUS coding region, except for untransformed control calluses, all G418-resistant calluses transformed with pLS201 showed amplification of the 0.86 kb fragment (Fig. 2A). It was confirmed in subsequent Southern hybridization that the PCR products agreed with part of the GUS coding region (Fig. 2B). This result indicates that antibiotics G418 inhibited entirely growth of untransformed control calluses, suggesting that 100 mg/L of G418 is proper concentration to select transformed calluses.

The GUS-expressing calluses cotransformed with pAct1D and pGA643, using primers for GUS and NPTII genes, respectively, were analyzed. In PCR analysis, the presence of amplification of the 0.86 kb and 0.7 kb fragments, representing GUS and NPTII coding regions, resepectively, was confirmed in all tested (Fig. 2C). This result indicates that GUS-expressing G418-resistant calluses were truly transformed with the two plasmids.

Quantitative analysis of GUS activity in transformed calluses with CaMV 35S-GUS or *Act1*-GUS

We previously analyzed the activity of constructs containing different promoter-sequences fused with the GUS gene in transformed rice cells through transient GUS assay, and ascertained that the rice *Act1* promoter showed the strongest expression among them (Jeon *et al.*, 1994). In this paper, we carried out fluorometric analysis using the substrate MUG to determine the level of GUS expression in the calluses stably transformed with either pLS201 or pAct1 D. Tables 1 and 2 show the results of these analyses in two cultivars. The calluses transformed with *Act1*-GUS construct showed that GUS activities were approximately 100- and 150-fold higher in all of the two cultivars than those transformed with CaMV 35S-GUS construct. It was also observed that no significant difference in GUS activity between two cultivars was shown. The untransformed control callus-

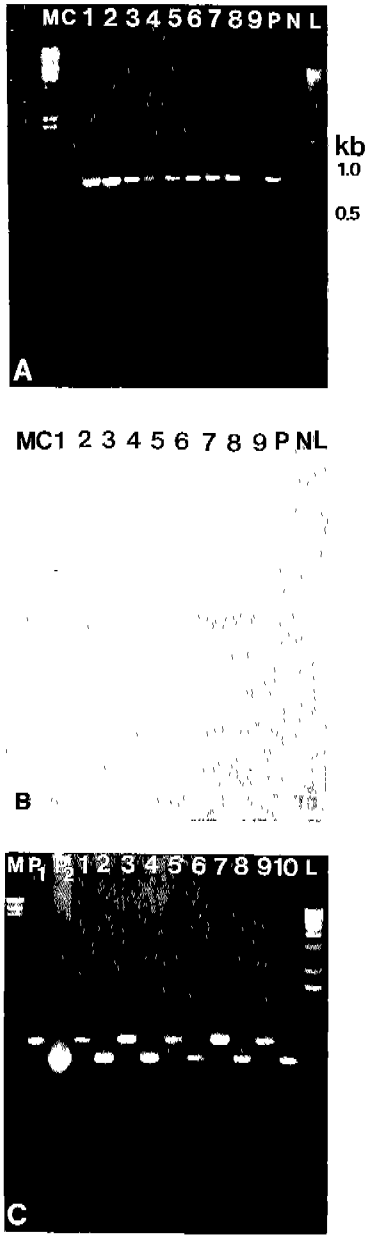


Fig. 2. A, Agarose gel electrophoresis of PCR-amplified fragment of calluses (cv. Scomjinbyeo) transformed with pLS201 using primers for the GUS gene. Lane M, lambda DNA digested with *Hin*dIII; Lane C, DNA from untransformed callus; Lanes 1-9, DNA from each transformed callus; Lane P, pLS201; Lane N, no DNA; Lane L, 1 kb DNA ladder. B, Southern blot analysis for PCR product separated in (A). Hybridization was carried out using DIG-labelled GUS probe. C, Agarose gel electrophoresis of PCR products of calluses (cv. Dobongbyeo) transformed with pAct1D and pGA643 using primers for either GUS gene or NPTII gene. Lane P1, pAct1D; Lane P2, pGA643; Lanes 1/2, 3/4, 5/6, 7/8 and 9/10, DNA from 5 transformed calluses. (Lanes P1, 1, 3, 5, 7 and 9 for GUS primers; Lanes P2, 2, 4, 6, 8 and 10 for NPTII primers). Lanes M and L are identical with in (A).

Table 1. GUS activities measured in protein extracts from stably transformed calluses transformed with pLS201 in rice cvs. Seomjinbyeo and Dobongbyeo

Callus line	cv. Seomjinbyeo	cv. Dobongbyeo
Untransformed	<10	<10[a]
1	1,109.47	1,043.15
2	293.58	226.12
3	831.83	151.68
4	545.47	343.06
5	370.67	642.11
Mean	630.20	481.22

[a]GUS activity is expressed as pmol MU·mg protein^{-1}·min^{-1}.

Table 2. GUS activities measured in protein extracts from stably transformed calluses cotransformed with pAct1D and pGA643 in rice cvs. Seomjinbyeo and Dobongbyeo

Callus line	Seomjinbyeo	Dobongbyeo
Untransformed	<10	<10[a]
1	178,401.21	40,210.40
2	8,806.44	38,309.18
3	92,897.66	20,789.61
4	11,688.71	26,310.28
5	27.09	232,136.75
Mean	58,364.22	71,551.24

[a]GUS activity is expressed as pmol MU·mg protein^{-1}·min^{-1}.

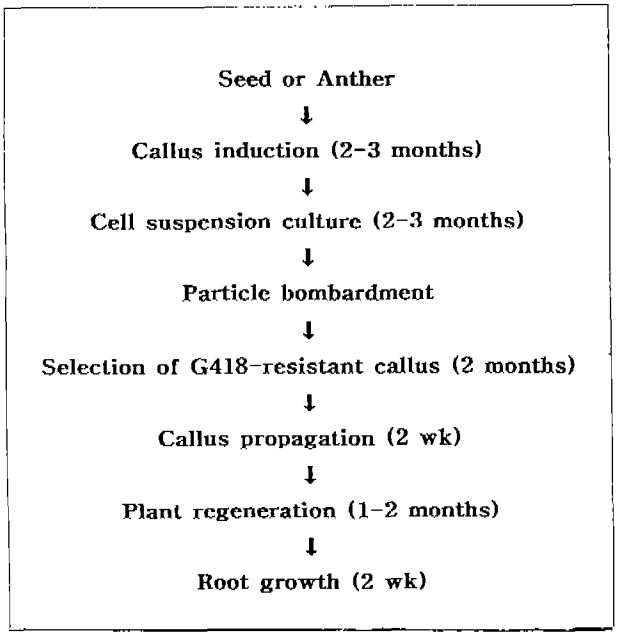


Fig. 3. Time frame for processes in the production of transgenic rice plants. The times shown are averages for performed in all experiments.

es hardly showed GUS activity. These results are consistent with those analyzed through transient GUS expression on the basis of GUS-expressing spots (Chibbar *et al.*, 1993; Jeon *et al.*, 1994). We showed obviously that the analysis of transient expression of a gene construct can be used instead of that of gene expression in stably transformed calluses.

Regeneration of rice plants from transformed calluses

We previously established the particle bombardment-mediated transformation system of rice embryogenic cell suspension culture using a simple homemade particle inflow gun (Jeon *et al.*, 1994). In this paper, to investigate expression patterns of CaMV 35S and rice *Act1* promoters *in vivo*, we produced transgenic rice plants from calluses transformed with CaMV 35S-GUS or *Act1*-GUS fusion construct by particle bombardment method.

Fig. 3 represented the steps of the transformation process producing transgenic rice plants, whose required time period was 8-11 months. It was very important to propagate the G418-resistant calluses in liquid medium prior to transferring them onto a regeneration medium, and to add 1.6% agar to the regeneration medium for obtaining many transgenic plants (data not shown). These steps in our procedures are different from those previously published (Abdullah *et al.*, 1986; Zhang and Wu, 1988; Battraw and Hall, 1992).

It is suggested that the calluses cultured in liquid medium without antibiotics, which showed a rapid growth and an active metabolism of cells, are maintained as embryogenic calluses having a high regeneration ability. Most of them showed a high activity in *in situ* GUS assay (Fig. 4A). It was observed that the calluses on a regeneration medium containing 1.6% agar formed multiple embryo-like structures and showed slow growth of calluses when compared to those on a medium containing 0.8% agar. We speculate that the osmotic stress on the calluses, which may have resulted from the high concentration of agar, non-penetrating osmotic agent, had a good effect on their regeneration.

Within 2 wk after the calluses were transferred onto a regeneration medium, green spots were first observed on some calluses. These spots developed

Fig. 4. A. *In situ* GUS staining of calluses (cv. Seomjinbyeo) transformed with pLS201 which were propagated in N6D liquid medium. B, GUS staining in the young leaf of untransformed (left) and transformed rice (right) (cv. Seomjinbyeo) containing *Act1*-GUS fusion construct. C, Green plantlets are regenerating from calluses (cv. Dobongbyeo) cotransformed with pAct1D and pGA643. D, Just before being transferred to soil, transgenic plants with strong roots were photographed. These plants which were induced in (C) were grown on the rooting medium for 2 weeks. Bar= 10 mm.

into tiny shoots 3-4 wk from initial culture (Fig. 4C). Most roots developed bilaterally and simultaneously from the bases of shoots, whose appearance was similiar to embryogenesis (Fig. 4C). We analyzed

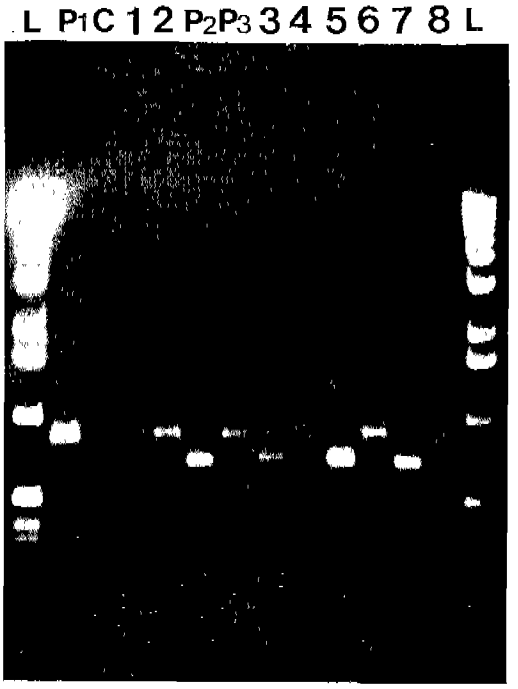
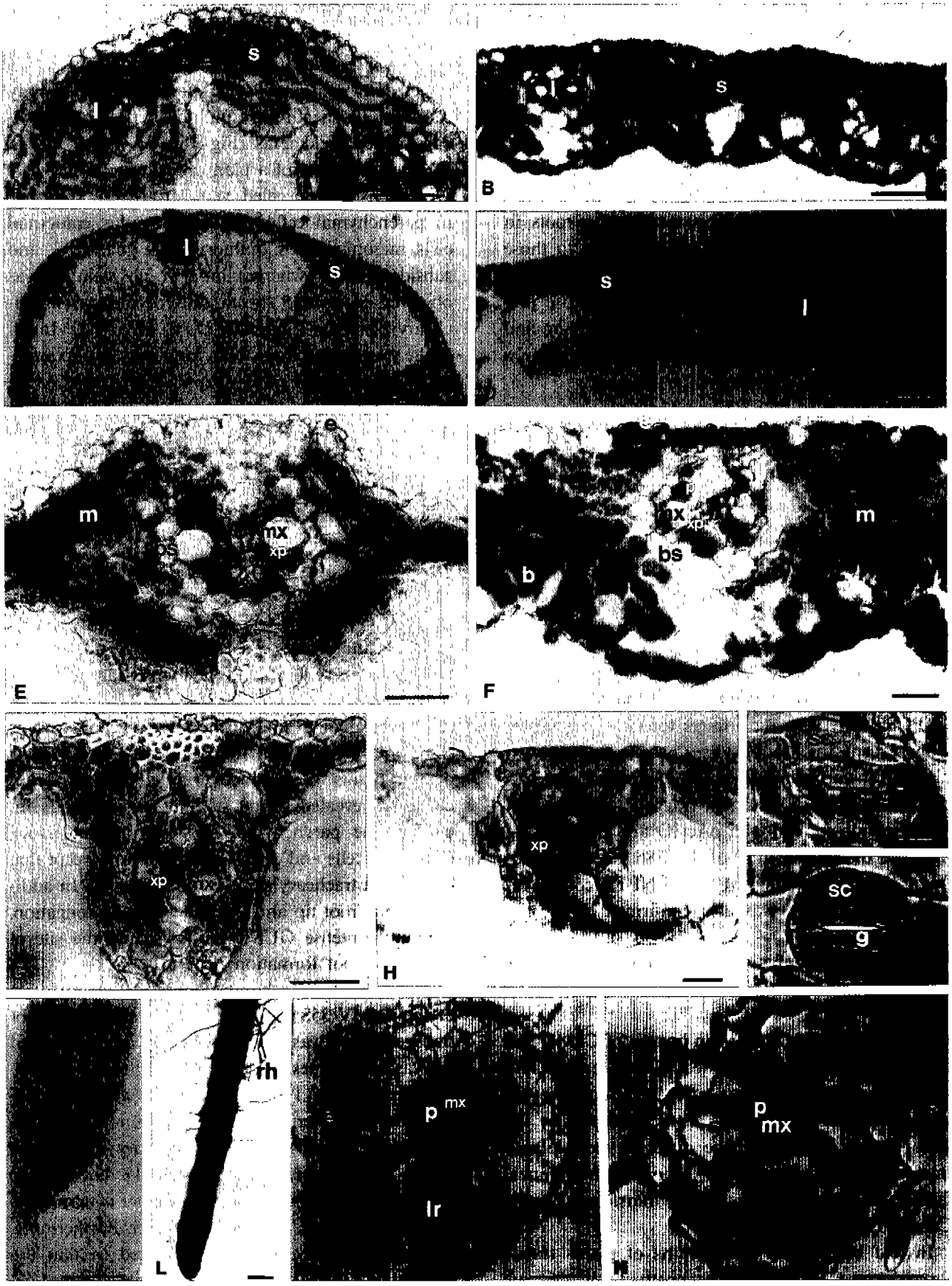


Fig. 5. Agarose gel electrophoresis of PCR-amplified fragment of plants transformed with pLS201 or pAct1D using primers for either the GUS or NPTII gene. Lane L, 1 kb DNA ladder; Lane P1, pLS201; Lane C, DNA from untransformed callus; Lanes 1-2, DNA from each plant transformed with pLS201; Lane P2, pGA643; Lane P3, pAct1D; Lanes 3/4, 5/6 and 7/8, DNA from 3 plants transformed with pAct1D and pGA643. (Lanes P1, C, 1, 2, P3, 4, 6 and 8 for GUS primers; Lanes P2, 3, 5 and 7 for NPTII primers).

Fig. 6. Histochemical localization of GUS activity in leaf blades, leaf sheaths and roots of rices transformed with CaMV 35S-GUS or *Act1*-GUS construct. A, Transverse section of leaf blade of rice transformed with CaMV 35S-GUS. B, Transverse section of leaf blade of rice transformed with *Act1*-GUS. C, Transverse section of leaf sheath of rice transformed with CaMV 35S-GUS. D, Transverse section of leaf sheath of rice transformed with *Act1*-GUS. E, Large vascular bundle of A. F, Large vascular bundle of B. G, Large vascular bundle of C. H, Large vascular bundle of D. I, Stomatal cell in leaf of rice transformed with CaMV 35S-GUS. J, Stomatal cell in leaf of rice transformed with *Act1*-GUS. K, Root of rice transformed with CaMV 35S-GUS. L, Root of rice transformed with *Act1*-GUS. M, Transverse section of K. N, Transverse section of L. b, bulliform cell; bs, bundle sheath cell; e, epidermis; g, guard cell; l, large vascular bundle; lr, lateral root; m, mesophyll cell; mx, metaxylem trecheary elements; p, phloem cell; rc, root cap; rh, root hair; s, small vascular bundle; sc, subsidiary cell; xp, xylem parenchymal cell. Bar=50 μm (A, B, C and D), 20 μm (E, F, G and H), 10 μm (I and J) and 50 μm (K, L, M and N).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the transgenic plants by *in situ* GUS assay at this stage (Fig. 4B). It was found that a leaf of a transgenic plant stained rapidly (right in Fig. 4B), whereas a leaf of an untransformed plant displayed no staining (left).

It is very important to obtain plants containing strong roots prior to transferring the regenerants to soil. The rooting medium containing 1 mg/L NAA used in our procedures developed strong roots in the regenerants (Fig. 4D) when compared to those grown without plant growth regulators (data not shown). This step is also different from those previously published (Abdullah *et al.*, 1986; Zhang and Wu, 1988; Battraw and Hall, 1992) which did not use plant growth regulators at this stage.

It was observed that albino plants were produced with a high frequency in cv. Seomjinbyeo, whose cell suspensions were originated from anther-derived calluses, compared to cv. Dobongbyeo whose cell suspensions were originated from seed-derived calluses. We have previously reported that albino plants were produced from anther-derived calluses with a high frequency (Kim *et al.*, 1993).

To confirm the presence of foreign genes in transgenic rices, PCR analyses were performed using genomic DNA isolated in each plant and primers for the coding regions in either the GUS or NPTII gene. In PCR analysis using primers for GUS coding region, except for untransformed control plant, all plants transformed with pLS201 showed amplification of the 0.86 kb fragment (Fig. 5). In case of all plants cotransformed with pAct1D and pGA643 the presence of amplification of the 0.86 kb and 0.7 kb fragments, representing GUS and NPTII coding regions, resepectively, was also confirmed (Fig. 5).

Histochemical analysis of transgenic plants containing CaMV 35S-GUS or *Act1*-GUS

To determine the expression pattern of CaMV 35S and rice *Act1* promoters, the leaf blades, leaf sheaths and roots of the transgenic rice, cv. Seomjinbyeo, were sectioned and stained histochemically by X-gluc (Fig. 6). In general, the materials from plants transformed with *Act1*-GUS fusion stained much faster than those from plants transformed with CaMV 35S-GUS.

In leaf blades and leaf sheaths of plants transformed with CaMV 35S-GUS construct it was found that strong GUS activity was detected in parenchymal cells of vascular tissue and mesophyll cells, whereas little or no CaMV 35S promoter activity was detected in epidermal cells, guard cells, subsidiary cells, bundle sheath cells, bulliform cells or metaxylem tracheary elements (Figs. 6A, C, E, G and I). In contrast, leaves of a plant transformed with *Act1*-GUS construct revealed very strong GUS activity in parenchymal cells of vascular tissue, epidermal cells, mesophyll cells, bulliform cells, guard cells and subsidiary cells, whereas little or no staining was observed in bundle sheath cells or metaxylem tracheary elements (Figs. 6B, D, F, H and J). In all of the transgenic plants, both large and small vascular bundles showed similar expression patterns of the GUS gene (Figs. 6A, B, C and D). No significant difference in the expression pattern of GUS between leaf blades and leaf sheaths was observed.

In the roots, early staining of tips in the incubation period was observed (Fig. 6K). In the materials transformed with CaMV 35S-GUS construct, strong GUS activity was detected mainly in parenchymal cells of vascular tissue whereas the weaker activity of the CaMV 35S promoter was detected in epidermal cells and cortex cells. However, little or no staining was observed in metaxylem tracheary elements (Fig. 6M). The lateral roots showed a pattern of staining similar to that of the larger sectioned roots (Fig. 6M). In contrast, transverse sections of roots of the plants transformed with *Act1*-GUS fusion construct revealed strong staining in most cells including the parenchymal cells of vascular tissue, epidermal cells and cortex cells (Fig. 6N) except that metaxylem tracheary elements little stained. In addition to the root tip and the zone of root elongation, we found intense GUS staining around the site of secondary root formation (Fig. 6L).

These results corroborate reports that the activity of the CaMV 35S promoter in vegetative organs of transgenic rice was localized mainly in and around the vascular tissues (Battraw and Hall, 1990; Terada and Shimamoto, 1990), and that the activity of the rice *Act1* promoter was detected in almost all types of cells except for metaxylem tracheary elements of leaves and roots (Zhang *et al.*, 1991). Therefore it is concluded that the rice *Act1* promoter is more constitutive than the CaMV 35S promoter. Furthermore, our results can be strongly supported in that the comparative analyses of GUS activity directed by

both promoters were performed for the first time in transgenic rice of an identical cultivar.

In conclusion, it is suggested that the rice *Act1* promoter can be utilized more successfully for the expression of a variety of foreign genes in rice than the CaMV 35S promoter. Presently we have obtained more than 70 green plants from the transformed calluses and some of them are now growing to set seeds in a greenhouse.

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形質轉換된 벼에서 CaMV 35S 프로모터 및 벼 *Act1* 프로모터에 의한 GUS의 發現

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적 요

벼에서 CaMV 35S 프로모터와 벼 *Act1* 프로모터의 발현양상을 조사하기 위하여 CaMV 35S-GUS 유전자와 *Act1*-GUS 유전자가 도입된 형질전환 식물체를 유도하고, 이들에서 형광 및 조직화학적 분석을 실시하였다. 안정하게 형질전환된 캘러스의 GUS 형광분석에서 벼 *Act1* 프로모터의 활성이 CaMV 35S 프로모터보다 높았다. 조직화학적 분석 결과, CaMV 35S 프로모터는 잎과 뿌리의 관다발 유조직세포 및 잎의 엽육세포에서 주로 발현되었다. 이 결과는 쌍자엽식물인 감자에서의 결과와 유사하였다. 이와는 대조적으로 벼 *Act1* 프로모터는 잎의 관다발 유조직세포, 엽육세포, 표피세포, 우두상 세포, 기공의 공변세포와 부세포 및 뿌리의 대부분의 세포에서 발현되었으며, 이는 벼 *Act1* 프로모터가 CaMV 35S 프로모터보다 더 다양한 세포에서 발현됨을 의미한다. 또한 잎 및 뿌리의 가도관에서는 두 가지 프로모터 모두 발현되지 않음을 확인하였다. 이러한 결과들은 벼에서 유용한 유전자의 발현을 위해서는 벼 *Act1* 프로모터가 CaMV 35S 프로모터보다 유용함을 의미한다.

주요어 : GUS, CaMV 35S 프로모터, 벼 *Act1* 프로모터, 형질전환된 벼

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