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Full Length Article

Regulation Behavior of *RSuS-1* Promoter using *uidA* Gene in Rice (*Oryza sativa*)

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Abstract

The main objective of this study was to characterize the expression pattern and strength of Rice Sucrose Synthase promoter in rice genome. The expression pattern and strength of Rice Sucrose Synthase-1 promoter (p*RSuS-1*) both qualitatively and quantitatively in different plant tissues was done by ligating to *gusA* gene. To confirm its tissue specific expression driven by *pRSuS-1*, in another construct, a constitutive promoter Cauliflower Mosaic Virus (CaMV) *35S* was also fused to the same reporter gene. The chimeric genes were introduced into a rice cultivar mediated by *Agrobacterium tumefaciens* while using hygromycin sequence as a selectable marker agent. Gus specific activity generated by the promoters was quantified using fluorometric method in the vegetative tissues such as leaves, stem, roots, seed coat and seed endosperm+embryo. The results demonstrated that the expression level produced by *CaMV 35S promoter* was roughly tenfold and twofold higher as per expected than p*RSuS-1 and* p*RSuS-2* sequences in the leaves, roots, seed coat and stem, respectively. Gus specific expression by p*RSuS-1 and pRSuS-2* in the seed endosperm+embryo was negligible. The overall activity of p*CaMV 35S* in the whole plant vegetative tissues was still three-fold greater. Also, a novel modification in the GUS staining technique of whole rice plant tissues has also been reported. The histochemical activity generated by *RSuS-1* was detected in all of the plant parts used except for the seed embryo and endosperm and in this way, confirmed the environmentally safe gene expression of this useful promoter from consumer's point of view.

Keywords: *Agrobacterium*; Gene expression; GUS staining; Rice; p*RSuS-1; uidA*

Introduction

Genetic engineering is now considered as a valuable way for incorporating the desirable traits into the crop plants. For improving the efficacy of gene transfer into the crop plants, various requirements like use of promoters, choice of specific promoter and their application in tissue specific expression are needed (Kim *et al.*, 1994; Park *et al.*, 2002). It is obvious from the fact that the promoters affect transcription pattern. A lot of new promoters have been isolated and among these the most extensively used constitutive promoter is the *35S* promoter of cauliflower mosaic virus (CaMV) that usually bring optimum limit of gene expression in crops i.e., in monocots and dicots respectively (Kay *et al.*, 1987; Benfey *et al.*, 1990; Mitsuhara *et al.*, 1996; Vain *et al.*, 1996). In this type of promoter, gene expression process continues in plant tissue during their development. So, lack of temporal and spatial regulation has a number of possible disadvantages for the use of genetically modified crops (Gittins *et al.*, 2000, 2003). Furthermore, frequent use of the same promoter tempts transgene silencing (Kumpatla *et al.*, 1998), and to achieve good results, the use of diverse promoters for gene expression in the same plant would overcome this problem.

Besides this, further dicot-based promoters used for monocot genetic transformation experiments, but their response tends to be lower than for the monocot promoters (Wilmink *et al.*, 1995). On the other hand, the use of monocot promoters resulted in the improved expression in monocots, including rice (Kyozuka *et al.*, 1993). Some of the promoters have been investigated and well characterized as alternatives for successful transgene expression in monocots, for example rice *Act 1* (McElroy *et al.*, 1991), the rice *RbcS* promoter (Kyozuka *et al.*, 1993; Jang *et al.*, 1999), maize *Ubi-1* promoter (Uchimiya *et al.*, 1993; Jang *et al.*, 2002). After the isolation of a promoter element, there should be its characterization of expression pattern and strength before induction in the transgenic system. Such a study would also

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be helpful for designing and planning proper strategies for successful and most appropriate use of a promoter. It has been reported that some of the studied promoters also have diverse characteristics in their structure and function and, therefore, they have diverse levels of activity in gene expression patterns. It is supposed to be due to the disparity in the genetic interaction of promoter elements with unidentified factors (Ellis *et al.*, 1987; Miao *et al.*, 1991; Ito *et al.*, 2000).

Although in existing situation, there is a scarcity of suitable and well-characterized promoters for transgenic expression in monocots. An efficiently characterized vascular tissue specific promoter might be useful in rice for environmentally safer expression of protein factors against insect pests that cause major economic losses. To make a more useful addition, this study was undertaken to characterize the relative efficacy in different plant parts with two plasmid constructs containing a vascular tissue-specific (phloem) promoter rice sucrose synthase-1 (*RSuS-1*) with a native (original) intron sequence at the 3' end and without intron, respectively, ligated to a widely used *uidA* gene by examining the expression, both qualitatively and quantitatively, in Japonica rice. The transformation process was mediated by the *Agrobacterium tumefaciens*, strain EHA-105 with the use of scutellum callus as target explant.

Materials and Methods

Plant Materials

Mature dehusked seeds of a Japonica rice variety, Xuishui-11 were surface sterilized with 70% ethanol for 2–3 min, followed by immersion in 0.1% $HgCl_2$ for 10–15 min and then washed 3–4 times repeatedly with autoclaved water. After this, these seeds were put on blot paper for some time. These sterilized seeds of rice were ready to be used for callus induction medium (basal N6 salts; 30 g/L sucrose, 2 mg/L 2, 4-D; and 8 g/L agar with pH 5.8) for a period of 10 days. Under *in vitro* conditions, embryogenic calli from mature seed of rice was initiated. After this, these calli were sub-cultured on pre-induction medium for 2–3 days to maintain optimum physiological activity (Khanna and Raina, 1999). Then these were used for co-cultivation process of gene transformation experiments.

Cloning and Sequencing of *RSuS-1* Promoter Segments

The fragments containing *RSuS-1* promoter were cloned from a Chinese Indica rice cultivar Minghui-63 using PCR. The primers for cloning were designed according to the published sequence (Wang *et al.*, 1992). The two PCR cloned fragments designated as *RSuS-1* (2897kb) with intron and *RSuS-2* (1715kb) without intron were cloned into *SmaI* site of the plasmid pBS SK (-) by blunt ligation. Direction was determined by enzyme digestion, and right direction clone was used to be sequenced. The sequenced promoter segments were compared with the already published sequence.

Transformation Vectors

The backbone of all three plasmid constructs containing different promoter regions was based on a pCAMBIA1301 binary vector as highlighted in (Fig. 1). In the original pCAMBIA1301 shown in Fig. 1a (kindly provided by Mr. Li Yongchun, Zhejiang University, China), 35S promoter of the GUS gene was excised by *Hind III* and *Nco I*, filled by *Klenow*, and circularized. Later, the promoter sequences were isolated from the cloning vector by *KpnI* and *BamHI* digestion. The vector (without 35S promoter) was also digested with the same pair of enzymes, and large fragment isolated. Finally, the promoter fragments were ligated to the large vector fragment to produce the final form of vector for *Agrobacterium* mediated transformation as shown in Fig. 1b, c. Hygromycin gene sequence was employed as selectable marker placed under the control of p*CaMV 35S* in all of the cases.

Transformation of Plasmid DNA into *Agrobacterium*

Competent bacterial cells for plasmid DNA transformation were prepared through $CaCl_2$ method as described by Sambrook and Russel (2001).

Transformation Strategy; Co-cultivation, Selection and Plant Regeneration

For transformation process, first the strain EHA-105 and then pCAMBIA1301 vectors were used. These were streaked on YEP medium for colony forming augmented with rifampicin, kanamycin and hygromycin antibiotics at concentration of 20, 50 and 50 mg/L, respectively. Bacterial colonies appeared, and then single colony was taken for further process of transformation. The primary calli sub-cultured for 3–4 days were immersed in the MS medium containing bacterial suspension for 10–15 min and then transferred to sterilized filter paper to remove excess of bacterial liquid. Then infected calli were shifted to co-cultivation medium (Basal N6 salts; 30 g/L sucrose, 2 mg/L 2, 4-D; 100 µM acetosyringone and 8 g/L agar with pH 6.0) at 26°C for 60–72 h in the dark. After the completion of co-cultivation time period, washing of infected calli (3–5 times) was performed with autoclaved water tracked by 2–3 times washing with liquid MS medium containing having carbenicillin antibiotic (500 mg/L) to remove all traces of bacteria. These were put on autoclaved filter paper for some time. After this step, these calli were shifted to selection medium (Basal N6 salts, 30 g/L sucrose, 2 mg/L 2,4-D; 50 mg/L hygromycin, 500 mg/L carbencillin and 8 g/L agar with pH 5.8) and put it in growth chamber at 26 °C in dark for proper selection time to grow under such condition. After some time (3–5) weeks on selection medium, resistant calli

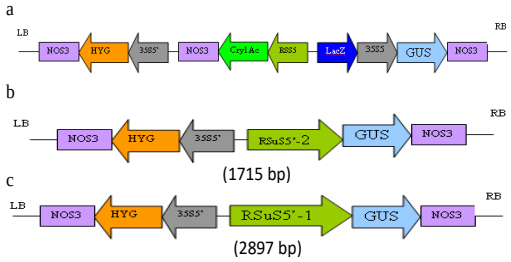

Fig. 1: Three vectors constructs employed in the transformation process

Fig. 2: Sequence comparison of Cloned RSuS-1 promoter fragment

were initiated their growth. Then these resistant calli were thoroughly shifted to regeneration medium (Basal MS, 30 g/L sucrose, 2 mg/L kinetin, 0.5 mg/L NAA, 50 mg/L hygromycin, 5g/L Phyta gel (Sangon), pH5.8) supplemented with hygromycin (50 mg/L). After 3–4 weeks, regeneration of shoots was started. To gain fully shoot size up to 2–3 cm, then these were shifted to rooting medium (Basal MS, 30 g/L sucrose, 0.5 mg/L NAA, 50 mg/L hygromycin, 3 g/L Phyta gel, pH 5.8) for additional time of 2–3 weeks. The fully developed resistant plantlets (having roots and shoots) then these were transferred to soil pots to acclimatize under glasshouse condition.

PCR Analysis

DNA samples extracted from the putative transgenic plants were subjected to PCR analysis to confirm the transgenic status by employing the primers specific for hygromycin sequence i.e. 5'TAGGAGGGCGTGGATATGGC 3' and 5' TACACAGCCATCGGTCCAGA 3'. The thermal conditions were as follows; pre-denaturation, 94°C for 5 min with one cycle, denaturation, 94°C for 1 min, annealing, 60°C for I min and DNA extension, 72°C for 1 min comprising 30 amplifications followed by a final post extension cycle at 72°C for 10 min. The amplified products were electrophorized on 1% agarose gel using the ethidium bromide staining.

Substrates

The substrates used were: 4-methyl umbelliferyl glucuronide (MUG; Sigma M-9130), X-Gluc (Research Organics Inc., Cleveland, OH, USA).

Histochemical Assay

For the localization of GUS activity to particular or all of the cell types, GUS staining with X-Gluc and histochemistry were performed with some novel modifications. The whole fresh plant tissues (leaves, stems, roots and seed parts) were frozen in liquid nitrogen for three times followed by thawing each time at 37°C. Later, the tissues were immersed in 80% cold acetone for 15 min at room temperature to facilitate the penetration of 5-bromo-4-chloro-3-indoly-glucuronide (X-Gluc), the substrate of GUS. Samples were rinsed with water, placed in a freshly prepared X-Gluc solution (100 mM sodium phosphate buffer (pH 7.0), 0.5% Triton *X*-100, 20% methanol and 0.5mg/mL X-Gluc fine powder) and vacuum infiltrated for 15–20 min. The tissues were later incubated at 37°C from 20 min to several h. When the incubation was completed, they were washed with ddH_2O and incubated in a mixture of 95% ethanol, 99% actone and pure acetic acid (1:3:1) for 1–2 h for the bleaching of chlorophyll (in case of leaf and stem tissues) contents to improve the contrast. The materials with GUS expression displayed blue color.

Fluorometric Assay

Lysis conditions: About 50 mg plant tissues were lysed for assays in 50 mM sodium phosphate buffer (pH 7.0), 10 mM EDTA, 0.1% Triton *X*-100, 0.1% sodium lauryl sarcosine (Sangon), 10 mM β-mercaptoethanol [extraction buffer] by freezing and grinding with liquid nitrogen in a 1.5 mL eppendorf tube. It was preferred to use the fresh extracts for assay and storage of extracts in this buffer at -20°C was avoided, as it seems to inactivate the enzyme. For leaf, stem and root, samples were collected before tiller stage while for seed coat and seed endosperm-embryo tissues were collected at milky stage and about one week after milky stage when the grain was just hardened.

Reaction: The fluorogenic reaction was carried out according to the method followed by Jefferson *et al.* (1987) except for a minor modification in 1 m*M* MUG with a reaction volume of 0.5 mL. Reaction was incubated 37°C and meanwhile 50 µL aliquots were removed after 10 min and the reaction was terminated with the addition of 0.45 mL 0.2 M Na_2CO_3. Addition of Na_2CO_3 served two functions: to stop enzyme reaction and to develop the fluorescence of MU (4-methyl umbelliferone), which is about seven times intense at alkaline pH. The amount of 4-MU product in the reaction was determined by fluorometeric measurement using a Hoefer DyNA Quant® 200 flourometer (excitation 365 nm/emission 455 nm). The fluorometer was calibrated with the freshly prepared MU standards of 50 nM and 1 µM in the

same buffers (0.2 M Na_2CO_3). The average amount of 4-MU product in three plants from every transgenic line was expressed as nm 4-MU/min/mg of fresh tissue weight.

Data Analysis

A standard regression line was established by using the different concentrations i.e. 0, 50, 100, 150 and 200 nM of freshly prepared 1 µM 4−MU (Sigma-M1381). The amount of 4-MU product in the reaction was calculated from the slope of regression line using Microsoft Office XP Excel.

Results

Comparison of Cloned RSuS-1 Promoter Sequences

The cloned rice sucrose synthase-1 (RSuS-1) promoter sequence as shown in Fig. 2 was compared with the published sequence. In Fig. 2, bases boxed are those different to the published/reported sequence, if the substitutes are not shown, it represents that the bases are lost. Bases in the triangles are those inserted; the sequence underlined by'----' is the first intron of the *RSuS 1* gene; the putative transcription and the expression initiation sites are indicated by the markers '.' under corresponding bases.

Production of Transgenic Rice Lines

The *RSuS-1* (with intron), *RSuS-2* (without intron) and *CaMV 35S* promoters were cloned in the pCAMBIA1301 binary vector to drive the expression of *gusA* reporter gene. Using these constructs, a number of transgenic lines were produced by *Agrobacterium tumefaciens*-mediated transformation. These lines were grown to maturity to get seeds for T_1 generation.

Molecular Analysis of Transgenic Clones

Seven T_1 transgenic lines, two containing p*CaMV 35S*, three for p*RSuS-1* and two harboring p*RSuS-2* promoters, were randomly selected for molecular analysis based on PCR to confirm the definite transgenic status before further biochemical analyses. An expected fragment of 852 bp for hygromycin gene sequence was amplified in all of the lines as has been shown in the Fig. 3.

Activity of Promoters in Tissues of Transgenic Lines

Strength of *CaMV 35S* promoter: The GUS specific activity generated under the influence of *CaMV 35S* promoter has been shown in Tables 1 and 2. The pattern of expression level in various tissues was similar in both of the lines, R-1 and R-5. The maximum activity was detected in the stem followed by leaves, root, seed coat and endosperm + embryo. However, the difference in activity between stem and leaves was remarkably lower than that of between stem and other tissues (roots, seed coat and endosperm + embryo) implying the differential strength of this promoter in different tissues.

Strength of tissue specific promoter: The GUS expression level obtained with the promoters *RSuS-1* and *RSuS-2*, in the leaves, stem, root and seed coat of three and two transgenic lines, respectively also followed the almost same expression pattern as in case of p*CaMV 35S* (Tables 1, 2). The activity in the seed endosperm + embryo was nearly negligible when the values compared with control (non-transgenic samples). All the transgenic lines exhibited almost similar level of expression irrespective of the presence of intron. Both the promoters directed a remarkably increased level of GUS specific activity in the stem that was roughly four to eightfold higher than that of other tissues. The expression levels were very similar for leaves and roots while in case of seed coat, it was relatively lower compared to these tissues (leaves and roots). A plausible explanation for the greater activity in the stem region might be the presence of higher mass of vascular tissue particularly phloem that proportionally gave rise to significant variation in the enzyme activity compared to leaves and roots. A negligible background activity was detected in the non-transgenic plants (data not shown).

Overall comparison of promoters activity: From the data obtained for the individual plant tissues, it is quite clear that p*CaMV 35S* is the strongest promoter (Tables 1, 2). The enzyme activity in the leaves and roots produced by p*CaMV 35S* was nearly tenfold higher than *RSuS-1* and *RSuS-2*. However, in the stem region, the expression induced by *RSuS-1* and *RSuS-2* was on an average six fold higher than that of leaves and roots as mentioned earlier and this brought down the difference figure with *CaMV 35S* to about twofold. *CaMV 35SP* even worked with strong potential in the seed tissues while the tissue specific promoters generated a comparatively weaker activity value in the seed coat with a negligible expression in the endosperm + embryo (Table 2). On the other hand, when the average expression level generated by the promoters for different transgenic lines in vegetative tissues was compared, it revealed a roughly threefold difference (Fig. 4).

Visualization of GUS Activity in Transgenic Plants/ Organs

Gus activity was localized by the examination of tissues that had been treated with X-Gluc and the results revealing the GUS expression patterns under the control of each promoter had been presented in the Fig. 4 and 5. The p*CaMV 35* produced a uniform strength of activity in all of the tissue (leaves, stem, roots, seed coat and endosperm + embryo). Apart from the tissue specific activity of *RSuS-1*, it also displayed variable activity within the tissues e.g. the activity was more pronounced near the root tip (root cap) than rest of the portion (Fig. 5f). In case of seed testa, more activity was concentrated in the central part (Fig. 6b). Furthermore, freezing of tissues with liquid nitrogen followed by subsequent thawing at 37°C and acetone was critical for the

Table 1: Gus specific activity in the different vegetative tissues of transgenic lines

Line	Promoter involved	Leaf	Stem	Root
		nm 4-MU/min/mg fresh tissue weight	nm 4-MU/min/mg fresh tissue weight	nm 4-MU/min/mg fresh tissue weight
R-1	*CaMV 35S*	494	552.5	379.8
R-5	*CaMV 35S*	442	466.0	276.7
Gus1-19	*RSuS-1*	77.3	149.72	35.2
Gus1-20	*RSuS-1*	72.4	252.4	67.6
Gus1-25	*RSuS-1*	70.8	273.8	68.4
Gus2-1	*RSuS-2*	62.3	288.8	50.0
Gus2-2	*RSuS-2*	62.7	315.1	44.4

Table 2: Gus specific activity in the different seed parts of transgenic lines

Line	Promoter involved	Seed coat	Endosperm+Embryo
		nm 4-MU/min/mg fresh tissue weight	nm 4-MU/min/mg fresh tissue weight
R-1	*CaMV 35S*	326.84	283.12
R-5	*CaMV 35S*	225.76	205.73
Gus1-19	*RSuS-1*	25.2	*NG
Gus1-20	*RSuS-1*	21.6	*NG
Gus1-25	*RSuS-1*	11.6	*NG
Gus2-1	*RSuS-2*	16.24	*NG
Gus2-2	*RSuS-2*	9.2	*NG

*NG-negligible (1–3), the fluorescence values being comparable to negative control (Xiushui-11)

staining process of whole rice plant tissues aside from vacuum infiltration. However, the longer exposure to acetone (>20 min) and its higher concentration (90–100%) was not beneficial for the tissues as it might be resulted in the loss of enzymatic activity. So, it was inferred that 80% acetone for 15 min was the optimum condition.

Discussion

In spite of isolating several promoters from a wide variety of plants and microorganisms, some are used in plant genetic transformation experiments. It is well studied that the most extensively used constitutive promoter is the *35S* promoter of cauliflower mosaic virus (CaMV) that normally possess optimum level of gene expression in dicot and monocot as well (Kay *et al.*, 1987; Benfey *et al.*, 1990; Mitsuhara *et al.*, 1996; Vain *et al.*, 1996).

The *RSuS-1* (rice sucrose synthase gene1) is an important gene in plant amylase synthesis pathway and the phloem-specific expression of its promoter in transgenic plants has been reported (Sudhakar *et al.*, 1998). However, according to our knowledge, there is a limited research on the characterization and relative strength of this useful tissue specific promoter in different plant organs/tissues that is indispensable for its more practical application in transgenic plant systems. In this study, we have performed this task along with that of p*CaMV 35S.* The purpose of use of p*CaMV 35S* was just to demarcate (differentiate) and quantify the spatial activity of *pRSuS-1*. As a reporter gene, *uidA* gene, which is well established for its qualitative and quantitative measurements in plants was used (Jefferson *et al.*, 1987). Uneven and patched GUS staining in rice tissues is a problem often faced during the study of *uidA* gene expression pattern. Although the staining methodology developed by Jefferson *et al.* (1987) was suitable for

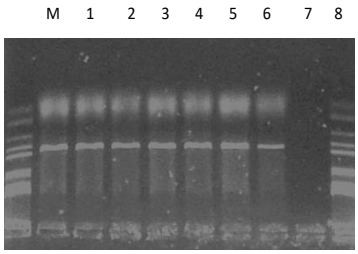

Fig. 3: PCR analysis- M; DL marker 2000+15000, Lane 1; R-1, Lane 2; R-5, Lane 3; Gus1-19, Lane 4; Gus1-20, Lane 5 Gus1-25, Lane 6; Gus2-1, Lane 7, Gus2-2, Lane 8, non-transgenic Xiushui-11.

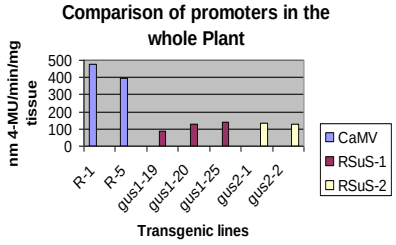

Fig. 4: Comparison of promoter average efficiency in the whole plant

transgenic diocts, but it did not function well with monocots. So, work has been continuous in the past to modify the protocol fit for monocots (Rueb and Henesgens, 1989; Tuominen *et al.*, 2000). In such an effort, we also attempted to refine further the protocol and were successful to attain more uniform and un-patched staining of whole plant tissue (including the use of liquid nitrogen and optimized concentration of acetone) compared to the previous protocols.

By considering the activity of two promoters (p*CaMV 35S* and p*RSuS*) in different vegetative tissues (leaf, stem, root), the *gusA* expression levels relative to those produced

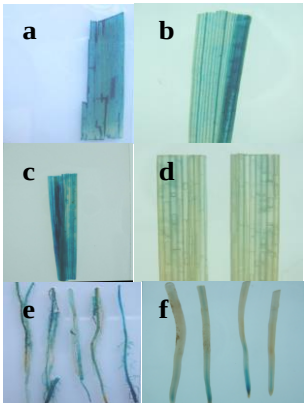

Fig. 5: GUS staining assay in vegetative parts; a–b, c–d and e–f are leaf, stem and root tissues expressing uidA gene under the control of *CaMV 35S* and *RSuS-1* promoters, respectively

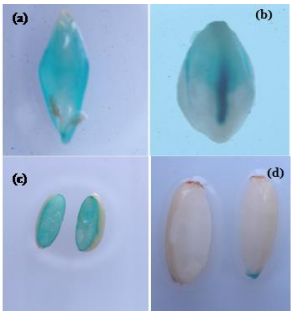

Fig. 6: GUS staining assay in seed parts; a-c and b-d are seed coat and seed endosperm + embryo expressing uidA gene under the control of *CaMV 35S* and *RSuS-1*promoters,

by the p*CaMV 35S* were roughly constant across only in the stem and leaf tissues but not in roots examined, confirming its semi-constitutive nature of expression. This is in contrast with those of Gittins *et al.* (2003) who observed a fairly constant level of GUS activity generated by cauliflower mosaic virus promoter across all of the vegetative tissues studied in apple. The p*RSuS*-1 and p*RSuS*-2 could only display the remarkable expression in the stem that was still approximately threefold lower than p*CaMV 35S*. In fact, there exist some obvious limitations to compare the quantitative data from the spatially regulated promoters with a constitutive promoter such as *CaMV 35S*. The only satisfactory way of doing this would be on per cell basis where the measurements were restricted to vascular tissues, which clearly impose severe practical constraints. Studies reported that the phloem expression driven under p*CaMV 35S* matches that from *RSuS-1* in dicots (Hider and Boulter, 1999) and, so, it can be hoped that the gene expression in stem driven by the tissue specific promoter in transgenic rice might be several times higher than p*CaMV 35S*. The reduced expression by p*RSuS-1* and p*RSuS-2* in the seed was an encouraging achievement from bio-safety point of view. The discrepancy in the activity levels between/among the transgenic lines harboring the same promoter element might be due to some other factors such as position effect, various copy number of integrated transgene etc.

The presence and processing of intron sequences in gene expression reported in animals and plants. The addition of introns may enhance the expression of genes in cereals (Callis *et al.,* 1987). The stimulating effect of the intron on expression of β-glucuronidase (GUS) activity was examined in transgenic rice calli, and maize plants in some earlier studies (Tanaka *et al.,* 1990; Vain *et al.,* 1996). Such an effect of the intron on GUS expression was suggested to be correlated with an efficient splicing of pre-mRNA and an increased level of mature mRNA. Keeping in view these findings, we designed two set of *RSuS*-promoter constructs, with and without native intron located in the transcriptional unit and attempted to demonstrate that there ispossibility for the existence of such a mechanism in our study while using *RSuS* promoter sequence. But unfortunately, we could not get the expected result. So, it is not always indispensable for an intron machinery to carry out the efficient splicing of mRNA. Apart from complex phenomenon of foreign expression in host cells, there might be some other factors responsible for such an inefficiency of intron splicing as sequence of the exon flanking the intron and location of the intron within a gene (Klinz and Gallwitz, 1985).

While observing the qualitative response, both the promoters (*RSuS*-1 and *CaMV 35S*) seemed to respond uniformly according to their expression nature in all of the tissues except for the root in case of *RSuS*-1. The enhanced strength of this promoter (*RSuS*-1) in the meristemic region near the root cap portion, perhaps, might be due to the increasing requirement of energy provided by carbon source for active cellular processes. Such an attribute can be useful while making strategies for the control of soil born pest as nematodes. Apart from the controversial reduced efficacy of *CaMV 35S* promoter in monocots (although we found p*CaMV 35S* still as stronger and the most efficient), recently some of the mounting potential hazards have been reported to be associated with transgenic plants containing *CaMV 35S* promoter (Kohli *et al*., 1999; Ho *et al.,* 1999 and 2000). It has a modular structure hosting a recombination hot spot, with parts common to and interchangeable with promoters of other plant and animal viruses resulting in the generation of new type of viruses (Ho *et al.*, 2000).

As the genetic engineering has moved from the initial euphoria to the course of correction, the development of transgenic rice using tissue specific promoter such as *RSuS-1* especially for effective insect resistance employing *Bt* genes will be useful as even the expression product in nanograms of such transgenes is enough to confer resistance (Wei *et al.*, 2000). Moreover, the development of transgenic crops with the minimized expression of foreign protein driven by *pRSuS-1* in the edible portions like seed (as demonstrated by our results) would be relatively safer for consumers. This spatial type of expression is meant to express in only those parts, where it is required. This would probably minimize the concerns of regulatory agencies or rice consumers.

Conclusion

The expression of Rice Sucrose Synthase-1 promoter (p*RSuS-1*) confirmed in various rice tissues by ligating to *gusA* gene. The transformation into rice with Gus assay confirmed the absence of expression in seed embryo and endosperm where phloem tissue is absent. This represents the effective use of this promoter for incorporation of insecticidal genes against sucking insect pests. Also, a novel modification in the GUS staining technique of whole rice plant tissues has also been reported.

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