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Original Article

Cloning and Characterization of a Tissue Specific Promoter *GluB-1* from *Nipponbare* by Transformation in Rice

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ABSTRACT: Tools of plant biotechnology can be applied as a routine procedure for improving rice grain quality or production of desired recombinant proteins in the rice seeds. For this purpose, endosperm specific promoter is a useful choice as desired gene expression would take place only in rice seed and not in root or shoot. Also, rice seed has become an attractive bioreactor for the production of recombinant proteins compared to other cereals. Glutelins are the most abundant storage proteins in rice grain which constitute up to 80% of the total protein content. The promoter region of *GluB-1*, one of the glutelin genes in rice, has been used as a model to study the regulation of seed-storage protein accumulation. In this study the upstream region (~2.4 kb) of the *GluB-1* gene was amplified from the genomic DNA of the *Nipponbare* cultivar of *Oryza sativa* (*japonica* group) and then cloned successively into an entry and promoter-characterization binary destination vector having the reporter gene *β-glucuronidase* (*GUS*) by applying Gateway Technology. Three plants generated by *Agrobacterium* mediated tissue culture were confirmed by PCR with *GluB-1* specific primer. In the transformed plants, histochemical *GUS* assay showed no expression in the root and shoot but was prominent in the endosperm of the T_1 seeds. Therefore this 2.4 kb promoter from *Nipponbare* rice can be successfully used to improve rice grain quality or express stable recombinant proteins in rice seeds for therapeutic purposes.

KEYWORDS: glutelin, Nipponbare cultivar, *β-glucuronidase* (*GUS*), *Agrobacterium*-mediated transformation.

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INTRODUCTION

Rice is one of the world's most important crops especially in the developing countries though the grain has lowest protein content (5 – 12%) compared to other cereals. Rice grains contain low levels of amino acids lysine and threonine[1,2] and is also a poor source of essential vitamins and micronutrients. Therefore there is a need to improve its nutritional value. Rice seeds are also considered a potential candidate for producing stable antibodies and vaccines. The endosperm of a seed forms an ideal storage for the production of recombinant protein due to low cost, control over the production rate, high level of safety during storage of the recombinant protein and low risk of pathogens.[3-6]

For improving the quality and quantity of rice proteins, conventional breeding has not been promising.[2] But rapid progress in molecular techniques like genetic engineering is now providing another possible option for improvement of the rice grain. By *Agrobacterium*-mediated transformation a soybean ferritin gene (soyferH-1) was transformed into rice under the control of a rice endosperm-specific promoter *GluB-1* (1.3 kb). It was found that transgenic rice accumulated ferritin protein in the seed and iron concentrations in transgenic rice seed were found to increase up to threefold over non-transformant seed.[7]

It has been reported that recombinant proteins, antibodies or vaccines that accumulated in rice seeds are very stable, can be administered orally without any need for further processing and purification and can be stored for years even at room temperature without decomposition.[7] Vaccines administered via seeds are thought to trigger antibody production by the mucosal immune system without processing or purification.[8] But the production of a desired protein using transgenic technology could be affected by lots of factors. Here choice of promoter plays a crucial role as it determines the temporal and spatial expression pattern and the transcript level of a gene.[9]

Promoters used in biotechnology are of three different types according to the intended type of control of gene expression. They are constitutive, inducible and tissue specific. The most commonly used constitutive promoter is *Cauliflower mosaic (CaMV) 35S* promoter derived from plant virus sources.[10] It can drive high levels of transgene expression in both dicots and monocots[11,12] and virtually all tissues. But constitutive over-expression of transgenes

may lead to undesirable pleiotropic effects in transgenic plants.[13,14] Thus, the use of inducible or tissue specific promoter is needed to attain temporal and tissue-specific expression of foreign genes.

Inducible promoters are modulated by abiotic factors such as salinity, drought, cold and wounding. A stress-responsive promoter *RD29A* promoter isolated from *Arabidopsis* has been shown to improve stress tolerance without causing growth defects.[15] *DREB1* gene under the control of the *RD29A* promoter has been expressed in several crops including tobacco,[16] chrysanthemum**,** potato, peanut, soybean, wheat and rice.[17] These transgenic plants showed improved stress tolerance without any growth defects. Another stress inducible promoter, *Oshox24* placed upstream of *OsNAC6* gene was used to generate transgenic rice plants, which showed increased stress tolerance with no growth defects.[18]

Tissue-specific or development-stage-specific promoters direct the expression of a gene in specific tissues or at certain stages of development. Over-expression of OsNAC10 under the control of a root-specific promoter, RCc3,[19] showed improved plant tolerance of transgenic rice to drought, high salinity, and low temperature during the vegetative stage of growth demonstrating the potential use of root-specific expression strategy for improving drought tolerance in rice.[20] Another promoter of fruit ripening-related genes has been targeted for their potential use in fruit-specific expression of transgenes. Promoters from the 1-aminocyclopropane-1-carboxylate (ACC) oxidase gene have been characterized from apple.[21] Analysis of the apple ACC oxidase under ACC oxidase promoter in transgenic tomato showed that this promoter was able to drive ripening-specific expression of antiporter gene in the tomato fruit, with no activity present in other tissues (roots, leaves, flowers) or in immature or mature green fruits.

Promoters specifically active in rice seeds are thus useful in transgenic approach and for production of recombinant proteins or improvement of rice grain quality. So tissue specific gene expression is desirable because they minimize the deleterious effects of transgene overexpression such as stunting, increased susceptibility to pathogen attack and reduction in yield.[22] Also foreign protein genes driven by an endosperm-specific promoter are more stable than those driven by ubiquitous promoters.[23] But the lack of characterization and availability of strong endosperm-specific promoters for driving the expression of recombinant protein genes in the cereal endosperm is still a major limitation[9] and the expression level of the target gene in the desired tissue is often not satisfactory.[24]

The glutelin gene promoters of rice are ideal candidates for isolating and obtaining strong promoters with endosperm specificity[9] as rice glutelin, accounts for ~80% of the total rice seed storage protein. *GluB-1* is a glutelin promoter which expresses specifically in the endosperm during seed ripening and is commonly used for foreign gene expression in transgenic rice seeds.[7]

GluB-1 promoter has already been used in various transgenic approaches such as to produce higher level of β-carotene in golden rice. Recently *GluB-1* promoter was used to improve bacterial carotene desaturase (CrtI) in the pathway of β-carotene synthesis.[25] Also the storage protein glycinin of soybean was expressed in transgenic rice plants under the control of the rice glutelin *GluB-1* promoter.[26] One of the most important applications of *GluB-1* promoter is in rice-based oral vaccine production. This vaccine expressed cholera toxin B subunit (CTB) under the control of the promoter *GluB-1* (2.3 kb) with codon usage optimization for expression in rice seed. This vaccine offers a highly practical and cost-effective strategy for orally vaccinating large populations against mucosal infections.[27]

GluB-1 promoter of different sizes (1.3 kb, 2.3 kb and 2.4 kb) was used previously in various transgenic researches. All these promoters showed seed specific expression in the aleurone and subaleurone layer.[2] Both 1.3 kb and 2.3 kb size *GluB-1* promoters showed high activity in the region of the endosperm close to the embryo. But *GUS* expression of the 2.3 kb *GluB-1* promoter was higher than 1.3 kb *GluB-1* in both transgenic rice seeds and after different days of flowering.[7] So in this study, the 2.4 kb of the *GluB-1* promoter was isolated from *Nipponbare* rice, then cloned and characterized in the rice variety Binnatoa, prior to its further use to express endosperm-specific proteins.

The transformation event was successful and high endosperm-specific expression of the reporter gene *GUS* downstream of the 2.4 kb *GluB-1* promoter in rice seed was confirmed. It was therefore shown that the 2.4 kb upstream region from *Nipponbare* was also the ideal size of promoter to drive successful expression of recombinant proteins in rice grain.

MATERIALS AND METHODS

Construction of pENTR_GluB-1 vector

DNA was extracted from Nipponbare rice variety using the CTAB method[28] and then quantified by Nanodrop® spectrophotometer ND-1000 (Thermo Fisher Scientific Inc. Waltham, MA, USA). Later the upstream region (2407 bp) of *GluB-1*gene (*GluB-1* promoter; Gene name OS02G0249800, Gene ID 4328883) was amplified with target specific primers (Table 1) by polymerase chain reaction (PCR). CACC overhang was added to the designed forward primer to ensure its compatibility with *pENTR/D-TOPO* vector. The PCR reaction program was optimized as follows: Initial denaturation was at 95°C for 5 min and 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 2 min 30 sec followed by a final extension at 72°C for 10 min. A final concentration of 2.3 mM $MgCl_2$, 0.1 mM dNTPs, 0.3 µM of each primer and 1 unit of recombinant Taq polymerase (Invitrogen, Carlsbad, CA, USA) was used. The desired *GluB-1* promoter of 2407 bp was extracted from the gel using Qiaquick Gel extraction kit (Qiagen, Hilden, Germany) and quantified through a nanodrop. The cloning reaction was initiated into *pENTR/D-TOPO* vector (Invitrogen, Carlsbad, CA, USA) following the

manufacturer's protocol. *E. coli DH5α* competent cells were transformed by the *pENTR_GluB-1* plasmid construct through heat shock employing standard protocols.[29] Then successful cloning into *pENTR/D-TOPO* was confirmed by PCR using *GluB-1* specific primers, restriction digestion with *EcoRV* restriction enzyme (NEBrinc, Ipswich, MA, USA). Final confirmation of the *pENTR_GluB-1* plasmid construct was done with gene specific primers by direct sequencing.

Construction of pHGWFS7.0_GluB-1_GUS vector and transformed into Agrobacterium

pENTR_GluB-1 vector was recombined into the destination vector (*pHGWFS7.0*) by the Gateway® LR recombination reaction (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). The destination vector (*pHGWFS7.0*) used in this study is efficient for analysis of promoter expression.[30] Site specific recombination properties of the Gateway system allowed recombination between *GluB-1* promoter from the *pENTR_GluB-1* and the target destination vector (*pHGWFS7.0*) where the *GluB-1* promoter would be placed successfully immediately upstream of the *GUS* gene. Positive colonies were confirmed by PCR with *GluB-1* specific primers and restriction digestion with *NcoI* (NEBrinc, Ipswich, MA, USA). Then using standard protocols,[31] *Agrobacterium tumefaciens* (*LBA4404*) was electroporated with cloned destination vector *pHGWFS7.0_GluB-1_GUS*. Finally positive colonies which contain desired *pHGWFS7.0_GluB1_GUS* vector were confirmed by PCR reactions with *GluB-1* promoter specific primers.

Table1. List of primers used in the study.

Primer	Sequence
GluB-1_2.4DF	5′-CACCCAGATTCTTGCTACCAAC-3′
GluB-1_SPR	5′-GGCCATAGAACCATGGCAT-3′

Generation of transgenic rice by tissue culture method

Callus induction, pre-incubation and co-cultivation: *Binnatoa* rice variety was used for transformation with desired *pHGWFS7.0_GluB-1_GUS* vector. Dehusked mature seeds were surface sterilized and plated directly on MS callus induction medium (MS salts, organic elements, 30 g/l sucrose, 500 mg/l casein hydrolysate, 100 mg/l myo-inositol, 1 mg/l thiamine hydrochloride, 2 mg/l 2,4-D, 3 g/l phytagel, pH 5.8). After 21-30 days, calli were pre-incubated for 72 h in semisolid explant pre-incubation medium (Khanna and Raina 1999). Then the calli were immersed in bacterial suspension containing desired *pHGWFS7.0_GluB-1_GUS* vector for 10 min. Excess bacterial suspension was removed by placing them on sterile filter paper before transferring to semisolid co-cultivation medium (explant pre-incubation medium, AS 500 µM, 3 g/l phytagel, pH 5.8). The cultures were incubated at 25°C for three days in the dark. After three days the calli were removed and transferred to callus selection media (callus induction medium, 50-100 mg/l hygromycin, 500 mg/l carbenicillin, 250-500 mg/l cefotaxime).

Regeneration of transformed callus: After co-cultivation, calli were incubated in the selection media with hygromycin. After 3-4 weeks, proliferating hygromycin-resistant microcalli were subcultured on plant regeneration medium (MS salts, organic elements, 30 g/l sucrose, 2 mg/l BAP, 0.2 mg/l NAA, 100 mg/l myo-inositol, 3 g/l phytagel, 50-100 mg/l carbenicillin, 50-100 mg/l cefotaxime and 1 mg/l thiamine hydrochloride, pH 5.8). Regenerants were transferred to rooting medium (one fourth strength MS salts (full strength Fe_2EDTA), 2 mg/l BAP, 0.2 mg/l NAA, 2 mg/l IBA, 500 mg/l casein hydrolysate, 100 mg/l myo-inositol, 30 g/l sucrose, 50-100 mg/l carbenicillin, 1 mg/l thiamine hydrochloride, 3 g/l phytagel, pH 5.8). After emergence of both shoot and root, seedlings were transferred to a hydroponic system of Yoshida solution[32] for hardening and finally transferred to soil. The transgenic plants were kept at a confined area in a net house. Positive transformed plants were confirmed by PCR analysis with *GluB-1* specific primers with PCR conditions exactly the same as above. After molecular confirmation, T_0 plants were advanced to T_1 generation.

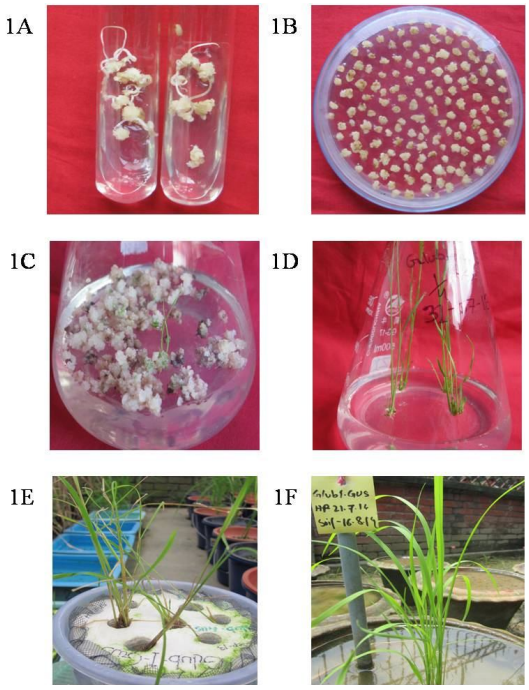

Figure 1. Regeneration of three transgenic plants with *GluB-1_GUS* constructs. **1A.** Callus in MS induction media, **1B.** Transformed calli in selection media, **1C.** Regenerated calli in regeneration media, **1D.** Transgenic plants in rooting media, **1E.** Transgenic plants in hydroponic system and **1F.** Transgenic plants in soil.

GUS assay in rice calli and transformed plants: For initiating Agrobacterium mediated transformation, Agrobacterium containing the *GluB-1_GUS* construct was streaked and cultured in YM media.[33] Rice calli were generated from Binnatoa variety and infected with *Agrobacterium tumefaciens* having the *GluB-1_GUS* construct. Infected calli were kept in dark for cocultivation at 28°C for 72 hrs. After 72 hrs of cocultivation, calli were treated with carbenicillin (250 mg/l) and then washed with double distilled H_2O.

Shoot, root and seed from transgenic (T_1) plants and transformed calli were assayed for beta-glucuronidase (*GUS*)[34] using the indigogenic substrate X-gluc (5-bromo-4-chloro-3-indolyl b-D-glucuronide). Here wild type Binnatoa was used as control. At first tissue segments were fixed at pH 5.6 by fixation solution containing 10 mM MES, 0.3% formaldehyde and 0.3 M mannitol, and vacuum infiltrated for 5–6 min. The tissues were then washed several times in 50 mM phosphate buffer (pH 7.0). After washing samples were immersed in ~200 µL X- gluc solution (0.1 M NaPO4 pH 7.0, 0.5 mM $K_3Fe(CN)_6$, 0.5 mM $K_4Fe(CN)_6$, 10mM EDTA, 5 bromo-4-chloro-3-indolyl-â-glucuronide; X-gluc) for 16–72 h at 37°C for color development. 10 mg X-gluc was dissolved in 1 mL methanol and made up to a final concentration of 1 mg/mL with 50 mM phosphate buffer at pH 7.0. After staining, sections were washed with 70% ethanol and then stored in 70% ethanol. After histochemical assay non transformed and transformed seeds were dissected longitudinally using a sharp blade. Samples were mounted on a glass slide and fixed with 65% glycerol before taking a photo. The photograph was taken under a light microscope at a resolution of 4X.

RESULTS

Confirmation of cloned pENTR_GluB-1 and pHGWFS7.0 _GluB-1_GUS vector

The 2.4 kb *GluB-1* promoter region was amplified from rice genomic DNA of *Nipponbare* and cloned into pENTR/ D-TOPO vector (Figure 2). Only two colonies were found positive which gave the two expected band sizes (3,299 bp and 1,687 bp) after restriction digestion with *EcoRV* (Figure 2C). Further confirmation of *GluB-1*cloning into *pENTR* was carried out by polymerase chain reaction (Figure 2B) with *GluB-1*specific primers followed by sequencing. M13 primers and *GluB-1*specific primers sets (Table 1) were used to obtain the database sequences and the BLAST hit indicated 100% match with the sequence of the amplified fragments.

Generation and selection of transformed plants

The positive *pHGWFS7.0_GluB-1_GUS* construct was electroporated into *Agrobacterium* and used for rice transformation.[35,36] In six individual experiments, ~600 calli were infected and after hygromycin (50 mg/L) selection three successfully transformed T_0 plants (P1, P2 and P3) were obtained. All three transformants were confirmed by PCR using *GluB-1* specific primers and the precise band of ~2.4 kb was observed (Figure 4).

Expression of GluB-1 promoter in transformed calli and stable transformed plants

For transient expression pattern of *GluB-1* promoter, histochemical *GUS* assay was performed in *GluB-1_GUS* infected rice calli. The *GluB-1_GUS* transformed rice calli showed relatively higher *GUS* expression compared to non-transgenic calli. The presence of blue color indicated successful insertion of the promoter and its ability to drive reporter gene (*GUS*) expression (Figure 5).

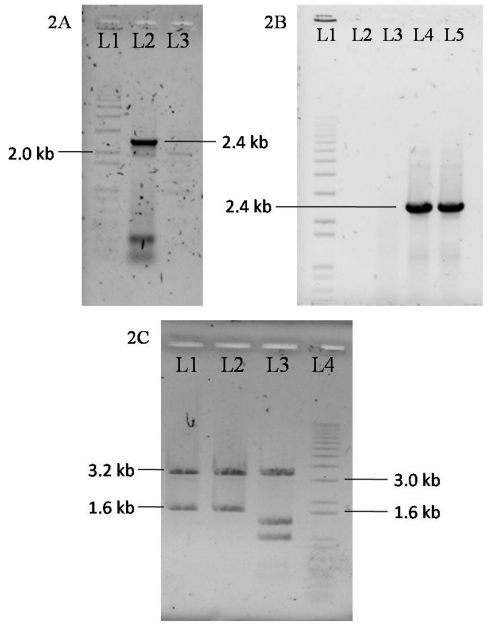

Figure 2. Amplification, cloning and confirmation of the *GluB-1* promoter in *pENTR* entry vector. **2A.** *GluB-1* promoter of 2.4 kb was amplified using sequence specific primers from Nipponbare. L1: 1kb$^+$ DNA ladder, L2: Amplified *GluB-1* promoter sequence. **2B.** *GluB-1* amplification from the cloned *pENTR* plasmid, L1: 1kb$^+$ DNA Ladder, L2: Negative control, L3-L4: Amplicons of the right size of *GluB-1* promoter. **2C.** Restriction digestion (RD) of cloned *pENTR_GluB-1* plasmid with *EcoRV*. L1-L2: showing correct size restriction fragments, L4: 1kb$^+$ DNA ladder.

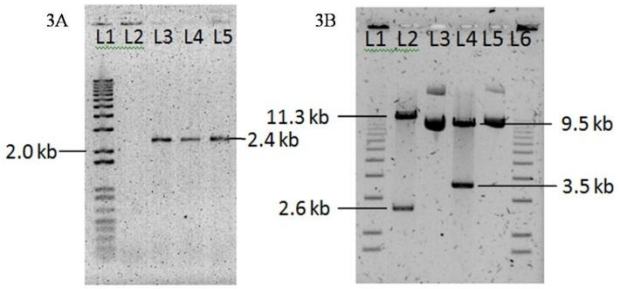

Figure 3. Confirmation of the *pHGWFS7.0_GluB-1_GUS* construct.
3A. Amplification of *GluB-1* promoter from *pHGWFS7.0_GluB-1_GUS* plasmid after LR recombination, L1: 1kb$^+$DNA Ladder, L2: Negative control, L3-L5: Amplicons of the right size *GluB-1* promoter.
3B. Restriction digestion of *pHGWFS7.0_GluB-1_GUS* constructs with *NcoI*, L1 and L6: 1kb$^+$ DNA ladder, L2:*pHGWFS7.0_GluB-1_GUS* digested with *NcoI* with fragments of expected sizes. L3: Uncut *pHGWFS7.0_GluB-1_GUS*. L4: Empty vector *pHGWFS7.0* digested with *NcoI*. L5: Uncut *pHGWFS7.0*.

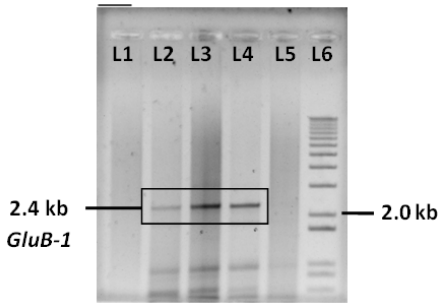

Figure 4. Amplification of the *GluB-1* promoter from three T_0 transformed plants (P1, P2 and P3), L1: Negative control, L2-L4: Amplicons showing the right size *GluB-1* promoter (P1, P2 and P3), L5: Binnatoa, L6: 1kb$^+$ DNA Ladder.

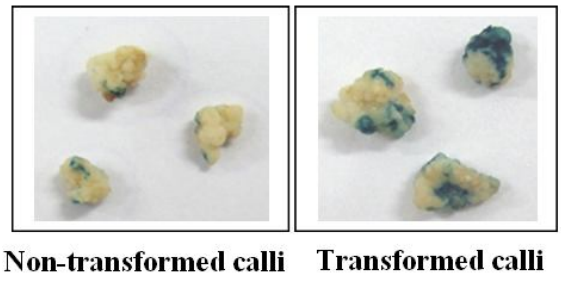

Figure 5. Transient *GUS* assay with *GluB-1_GUS* in non-transformed and transformed rice calli.

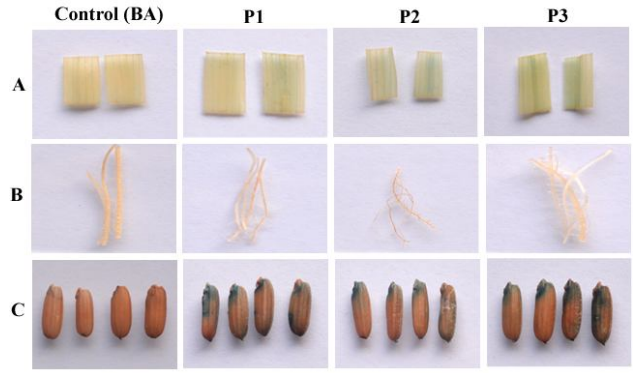

Figure 6. Histochemical analysis for the expression of *GUS* gene under *GluB-1* promoter in different parts of transgenic plants (A) Shoot (B) Root (C) Seed compared to Control, Binnatoa. In positive (transformed) plants (P1, P2 and P3) little expression was found in the shoot but there was no expression in the root. In seeds, *GUS* gene expression was found predominantly in the endosperm.

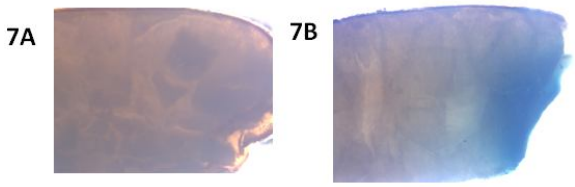

Figure 7. Microscopic analysis of hand cut longitudinal section of transgenic rice seeds. Only half of the seed, from the bottom is shown (portion near the embryo). **7A**. Non transformed seed. **7B.** *GluB-1_GUS* transformed seed.

Histochemical assay was also performed in *GluB-1_GUS* transformed T_1 plants. Very little expression of *GUS* gene was found in shoots and no expression was detected in the roots. But in transgenic seed *GUS* expression was observed only in the endosperm (Figure 6).

To find the *GUS* gene expression pattern under *GluB-1* promoter, seeds were dissected and analyzed under a microscope. In non transformed seeds blue color was not found. But in transformed seeds a strong blue color was produced around the endosperm area near the embryo (Figure 7). Thus it can be concluded that the 2.4 kb *GluB-1* promoter from *Nipponbare* rice mediates *GUS* gene expression in an endosperm specific manner.

DISCUSSION

Over the years, numerous promoters have been isolated and applied to plant genetic engineering systems. It is known that promoters control the level and specificity of gene expression in different developmental stages, tissues, cells and thus affects the transcription of a gene both quantitatively and qualitatively. To employ this important feature of a promoter and to use it in modern biotechnology chimeric *GUS* reporter gene fusion is used for expression studies not only to better understand promoter-specified gene expression patterns but also for functional analysis.

In rice, the starchy endosperm is the major site of nutrient deposition which is necessary for early seedling growth and development. Accumulation of seed storage proteins in rice occurs during grain development and seed storage proteins are normally expressed at high levels and specifically in the endosperm. Glutelin is the major seed storage protein of rice endosperm, accounting for ~80% by weight of total seed protein. So for improvement of the grain quality as well as producing foreign protein in rice seed, glutelin gene promoter has been studied with interest. Various promoters of rice glutelin gene family were isolated and tested for their expression pattern in different transgenic rice,[37,38] maize[39] and tobacco.[40] All the promoters showed the expected spatial expression within the endosperm but the potential expression activities of glutelin gene promoters were limited to a few (*GluA-2*, *GluA-3*, *GluB-1*, *GluB-2*, and *GluB-4*).[2,7,37,39] Among them the *GluB-1* promoter showed higher expression only in the endosperm area compared to the other glutelin promoters.

GluB-1 promoter was previously used in transgenic technology and some prominent results were found. An engineered peptide RPLKPW under the control of *GluB-1* promoter was expressed in transgenic rice which specifically accumulates in seeds. It was found that oral administration of transgenic rice seeds to spontaneously hypertensive rats significantly reduced systolic blood pressure. These results suggest the possible application of transgenic rice seed as a nutraceutical delivery system and specifically for administration of active peptides in hypertension.[41] Another industrial microbial enzyme fungal xylanase was produced in barley in the developing endosperm under the control of the *GluB-1* promoter which provided an apparently higher expression of recombinant proteins in barley grain in both transient and stable expression experiments.[42]

In rice seed storage protein, 5′ distal and proximal cis acting transcriptional regulatory elements are required for developmental control.[37] The proximal 5′ flanking region of *GluB-1* contains four motifs, GCN4, AACA, ACGT and prolamin-box that are conserved in many seed storage protein genes. Among them GCN4 motif acts as an essential element determining endosperm specific expression.[43] But in *GluB-1* promoter the region -245 bp of transcription site is more preferable regarding expression efficiency. In the -245 bp transcription region *GluB-1* promoter contains another AACA motif that exists upstream of -197 bp and deletion of this motif resulted in an eight fold reduction in promoter activity.[38] Moreover, it was previously reported that activity of 2.3 kb *GluB-1* was about 10 fold higher than 1.3 kb *GluB-1* promoter.[7] The reason behind this difference in activity was due to the presence of one more ACGT motif in the additional 1 kb fragment of the 2.3 kb promoter (Figure 8).

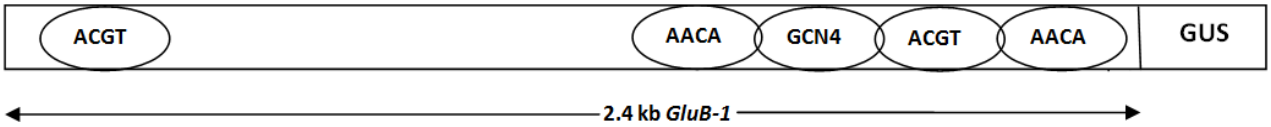

Figure 8. 2.4 Kb *GluB-1* promoter_GUS construct.

GluB-1 promoter has important significance as it shows seed specific expression. In this study 2.4 kb *GluB-1* promoter was isolated from Nipponbare rice variety and transformed into Binnatoa. The reason behind choosing 2.4 kb *GluB-1* was that it showed very specific and high level of expression only in the endosperm area of rice seed compared to the shorter length (1.3 kb) *GluB-1* promoter.[7]

A 2.4 kb *GluB-1* promoter which is nearly identical to the 2.4 kb *GluB-1* promoter isolated from *Nipponbare* was used in the production of the CTB vaccine for its high efficiency (unpublished data). However, this 2.4 kb *GluB-1* was not characterized previously for its precise expression pattern. So the current work shows that this promoter could be effective for use in transgenic research for the improvement of rice.

To visualize the expression pattern of the 2.4 kb *GluB-1* promoter from *Nipponbare* rice, histochemical *GUS* assay was performed in root, shoot and seed of the transformed T_1 seeds. No *GUS* expression was observed in the root and a very low expression was found in leaves. Little GUS expression was also found in nodal portion of shoot of transgenic plants (not shown here). But in seed *GUS* expression was found to be higher specifically in the endosperm area whereas no color was observed in non-transformed plants.

CONCLUSION

From this study it can be concluded that for promoter characterization transformation of Binnatoa rice variety by *Agrobacterium* mediated tissue culture method is suitable and efficient. The *GluB-1* promoter of *Nipponbare* rice glutelin protein was found to be suitable for expression of downstream genes only in endosperm specific area of rice seed and not in root and leaves.

In future, this *GluB-1* promoter can be used for further improvement of protein content as well as nutritional value in rice grain. Also different components like pharmaceutical products, important enzymes, various peptides of medical importance, edible vaccine that are functional *in vivo* condition can be produced in rice seed specifically using this promoter. Thus genetic engineering of rice with endosperm specific *GluB-1* promoter could provide a dependable approach for bettering rice quality and make it a strong candidate for transgenic research where tissue specific expression is most needed.

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