

Pollen-Specific Expression of *Oryza sativa Indica* Pollen Allergen Gene (*OSIPA*) Promoter in Rice and *Arabidopsis* Transgenic Systems

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Abstract Earlier, a pollen-specific *Oryza sativa indica* pollen allergen gene (*OSIPA*), coding for expansins/pollen allergens, was isolated from rice, and its promoter—upon expression in tobacco and *Arabidopsis*—was found active during the late stages of pollen development. In this investigation, to analyze the effects of different putative regulatory motifs of *OSIPA* promoter, a series of 5' deletions were fused to β -glucuronidase gene (*GUS*) which were stably introduced into rice and *Arabidopsis*. Histochemical GUS analysis of the transgenic plants revealed that a 1631 bp promoter fragment mediates maximum GUS expression at different stages of anther/pollen development. Promoter deletions to −1272, −966, −617, and −199 bp did not change the expression profile of the pollen specificity. However, the activity of promoter was reduced as the length of promoter decreased. The region between −1567 and −199 bp was found adequate to confer

pollen-specific expression in both rice and *Arabidopsis* systems. An approximate 4-fold increase in the GUS activity was observed in the pollen of rice when compared to that of *Arabidopsis*. As such, the *OSIPA* promoter seems promising for generation of stable male-sterile lines required for the production of hybrids in rice and other crop plants.

Keywords *Arabidopsis* · Cis-regulatory element · GUS · Pollen-specific promoter · Rice · Transgenics

Introduction

Development of male gametophyte plays a vital role in plant fertility and normal seed development. In monocotyledonous and dicotyledonous plant species, the differentiation and development of pollen within the anther sacs is mediated by different molecular and cellular events which share common components [1, 2]. In recent times, substantial progress has been made in understanding the process of male gametophyte development and significant information has been accumulated from the analyses of male-sterile mutants in the model plant *Arabidopsis*. In the rice plant, the formation of male gametophyte appears to follow a developmental pathway similar to that of *Arabidopsis* [3, 4]. Accordingly, the rice can now serve as a model monocot system to analyze the gene regulation on a par with that of dicot *Arabidopsis* and tobacco [5]. In rice, several cDNA microarray and proteome analyses have been performed to understand the complex nature of gene expression during anther and pollen development. Through these studies, certain genes specifically involved in the male gametophyte development have been identified [6–13].

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transformation of *Arabidopsis* (ecotype Columbia) was achieved by floral dip method [50] employing pBI101 plasmid carrying different expression units of *OSIPA* promoter.

PCR and Segregation Analysis of Transgenic Plants

Genomic DNA was isolated from Basta tolerant and untransformed control plants according to the method of McCouch et al. [51]. PCR analysis for the detection of *GUS-OSIPA* and *bar-nos* were carried out using the primers: *OSIPA*-F 5'-gcacaaattacgtacaaaacg-3', *GUS*-R 5'-gagtgaccgcacgcgacagcag-3' and *bar*-F 5'-ctaccatgagcccaaacg-3', Nos-R 5'-gcgatctagtaacatagatgac-3', respectively. Similarly, after selection on kanamycin the transformed *Arabidopsis* lines were checked for the presence of transgene by PCR amplification of a region coding *GUS* by using the gene-specific primers (*GUSR* 5'-tcattgttgctccctgc-3' and *GUSF* 5'-atgttacgtcctgtagaaacc-3').

Seeds from the primary transformants were harvested to carry out the segregation analysis. Collected seeds of rice and *Arabidopsis* were germinated on the selection medium containing PPT (5 mg/l) and kanamycin (50 mg/l), respectively. The number of sensitive and resistant seedlings was counted after 2 weeks of germination. Chi-square values were calculated by the formula $\chi^2 = \sum (O - E)^2/E$, where O and E are the observed and expected values, respectively. The probability was calculated based on Chi-square distribution table [52].

Histochemical Localization of β -Glucuronidase (GUS) Activity

Histochemical analysis of GUS activity was performed, essentially as described by Jefferson et al. [53] with minor modifications. T_1 and T_2 plants of rice and *Arabidopsis* *OSIPA* transformants were grown to flowering stage under controlled conditions. Inflorescences at different developmental stages (~6.0–25 cm) were collected from rice transgenics and control plants; glumes were opened into two halves with the help of fine forceps and anthers were used for GUS assay. Root, leaf, and gynoecium of transgenic as well as control rice plants were also checked for *GUS* expression. Similarly, *Arabidopsis* floral buds were collected. Anthers, gynoecium, petals, and sepals were separated, and were used for GUS assay. All the explants were incubated at 37°C for different durations (30 min to 24 h), in a reaction buffer containing 1 mM 5-bromo-4-chloro-3-indolyl- β -D-glucuronide (X-gluc), 100 mM sodium phosphate buffer (pH 7.0), 2 mM potassium ferri-cyanide and potassium ferrocyanide, and 0.1% Triton

X-100. After staining, explants were rinsed in 70% ethanol and were observed under the microscope.

Quantification of GUS Activity

For quantitative measurement of GUS activity in transgenic rice and *Arabidopsis*, the protocol described by Jefferson et al. [53] was followed. Plant tissue was homogenized in GUS extraction buffer containing 50 mM NaH_2PO_4 (pH 7.0), 10 mM EDTA, 0.1% Triton X-100, 0.1% sodium lauryl sarcosine, and 10 mM β -mercaptoethanol. Protein concentration of the plant extracts was estimated using Bradford reagent as per the manufacturer's instructions. GUS assay buffer containing the substrate MUG (4-methylumbelliferyl β -D-glucuronide) was added to protein samples and the reaction was incubated at 37°C. The specific activity of GUS was expressed as nmole 4-MU formed per hour per milligram of the total protein.

Results

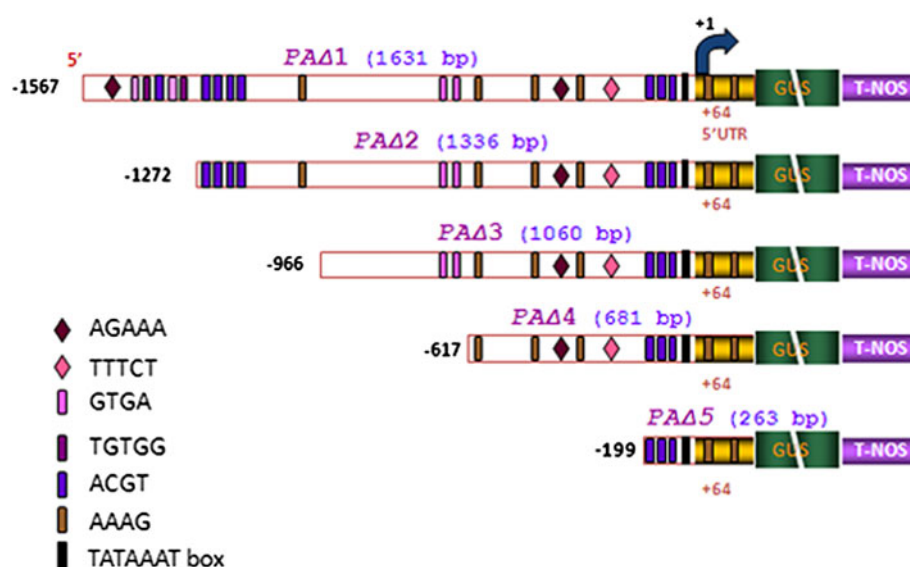
Dissecting the Regulatory Region of *OSIPA* Gene

In order to delineate the minimal promoter region of *OSIPA* conferring pollen specificity and the role of various conserved motifs present in the full-length promoter, a series of 5' deletions containing 1631, 1336, 1060, 681, and 263 bp—designated as *PAA1*, *PAA2*, *PAA3*, *PAA4*, and *PAA5* (Fig. 1)—were generated, and were fused to the *GUS* coding sequence followed by their mobilization into plant expression vectors (pBI101 and pSB11). These vectors contained upstream region with variable number of regulatory elements known to be involved in gene expression, and were subsequently used for *Arabidopsis* and rice transformation.

Analysis of Transgenic Rice and *Arabidopsis* Plants

Five different *OSIPA* promoter expression cassettes were independently introduced into rice and *Arabidopsis* by *Agrobacterium*-mediated transformation. A total number of 120 rice transformants were obtained from 12116 infected calli. For each construct, 4–6 independent transformants were selected based on level of their tolerance to herbicide Basta (Fig. 1SA) and were used for further analysis. Transgenic *Arabidopsis* plants were selected based on resistance to kanamycin (Fig. 1SB). More than 20 lines for each deletion construct were obtained and five independent lines for each construct were selected for subsequent analysis.

Fig. 1 Schematic representation of different *OSIPA* deletion constructs used to transform rice and *Arabidopsis* plants. The numbers at 5' end indicate the upstream end of the promoter fragment present in each construct, the downstream end was at +64 for all constructs. Different colored symbols represent the positions of the *cis*-acting elements



The genomic DNA isolated from Basta tolerant transgenic rice plants, when analyzed by PCR, showed amplifications of 750 and 600 bp products representing *bar* and *GUS* coding sequences, while control plants failed to show such amplification (Fig. 2S). Rice transgenics were also confirmed by Southern hybridization (data not shown). A PCR product of ~2 kb corresponding to the *GUS*-specific amplification product was detected in all the *Arabidopsis* lines, thereby confirming their transgenic nature (Fig. 3S). Untransformed wild-type plants were used as negative control, whereas binary vectors harboring various deletion constructs were used as positive controls. In the subsequent generation, the segregation pattern for transgenic rice and *Arabidopsis* lines carrying various deletion constructs was examined (Tables 1S and 2S). Lines homozygous for transgenes were selected and used for GUS histochemical and fluorometric assay.

Histochemical GUS Analysis in Transgenic Plants

Selected *OSIPA* transgenic rice and *Arabidopsis* lines were analyzed for GUS activity. A visible expression of GUS was noticed in the anthers of transgenic rice after 1–3 h of incubation in X-gluc containing buffer, and GUS was predominantly localized in pollen grains. Rice transformants containing *PAΔ1*, *PAΔ2*, *PAΔ3*, *PAΔ4*, and *PAΔ5* promoter deletion constructs showed varied levels of GUS expression (Fig. 2). Furthermore, a detailed analysis of the temporal expression of GUS was done at different stages of pollen grains of developing panicles ranging from 6.0 ± 1.0 to 25.0 ± 2.0 cm size. In *OSIPA* rice transformants, initial expression of GUS was observed at the pre-pollination stage of anthers from developing panicles (10.0 ± 2.0 cm) and reached maximum levels in mature pollen of dehiscent anthers (Fig. 3). No GUS activity could

Fig. 2 Histochemical analysis of *GUS* expression in florets of transgenic rice plants harboring various promoter deletion constructs. Control (a), *PAΔ1* (b), *PAΔ2* (c), *PAΔ3* (d), *PAΔ4* (e), and *PAΔ5* (f). GUS expression was observed only in the anthers with the maximum intensity in *PAΔ1* and minimum in *PAΔ5*

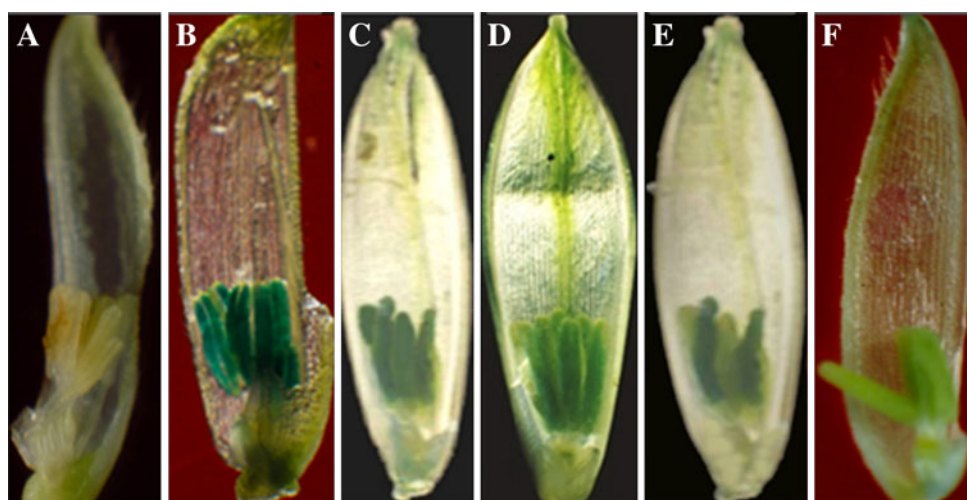
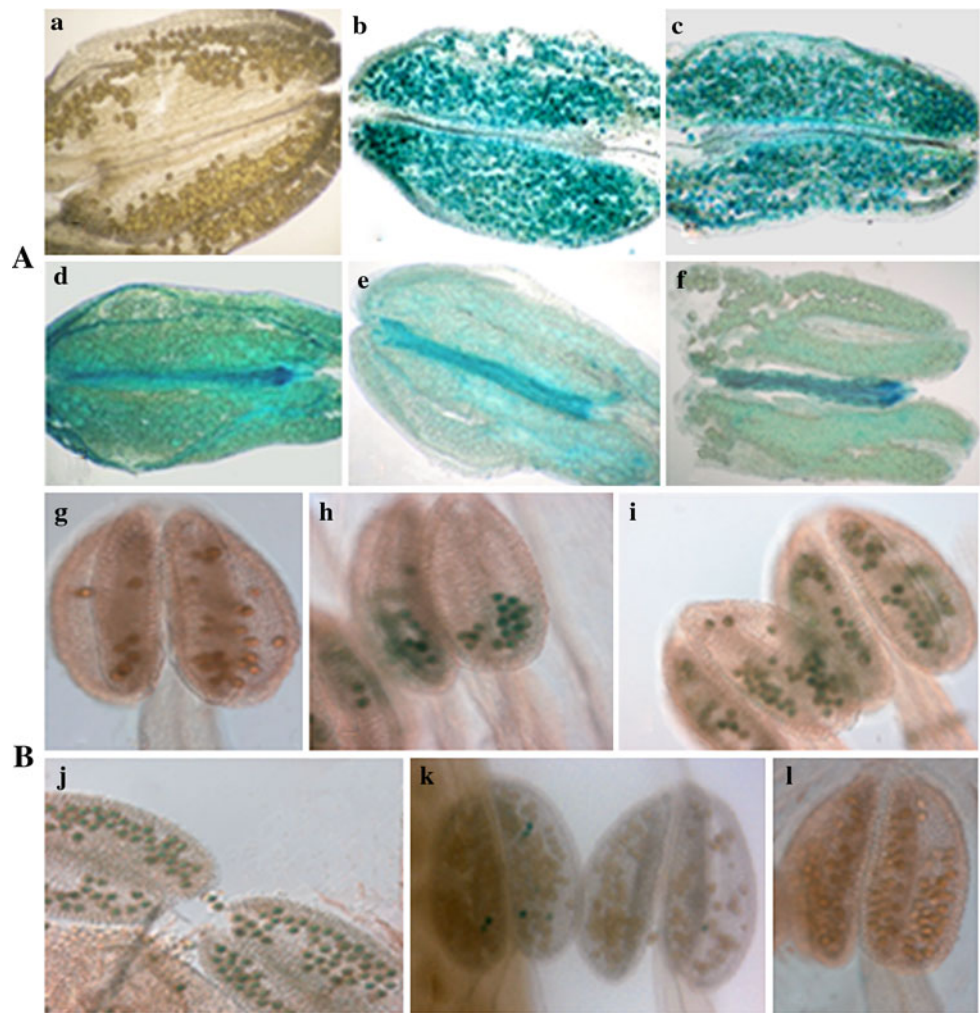


Fig. 3 Histochemical analysis of *GUS* expression in transgenic **a** rice and **b** *Arabidopsis* anthers containing promoter deletion constructs of *OSIPA* fused to *GUS* gene. Control anther (**a** and **g**), *PAA1* (**b** and **h**), *PAA2* (**c** and **i**), *PAA3* (**d** and **j**), *PAA4* (**e** and **k**), and *PAA5* (**f** and **l**)



be observed in any other organ of transgenic plants as well as in different explants (including anthers) of untransformed rice plants (Fig. 4). A similar trend was noticed in different *Arabidopsis* transformants containing *OSIPA* promoter deletions, but for *PAA4* and *PAA5* transformants which failed to show any detectable *GUS* activity (Fig. 3b). In *Arabidopsis*, the *OSIPA* promoter activity was detected during late stages of pollen development. *GUS* expression was absent in root, leaf, stem, sepals, petals, and gynoecium of transgenic *Arabidopsis* plants (data not shown). The untransformed wild-type plants did not show *GUS* activity in any of the organs tested. It was noticed that the intensity of *GUS*-specific blue color in anthers of transgenic rice was much higher than that of *Arabidopsis* (Fig. 3).

GUS Fluorometric Analysis to Determine *OSIPA* Promoter Strength

Quantitative *GUS* assays were performed to determine the strength of different *OSIPA* promoter fragments by

measuring the specific *GUS* activity in rice and *Arabidopsis*. Three to five homozygous lines for each construct of rice and *Arabidopsis* were used for quantification of *GUS* activity. Among *OSIPA* rice transformants, anthers of *PAA1* lines showed maximum *GUS* activity (18.16 ± 0.31 to 27.31 ± 0.50 nmole 4MU/h/mg of protein) when compared to other *OSIPA* promoter deletion lines. The *GUS* activity observed in the anthers of *PAA2*, *PAA3*, *PAA4*, and *PAA5* transformants varied from 13.50 ± 0.17 to 16.01 ± 0.37 , 11.26 ± 0.11 to 12.54 ± 0.27 , 9.10 ± 0.06 to 10.60 ± 0.18 , and 6.81 ± 0.62 to 8.52 ± 0.18 nmole 4MU/h/mg of protein, respectively (Fig. 5a). In *Arabidopsis*, maximum *GUS* activity was observed in the anthers of plants carrying *PAA1* (4.13 ± 0.13 to 6.84 ± 0.16) followed by *PAA2* (3.98 ± 0.12 to 4.31 ± 0.18), *PAA3* (2.71 ± 0.18 to 3.56 ± 0.26) and *PAA4* showed minimum (1.47 ± 0.05 to 1.7 ± 0.10) *GUS* activity. In the anthers of *PAA5*, though histochemical staining did not show any *GUS* expression, yet the fluorometric analysis revealed some (0.51 ± 0.11 to 0.85 ± 0.03) *GUS* activity (Fig. 5b). Moreover, sepals, petals, and gynoecium of *PAA1*, 2, 3,

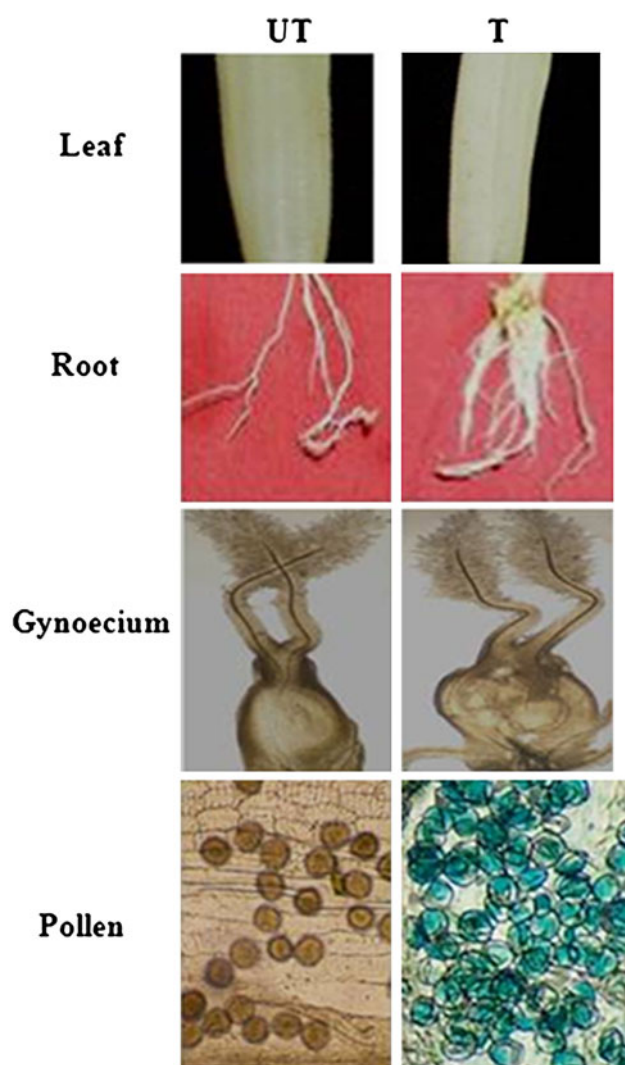


Fig. 4 Analysis of *OSIPA* promoter activity in different organs of untransformed control (UT) and $P\Delta 1$ transgenic (T) rice plants. GUS activity was analyzed in leaf, root, gynoecium, and pollen of control and rice transformants containing $P\Delta 1$ -*OSIPA* promoter. Control and transgenic (except pollen) plants did not show histochemical GUS staining in any of the organs tested

and 4 transformants and gynoecium alone in $P\Delta 5$ showed negligible fluorometric values in comparison with the values observed in anthers (Fig. 4S). The overall GUS-specific activity in the anthers of different *OSIPA* transgenic rice lines was 4-fold higher than that of *Arabidopsis* transformants (Fig. 5).

Discussion

Tissue-specific expression of a promoter is governed by the combinatorial action of various *cis*-acting elements present in its 5' region and the presence of different nuclear proteins interacting with these motifs. In this investigation,

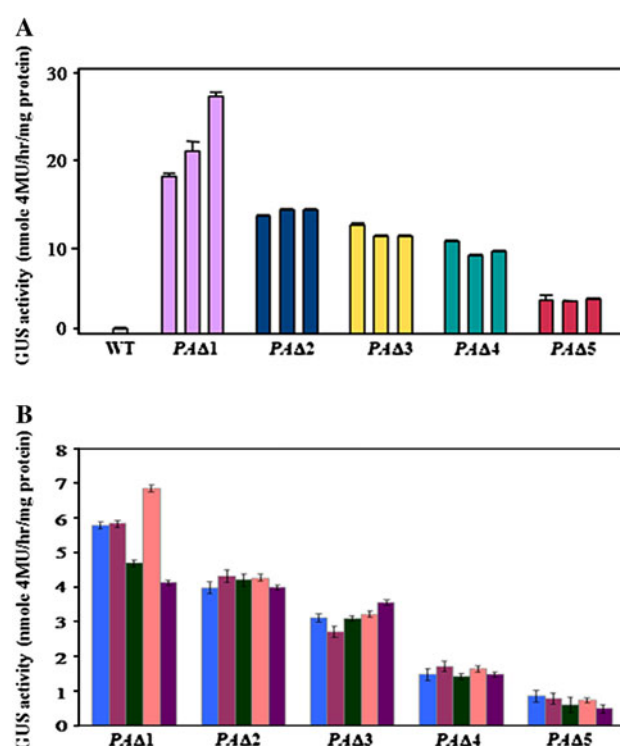


Fig. 5 **a** Fluorometric analysis of GUS activity in anthers of wild-type and transgenic rice plants harboring $P\Delta 1$, $P\Delta 2$, $P\Delta 3$, $P\Delta 4$, and $P\Delta 5$ constructs. The activity is expressed as nmole 4-methylumbelliferone/h/mg protein. Each column represents the mean GUS activity from three plants of three independent transgenic lines. Standard error bars are shown. **b** Fluorometric analysis of GUS activity in anthers of transgenic *Arabidopsis thaliana* plants containing $P\Delta 1$, $P\Delta 2$, $P\Delta 3$, $P\Delta 4$, and $P\Delta 5$ constructs. The activity is expressed as nmole 4-methylumbelliferone/h/mg protein. Each column represents the mean GUS activity from three plants of five independent transgenic lines. The value was obtained after subtracting any background specific GUS activity observed in anthers of wild-type *Arabidopsis* from the activity observed in anthers of transgenic *Arabidopsis* plants. Standard error bars are shown

OSIPA regulatory region has been functionally dissected by means of progressive 5' deletion analysis in homologous as well as in heterologous plant species. The *OSIPA* promoter has been shown to express within the anther specifically in the pollen. Although the deletion containing minimal 199 bp promoter region showed pollen-specific expression, yet the 1567 bp region carrying maximum number of in silico-identified elements is required for conferring high-level pollen-specific expression. Various *cis*-acting regulatory elements such as AGAAA, GTGA, and LAT enhancer elements (TGTGA & TGTGG) [54], TTTCT, ACGT, and AAAG involved in the pollen-specific expression were detected in the *OSIPA* promoter region (Table 1). Different pollen-specific *cis*-element motifs, viz., TGTGGTT (PB core motif) [24], AGAAA [55], TCCACCATA [56], and GTGA [31] were identified in anther-/pollen-specific promoters. Comprehensive

Table 1 Different *cis*-acting elements identified in the 1631 bp regulatory region of *Oryza sativa indica* pollen allergen gene (*OSIPA*) from −1567 to +64 bp

Name of the site (Sequence)	Position of the motifs	Function
ACGT MOTIF (ACGT)	−25, −127, −196, −1198, −1239, −1250, −1262, −1350	This motif is the target sequence for bZIP DNA-binding regulatory proteins
DOFCOREZM (AAAG)	−407, −433, −617, −1081, −1258, −1423, +21, +56	Binds dof proteins and enhances transcription in many promoters
POLLENILELAT52 (AGAAA)	−424, −1425	Transcriptional activation pollen-specific <i>cis</i> -regulatory elements which cooperate to achieve maximum levels of gene expression throughout pollen maturation.
GTGA MOTIF (GTGA)	−661, −668, −1371, −1376	Important in directing pollen-specific expression
Sequence motifs of the LAT enhancer element (TGTGA; TGTGG)	−662, −669, −1318 −1305, −1360	Required for specific expression of mature pollen

promoter analyses of pollen-specific genes were carried out in *LAT52*, *LAT56*, and *LAT59* tomato genes [23, 57]. Alignment of their promoter sequences revealed the presence of two key regulatory elements required for the late pollen gene expression. Minor variations were also found in the elements (LAT52/56) of pollen-specific promoters of *EIF4a8* [58], *G10* [31], *Npg1* [59], and *NTP303* [60] genes. LAT52/56 (TGTGGTTATATA) and LAT56/59 (GAAATTGTGA) boxes of *LAT* promoters were found essential for directing a high-level expression in the pollen [24]. Recently, promoters—which specifically expressed in the generative and sperm cells of the pollen grain—have been identified. The promoter of *LGC1* gene of lily, for example, could direct the reporter gene expression in the generative and sperm cells of transgenic tobacco [61], while *AtGEX2* promoter directed expression specifically in the sperm cells [62]. The ACGT core binding site of *LGC1*, *AtGEX2*, and *OsGEX2* promoters and AAAG core binding site of *AtGEX2*, *OsGEX2*, and *PtGEX2* promoters were shown to act as target sequences for bZIP DNA-binding regulatory proteins [63, 64] and for Dof transcription factors [65], respectively.

Deletions of *OSIPA* promoter were made keeping in view the systematic exclusion of different pollen-specific motifs (Fig. 1). Transgenic plants of rice and *Arabidopsis* disclosed pollen-specific GUS activity as evidenced by both histochemical and fluorometric assays. Varied levels of *GUS* expression were noticed in *PAA1*, *PAA2*, *PAA3*, *PAA4*, and *PAA5* rice transformants. Histochemical analysis of *GUS* expression at different developmental stages of pollen showed that *GUS* expression starts at the late P4 stage (~10 cm in size) of panicle development, followed by gradual increase as the flowers matured, and revealed maximum expression at P5 & P6 stages (dehiscid anthers). Similarly, the *OSIPA-Arabidopsis* transformants showed maximum *GUS* expression at the late stages of pollen development, corresponding to the 11 & 12 stages described by Sanders et al. [66]. Maximum *GUS* expression was

observed in the pollen of *PAA1*, *PAA2*, and *PAA3* *Arabidopsis* transformants, while *PAA4* and *PAA5* showed negligible/no *GUS* expression. Conversely, *GUS* activity was not detected in various other vegetative and reproductive organs of rice tested (Fig. 4). Since *OSIPA* is a native promoter of rice, the minimal promoter regions of *PAA4* and *PAA5* rice transformants were able to drive the *GUS* expression up to the pollen stage (Fig. 3a). However, the same *OSIPA* minimal regions were unable to drive any visible expression of *GUS* in the pollen of *Arabidopsis* (Fig. 3b). In rice, *GUS* expression was found highly specific to the pollen alone in *PAA1* and *PAA2*, presumably, because of the presence of suppressor elements in the longer promoter regions of *OSIPA*, causing the suppression of *OSIPA* activity in the connective tissues when compared to *PAA3*, *PAA4*, and *PAA5* transformants (Fig. 3a). The leaky expression of *GUS* observed in various other tissues of *Arabidopsis*, besides anthers, may be attributed to the monocot specificity of the *OSIPA* suppressor elements. A number of monocot-derived tissue-specific promoters, when tested in homologous and closely related transgenic plants showed expected levels of activity [67, 68]. However, in the heterologous systems, the promoter activity was less than expected, and was sometimes detectable even in other organs, suggesting that the regulatory sequences of monocots and dicots are different, and/or some sequence elements required for proper regulation are located outside the promoter fragments [68]. Mitsuhashi et al. [69] reported that the most efficient promoters in tobacco cells were not the same in rice cells owing to differences in the specificity of gene expression between monocot and dicot species.

Quantitative analysis of *GUS* activity in *OSIPA* transgenic rice and *Arabidopsis* plants amply suggest that the *OSIPA* promoter directs a high reporter gene activity in the pollen. Variation observed in the *GUS* activity in anthers of different transformants containing the same deletion construct is attributable to the insertional position effect. The average specific *GUS* activity observed in *PAA1*, *PAA2*,

PAA3, *PAA4*, and *PAA5* rice transformants were 22.19 ± 0.62 , 14.60 ± 0.24 , 11.69 ± 0.61 , 9.72 ± 0.14 , and 7.71 ± 0.30 nmole 4-MU/h/mg total protein, respectively (Fig. 5a). Decline in the specific GUS activity of different *OSIPA* deletion transformants indicates that the number and arrangement of pollen-specific regulatory motifs play critical role(s) in the determination of promoter strength and its specific expression. Earlier results indicated that *cis*-acting elements, responsible for the tissue specificity of pollen-specific genes, are located in the 5' flanking region adjacent to the transcription start point [21, 28, 55, 70]. It was reported that the deletion fragments of about -500 and -300 bp 5' regulatory region of rice *YY2* gene, fused to *GUS* coding sequence and expressed in rice and *Arabidopsis* plants, conferred anther-specific expression in both the systems. Whereas, plants carrying the smallest fragment of -200 bp *YY2* promoter region failed to show GUS activity. Based on these results, it was concluded that the *cis*-elements responsible for expression and tissue-specific regulation are present in the 300 bp region of the *YY2* promoter [71]. The rice arabinogalactan (*OSIAGP*) promoter also showed similar decreases in the specific GUS activity when the promoter region was deleted from -1000 to -300 bp, and a further deletion to -100 bp resulted in the complete loss of GUS activity [72]. Zhou et al. [73] carried out 5' deletion analysis of the potato *SBgLR* gene promoter and reported that several *cis*-regulatory motifs contribute to the promoter activity.

An overview of the results indicates that among *OSIPA* rice transformants, *PAA1* lines exhibit maximum pollen-specific GUS activity when compared to other transformants (Fig. 5a). The high-level GUS activity (27.31 ± 0.50 nmole 4-MU/h/mg protein) of *PAA1* may be attributed to the presence of maximum number of pollen-specific motifs, viz., 8 copies each of ACGT, AAAG, 4 copies of GTGA, 3 copies of TGTGA, and 2 copies each of TGTGG, AGAAA present in the *OSIPA* promoter (Table 1). Also, these multiple *cis*-acting elements of the full-length promoter most likely play crucial role for driving the maximum expression of *GUS* specifically in the pollen. When compared to *PAA1* transformants, *PAA2* and *PAA3* exhibited 1.70- to 2.17-fold decreased GUS activity, while *PAA4* and *PAA5* showed 2.57- to 3.20-fold decreased GUS activity. In *Arabidopsis* transformants, maximum GUS activity of 6.84 ± 0.16 nmole 4-MU/h/mg total protein was observed in *PAA1* anthers followed by *PAA2* and *PAA3* lines; while minimum activity was found in *PAA4* and *PAA5* transgenics. Apparently, deletion of sequences from the 5' region of *OSIPA* promoter resulted in the decreased number of pollen-specific motifs, thereby reducing the strength and specificity of the promoter expression. Similar pollen-specific *cis*-elements, viz., sequence motifs of enhancer elements (TGTGG, TGTGA)

of *LAT52/LAT56*, quantitative element (TGGTTA) of *LAT52*, pollen-specific activation element (AGAAA) of tomato *LAT52*, and late pollen gene element (GTGA) of tobacco *G10*, were found essential for their pollen-specific expression [31, 55, 56, 74].

Rice transformants, *PAA1*, *PAA2*, *PAA3*, *PAA4*, and *PAA5*, containing different fragments of *OSIPA* promoter exhibited varied levels of pollen-specific *GUS* expression. Whereas, *PAA4* and *PAA5* transformants, containing the minimal promoter regions, showed significant reduction in *GUS* expression. However, both the minimal promoter regions of *PAA4* and *PAA5* contained a few copies of in silico-identified motifs, viz., TTTCT, ACTG, AAAG, and AGAAA, to drive *GUS* expression up to the pollen stage. Absence of TGTGG & TGTGA enhancer elements, GTGA, and other potential pollen-specific motifs in the minimal promoter regions are responsible for reduction in the strength of *OSIPA* promoter as well as the specificity (Fig. 3a). Kyojuka et al. [68] reported that pollen-specific expression of maize *Adh1* gene requires a sequence element outside the promoter region in the untranslated leader sequences. Moreover, the putative promoters of *OsGEX2-japonica*, *OsGEX2-indica*, and *PtGEX2-Poplar*—each containing an AAAG sequence located upstream to the translational start site—were found essential for the generative cell-specific expression [62]. In *OSIPA*, most of the pollen-specific motifs were located upstream to the 5' transcriptional start site, while two copies of AAAG (transcription factor binding site) were identified between +21 and +56 bp in the untranslated leader sequence.

To sum up, the rice promoter *OSIPA*, containing all the pollen-specific motifs, yielded maximum pollen-specific *GUS* expression followed by other fragments of *OSIPA* in both rice and *Arabidopsis* systems. Histochemical and quantitative GUS analyses of transformants revealed that the promoter regions from -966 to -199 bp contain the necessary elements for expression in the pollen of rice and *Arabidopsis*, while other sequences present between -1567 and $+64$ bp amplify the expression levels. The overall quantitative GUS analysis revealed an approximate 4-fold increase in GUS activity in the pollen of transgenic rice as compared to the *Arabidopsis* transgenics. For the production of hybrid rice, development of stable male-sterile lines is an essential prerequisite. Since the 1060 bp region of *OSIPA* promoter contained all the key elements for conferring pollen-specific expression, it may be exploited as a potential candidate for the generation of male-sterile lines.

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