## ABSTRACT

## RICE PROMOTERS AND USES THEREOF

Three anther-specific promoters isolated from rice are provided herein. The promoters as described in the present invention have been shown to confer anther-specific expression in rice. The present invention provides a recombinant DNA expression cassette comprising the promoter as disclosed in the present invention. The present invention further provides a host cell comprising the recombinant DNA expression cassette and transgenic plant comprising the recombinant DNA expression cassette.

Application No: /DEL/2012



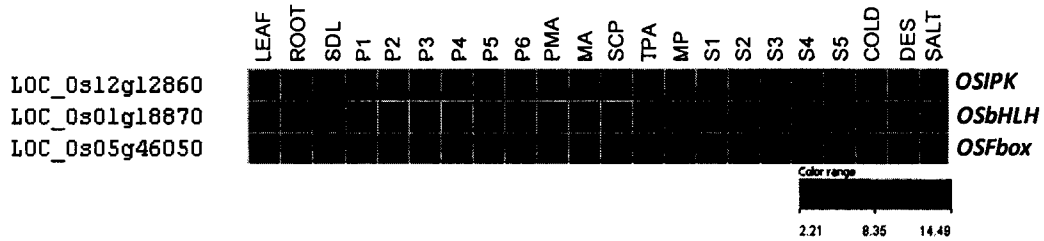



**Figure 1**

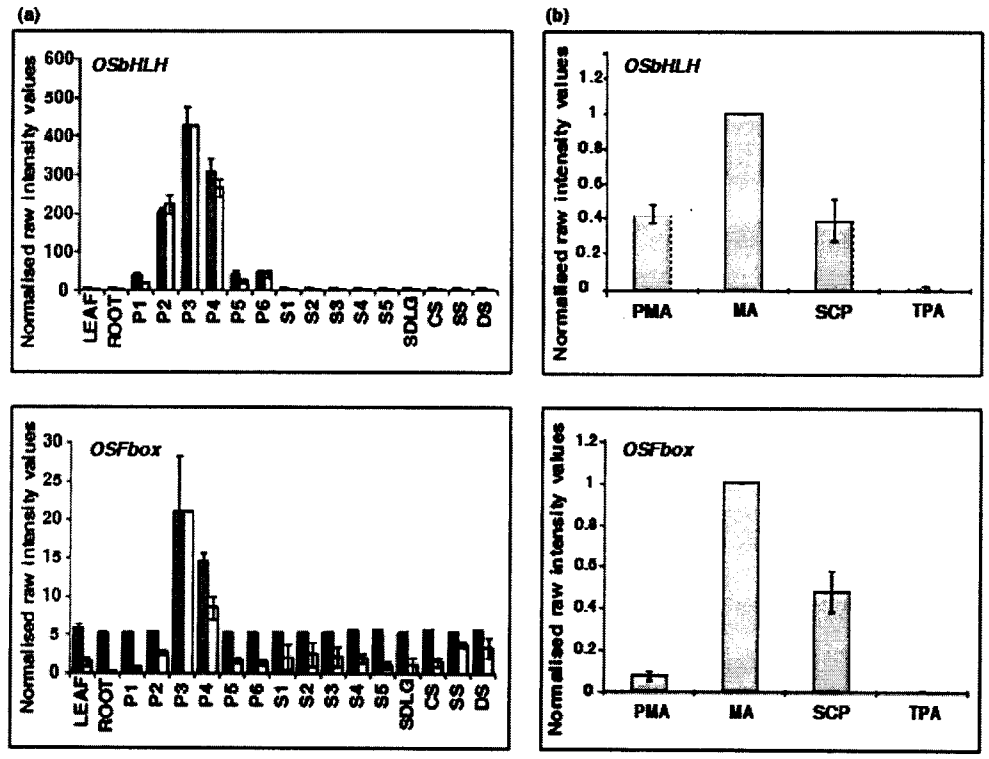



**Figure 2**

Rajani Jaiswal
IN/PA-1463
Agent for the Applicant


**Figure 3**

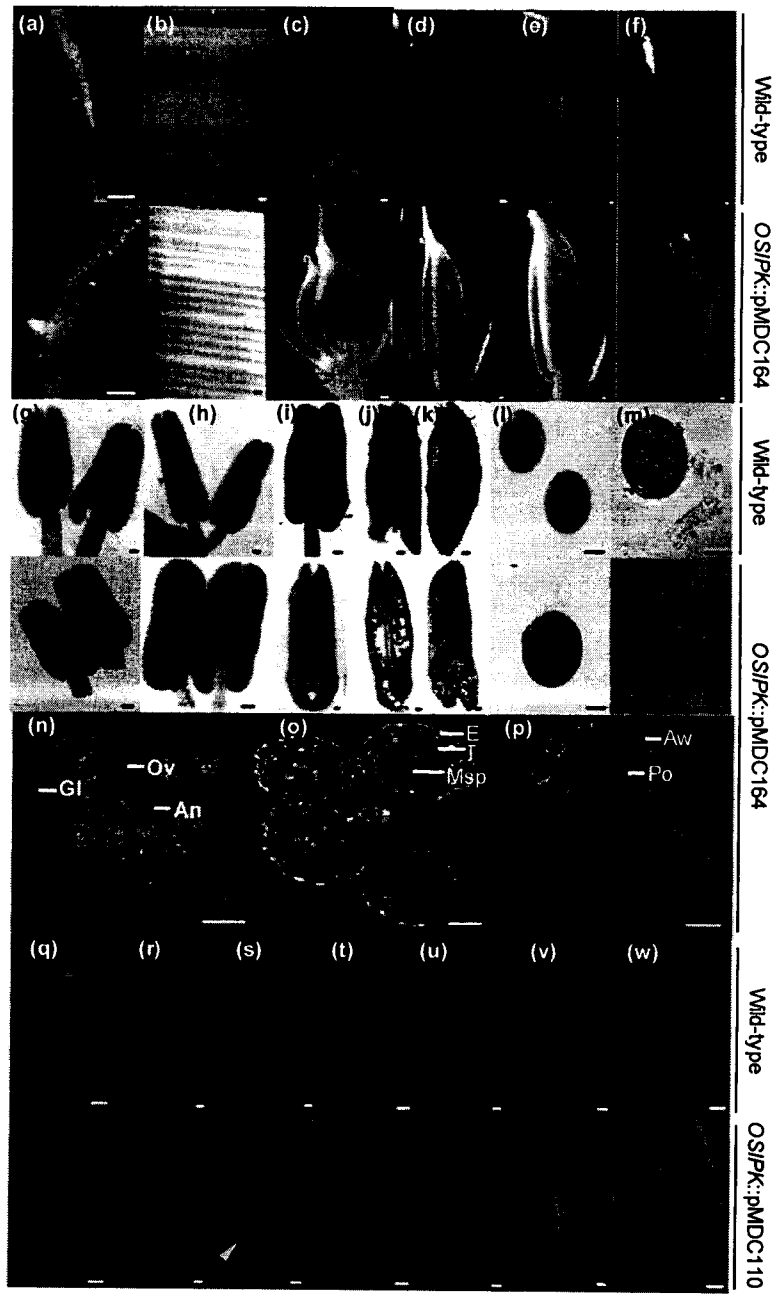



**Figure 4**

Rajani Jaiswal
IN/PA-1463
Agent for the Applicant

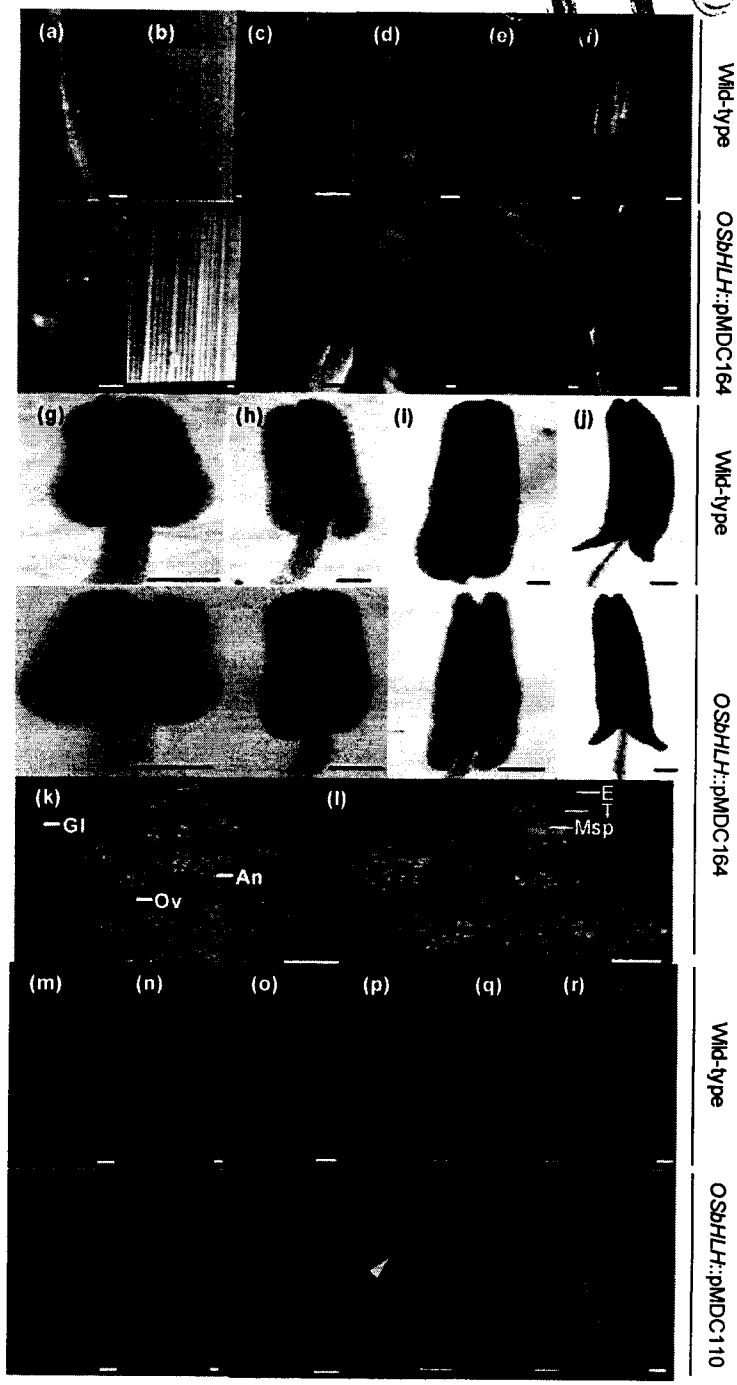



**Figure 5**

Rajani Jaiswal
IN/PA-1463
Agent for the Applicant

ORIGINAL



11 APR 2012

1103DEL12

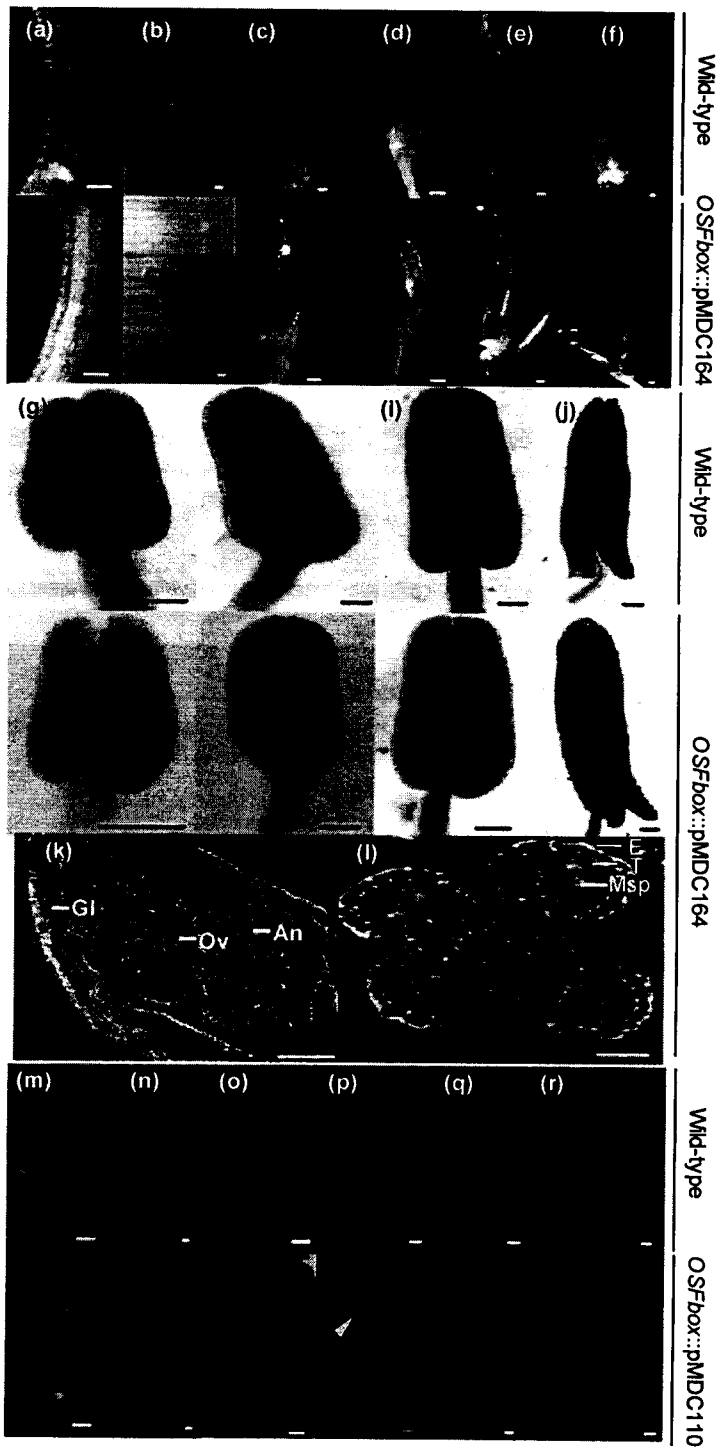



**Figure 6**

Rajani Jaiswal
IN/PA-1463
Agent for the Applicant

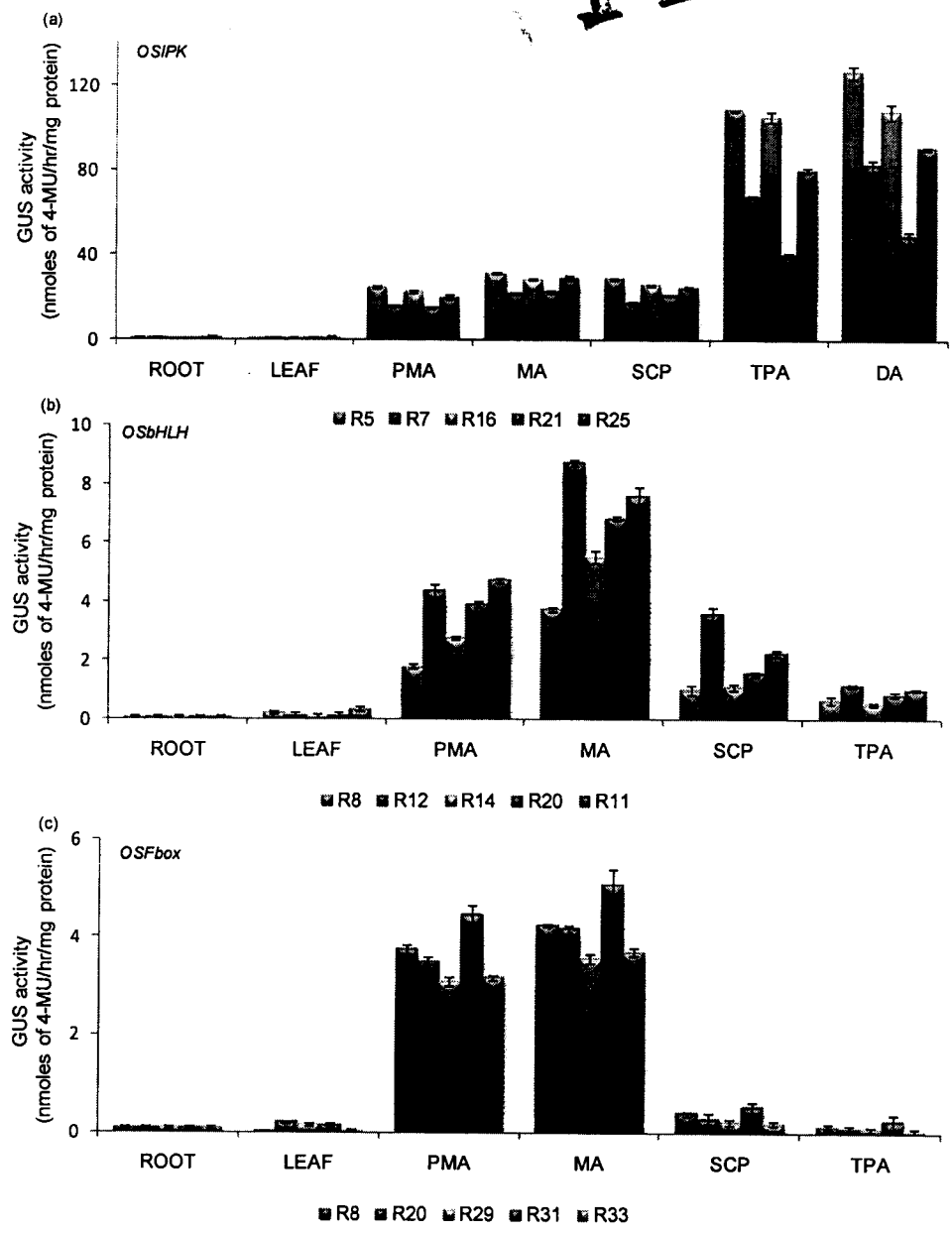



**Figure 7**

Rajani Jaiswal
IN/PA-1463
Agent for the Applicant

## FIELD OF INVENTION

The present invention relates to the field of plant molecular biology, in particular to a tissue specific promoter from rice.

## BACKGROUND OF THE INVENTION

Anther is an important plant organ responsible for male gamete formation. Development of anther begins with the initiation of founder cells also known as archesporial cells at the four corners of the anther primordium. These cells divide periclinally and give rise to parietal layer and sporogenous cell layers, which subsequently form various cell layers of anther namely, endothecium, middle layer, tapetum and microspore mother cells. Pollen mother cells undergo a meiotic and a mitotic division, giving rise to mature bicellular pollen. Tapetum is the most important cell layer that provides nutrients to the developing microspores and sporopollenin precursors for the formation of the pollen exine (Goldberg, R.B., Beals, T.P., and Sanders, P.M. (1993). Anther development: basic principles and practical applications. Plant Cell 5, 1217-1229; McCormick, S. (1993). Male gametophyte development. Plant Cell 5, 1265-1275). The normal development of anther housing the pollen is required for determining fertility. Therefore, it is essential to identify genes expressed in male gametophyte that regulate male fertility.

Rice is the main cereal crop in the world. Owing to its small genome that is completely sequenced and ease of genetic transformation, it is considered as the model cereal crop. Sequencing of the rice genome has unraveled a large number of genes that are required for successful completion of the plant's life cycle (International Rice Genome Sequencing Project (2005). The map-based sequence of the rice genome. Nature 436, 793–800.Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. (1987). GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6, 3901-3907). The task ahead is to annotate these genes for function and analyze their promoters to gain insights into the regulatory aspects of plant development. Each rice floret has six anthers arranged in a whorl around the pistil. Significant progress has been made to characterize the molecular repertoire of male organ in plants. Transcriptome analysis of rice anther has revealed large number of genes specifically or

preferentially expressed in anther (Deveshwar, P., Bovill, W.D., Sharma, R., Able, J.A. and Kapoor, S. (2011). Analysis of anther transcriptomes to identify genes contributing to meiosis and male gametophyte development in rice. BMC Plant Biol 11, 78).

To a great extent, expression of a gene can be correlated with the activity of its promoter. Promoters function like a "switch" and control the expression of gene. With the help of promoter-reporter gene fusion approach, the promoter regions of anther-/pollen-specific genes have also been investigated (Huang, Z., Gan, Z., He, Y., Li, Y., Liu, X. and Mu, H. (2011). Functional analysis of a rice late pollen-abundant UDP-glucose pyrophosphorylase (*OsUgp2*) promoter. Mol Biol Rep 7, 4291-4302). Site directed mutagenesis, 5' deletion analysis and gain of function experiments provide further information about the precise region required for conferring anther-/pollen-specificity.

The immediate challenge ahead is to isolate and functionally characterize anther-specific promoters for suitable spatial and temporal activity of target genes for genetic enhancement or cell/tissue ablation to control male fertility and investigate mechanisms of developmental regulation.

**BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS**

The following drawings form part of the present specification and are included to further illustrate aspects of the present invention. The invention may be better understood by reference to the drawings in combination with the detailed description of the specific embodiments presented herein.

Figure 1 shows heat map of *OSIPK*, *OSbHLH* and *OSFbox* genes drawn using microarray data from leaf, root, seedling (SDL), six panicle developmental stages (P1-P6), premeiotic anther (PMA), meiotic anther (MA), single cell pollen (SCP), trinucleate pollen containing anther (TPA), mature pollen (MP), five stages of seed development (S1-S5) and three abiotic stress conditions (cold, desiccation and salt).

Figure 2 shows validation of microarray based expression profiles by QPCR.

Figure 3 shows recombinant vector used to express *GUS/GFP* gene using *OSIPK, OSbHLH and OSFbox* promoter.

Figure 4 shows GUS histochemical assay of root (a), leaf (b), floret and anther of PMA (c, g), MA (d, h), SCP (e, i), TPA (f, j) stage, dehisced anther (k), pollen (l) and in vitro germinated pollen (m) from wild-type and *OSIPK*::pMDC164 transgenic rice plants. The scale bar is equal to 0.05 mm; Tissue-specific expression pattern of *OSIPK* URR::reporter gene construct in rice. GUS stained P4 stage floret and anthers of transgenic rice plants harboring *OSIPK* URR (SCP, n, o; dehisced anther, p), were sectioned using microtome and observed under dark field microscope (An; anther, Aw; anther wall, E; epidermis, Gl; glume, Msp; microsporocytes, Ov; ovary, Po; pollen, T; tapetum). Red colour under dark field microscope depicts *GUS* expression. The scale bar is equal to 0.1 mm; *GFP* expression pattern in various tissues of transgenic rice plants. Expression of *GFP* was monitored in root (q), leaf (r), floret of PMA (s), MA (t), SCP (u) and TPA stage (v) and dehisced anther (w) of wild-type and *OSIPK*::pMDC110 transgenic plants. The scale bar is equal to 0.05 mm.

Figure 5 shows spatio-temporal expression patterns of *OSbHLH*::*GUS/GFP* in transgenic rice. GUS histochemical assay of root (a), leaf (b), floret and anther of PMA (c, g), MA (d, h), SCP (e, i), TPA (f, j) stage from wild-type and *OSbHLH*::pMDC164 transgenic rice plants. The scale bar is equal to 0.05 mm; Transverse section of GUS stained floret of P3 stage of panicle (k) and MA (l) under dark field microscope are also shown (An, anther; E, epidermis; Gl, glume; Msp, microsporocytes; Ov, ovary; T, tapetum). The scale bar is equal to 0.1 mm; GFP-based analysis of *OSbHLH* URR activity in rice. *GFP* expression was analyzed in root (m), leaf (n), floret of PMA (o), MA (p), SCP (q) and TPA (r) of wild-type and *OSbHLH*::pMDC110 transgenic plants. The scale bar is equal to 0.05 mm.

Figure 6 shows localization of *OSFbox* URR activity in transgenic rice. GUSs histochemical assay of root (a), leaf (b), floret and anther of PMA (c, g), MA (d, h), SCP (e, i), TPA (f, j) stage from wild-type and *OSFbox*::pMDC164 transgenic rice plants. The scale bar is equal to 0.05 mm; Transverse section of GUS stained floret of P3 stage of panicle (k) and MA (l) under dark field microscope are also shown (An, anther; E, epidermis; Gl, glume; Msp, microsporocytes; Ov, ovary; T, tapetum). The scale bar is equal to 0.1 mm; *GFP* expression profile in various tissues of transgenic rice plants. *GFP* expression was investigated in root (m), leaf (n), floret of PMA (o),

MA (p), SCP (q) and TPA (r) of wild-type and *OSFbox*::pMDC110 transgenic plants. The scale bar is equal to 0.05 mm.

Figure 7 shows fluorometric analysis of GUS activity in anthers of transgenic rice plants containing (a) *OSIPK*::pMDC164 construct, (b) *OSbHLH*::pMDC164 construct and (c) *OSFbox*::pMDC164 construct. GUS activity was determined using protein extracts from roots, leaves, PMA, MA, SCP, TPA and dehisced anther (in case of *OSIPK*::pMDC164) of transgenic plants as well as wild-type plants.

**BRIEF DESCRIPTION OF SEQUENCE LISTING**

SEQ ID NO: 1 shows nucleotide sequence of *OSIPK* promoter (1568 bp)

SEQ ID NO: 2 shows nucleotide sequence of *OSbHLH* promoter (1927 bp)

SEQ ID NO: 3 shows nucleotide sequence of *OSFbox* promoter (1959 bp)

SEQ ID NO: 4 shows cDNA sequence of *OSIPK* (LOC_Os12g12860) (1692 bp)

SEQ ID NO: 5 shows cDNA sequence of *OSbHLH* (LOC_Os01g18870) (1140 bp)

SEQ ID NO: 6 shows cDNA sequence of *OSFbox* (LOC_Os05g46050) (1143 bp)

SEQ ID NO: 7 shows amino acid sequence of OSIPK (563 a.a.)

SEQ ID NO: 8 shows amino acid sequence of OSbHLH (379 a.a.)

SEQ ID NO: 9 shows amino acid sequence of OSFbox (380 a.a.)

SEQ ID NO: 10 shows RT Forward Primer of *OSbHLH* (19 bp)

SEQ ID NO: 11 shows RT Reverse Primer of *OSbHLH* (22 bp)

SEQ ID NO: 12 shows RT Forward Primer of *OSFbox* (21 bp)

SEQ ID NO: 13 shows RT Reverse Primer of *OSFbox* (18 bp)

SEQ ID NO: 14 shows RT Forward Primer of *ACTIN* (21 bp)

SEQ ID NO: 15 shows RT Reverse Primer of *ACTIN* (20 bp)

SEQ ID NO: 16 shows Forward Primer of *OSIPK* promoter (23 bp)

SEQ ID NO: 17 shows Reverse Primer of *OSIPK* promoter (22 bp)

SEQ ID NO: 18 shows Forward Primer of *OSbHLH* promoter (24 bp)

SEQ ID NO: 19 shows Reverse Primer of *OSbHLH* promoter (22 bp)

SEQ ID NO: 20 shows Forward Primer of *OSFbox* promoter (20 bp)

SEQ ID NO: 21 shows Reverse Primer of *OSFbox* promoter (19 bp)

SEQ ID NO: 22 shows M13 Forward Primer (18 bp)

SEQ ID NO: 23 shows M13 Reverse Primer (18 bp)

SEQ ID NO: 24 shows Primer of *OSIPK* (22 bp)

SEQ ID NO: 25 shows Primer of *OSbHLH* (21 bp)

SEQ ID NO: 26 shows Primer of *OSFbox* (24 bp)

SEQ ID NO: 27 shows *GUS* Reverse Primer (22 bp)

SEQ ID NO: 28 shows *GFP* Reverse Primer (24 bp)

SEQ ID NO: 29 shows pollen-specific element GTGANTG10 (4 bp)

SEQ ID NO: 30 shows pollen-specific element POLLEN1LeLAT52 (5 bp)

SEQ ID NO: 31 shows extra element QELEMENTZMZM13 (6 bp)

**DETAILED DESCRIPTION OF THE INVENTION**

Those skilled in the art will be aware that the invention described herein is subject to variations and modifications other than those specifically described. It is to be understood that the invention described herein includes all such variations and modifications. The invention also includes all such steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

*Definitions*

For convenience, before further description of the present invention, certain terms employed in the specification, examples and appended claims are collected here. These definitions should be read in light of the remainder of the disclosure and understood as by a person of skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art.

The articles "a", "an" and "the" are used to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article.

The terms "comprise" and "comprising" are used in the inclusive, open sense, meaning that additional elements may be included. It is not intended to be construed as "consists of only". Similarly, "comprise", "comprises", "comprising", "include", "includes", and "including" are interchangeable and not intended to be limiting.

The use of "or" means "and/or" unless stated otherwise.

The terms "nucleotide of interest", "gene of interest" and "target gene" are used interchangeably and not intended to be limiting.

The term "gene" is used broadly to refer to any segment of nucleic acid associated with a biological function. Thus, genes include coding sequences and/or the regulatory sequences required for their expression. For example, gene refers to a nucleic acid fragment that expresses mRNA or functional RNA, or encodes a specific protein, and which includes regulatory sequences. Genes also include non-expressed DNA segments that, for example, form recognition sequences for other proteins. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and may include sequences designed to have desired parameters.

A "transgene" refers to a gene that has been introduced into the genome by transformation and is stably maintained. Transgenes may include, for example, genes that are either heterologous or homologous to the genes of a particular plant to be transformed. Additionally, transgenes may comprise native genes inserted into a non-native organism, or chimeric genes. The term "endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene refers to a gene not normally found in the host organism but that is introduced by gene transfer.

"Coding sequence" refers to a DNA or RNA sequence that codes for a specific amino acid sequence and excludes the non-coding sequences. It may constitute an "uninterrupted coding sequence", i.e., lacking an intron, such as in a cDNA or it may include one or more introns bounded by appropriate splice junctions. An "intron" is a sequence of RNA which is contained in the primary transcript but which is removed

through cleavage and re-ligation of the RNA within the cell to create the mature mRNA that can be translated into a protein.

The term "transformation" means the transfer of nucleic acid (i.e., a nucleotide polymer) into a cell. As used herein, the term "genetic transformation" means the transfer and incorporation of DNA, especially recombinant DNA, into a cell.

The term "transgenic" means cells, cell cultures, plants, and progeny of plants which have received a foreign or modified gene by one of the various methods of transformation, wherein the foreign or modified gene is from the same or different species than the species of the plant receiving the foreign or modified gene.

"Operably-linked" refers to the association of nucleic acid sequences on single nucleic acid fragment so that the function of one is affected by the other. For example, a regulatory DNA sequence is said to be "operably linked to" or "associated with" a DNA sequence that codes for an RNA or a polypeptide if the two sequences are situated such that the regulatory DNA sequence affects expression of the coding DNA sequence (i.e., that the coding sequence or functional RNA is under the transcriptional control of the promoter). Coding sequences can be operably-linked to regulatory sequences in sense or antisense orientation. The particular sequences interact either directly or indirectly to carry out an intended function, such as mediation or modulation of gene expression.

The term "promoter" means the sequence of a DNA molecule that directs transcription of a downstream gene to which it is operatively linked or that, when fused to a particular gene and introduced into a cell, causes expression of the gene at a level higher than is possible in the absence of a DNA sequence. Such promoters can be full length promoter or active fragments thereof. "Active fragment" means a fragment that has at least about 0.1%, preferably to at least about 10%, and more preferably at least about 25% of the activity of a reference promoter sequence as tested via methods known to those skilled in the art, for detecting promoter activity, *e.g.*, measurement of GUS reporter gene levels.

"Tissue-specific promoter" refers to regulated promoters that are not expressed in all plant cells but only in one or more cell types in specific organs (such as leaves, seeds or flowers), specific tissues (such as embryo or cotyledon), or specific cell types (such as

leaf parenchyma or seed storage cells). These also include promoters that are temporally regulated, such as in early or late embryogenesis, during fruit ripening in developing seeds or fruit, in fully differentiated leaf, or at the onset of senescence.

"Expression" refers to the transcription and/or translation of an endogenous gene, ORF or portion thereof, or a transgene in plants. For example, in the case of antisense constructs, expression may refer to the transcription of the antisense DNA only. In addition, expression refers to the transcription and stable accumulation of sense (mRNA) or functional RNA. Expression may also refer to the production of protein.

"Specific expression" is the expression of gene products which is limited to one or a few plant tissues (spatial limitation) and/or to one or a few plant developmental stages (temporal limitation). It is acknowledged that hardly a true specificity exists: promoters seem to be preferably switch on in some tissues, while in other tissues there can be no or only little activity. This phenomenon is known as leaky expression. However, with specific expression in this invention is meant preferable expression in one or a few plant tissues.

The "expression pattern" of a promoter (with or without enhancer) is the pattern of expression levels which shows where in the plant and in what developmental stage transcription is initiated by said promoter. Expression patterns of a set of promoters are said to be complementary when the expression pattern of one promoter shows little overlap with the expression pattern of the other promoter.

"Expression cassette" as used herein means a DNA sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operably linked to a nucleotide sequence of interest, which is optionally operably linked to termination signals and/or other regulatory elements. An expression cassette may also comprise sequences required for proper translation of the nucleotide sequence. The coding region usually codes for a protein of interest but may also code for a functional RNA of interest, for example antisense RNA or a nontranslated RNA, in the sense or antisense direction. The expression cassette may also be one, which is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. An expression cassette may be assembled entirely extracellularly (e.g., by

recombinant cloning techniques). However, an expression cassette may also be assembled using in part endogenous components. For example, an expression cassette may be obtained by placing (or inserting) a promoter sequence upstream of an endogenous sequence, which thereby becomes functionally linked and controlled by said promoter sequences. Likewise, a nucleic acid sequence to be expressed may be placed (or inserted) downstream of an endogenous promoter sequence thereby forming an expression cassette. The expression of the nucleotide sequence in the expression cassette may be under the control of a constitutive promoter or of an inducible promoter which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, the promoter can also be specific to a particular tissue or organ or stage of development. The gene constructs described herein can further include enhancers, either translation or transcription enhancers, as may be required. These enhancer regions are well known to the persons skilled in the art and can include the ATG initiation codon and adjacent sequences. The sequence can also be derived from the promoter selected to express the gene and can be specifically modified to increase translation of the mRNA.

"Vector" is defined to include, inter alia, any plasmid, cosmid, phage or Agrobacterium binary vector in double or single stranded linear or circular form which may or may not be self transmissible or mobilizable, and which can transform prokaryotic or eukaryotic host either by integration into the cellular genome or exist extrachromosomally (e.g. autonomous replicating plasmid with an origin of replication). Specifically included are shuttle vectors by which is meant a DNA vehicle capable, naturally or by design, of replication in two different host organisms, which may be selected from actinomycetes and related species, bacteria and eukaryotic (e.g. higher plant, mammalian, yeast or fungal cells).

Preferably the nucleic acid in the vector is under the control of, and operably linked to, an appropriate promoter or other regulatory elements for transcription in a host cell such as a microbial, e.g. bacterial, or plant cell. The vector may be a bi-functional expression vector which functions in multiple hosts. In the case of genomic DNA, this may contain its own promoter or other regulatory elements and in the case of cDNA

this may be under the control of an appropriate promoter or other regulatory elements for expression in the host cell.

"Cloning vectors" typically contain one or a small number of restriction endonuclease recognition sites at which foreign DNA sequences can be inserted in a determinable fashion without loss of essential biological function of the vector, as well as a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance, hygromycin resistance or ampicillin resistance. Various cloning vectors are available in the prior art.

A "transgenic plant" is a plant having one or more plant cells that contain an expression vector.

"Plant tissue" includes differentiated and undifferentiated tissues or plants, including but not limited to roots, stems, shoots, leaves, pollen, seeds, tumor tissue and various forms of cells and culture such as single cells, protoplast, embryos, and callus tissue. The plant tissue may be in plants or in organ, tissue or cell culture.

The gene constructs described herein can further include a 3' untranslated (or terminator) region that contains a polyadenylation signal and other regulatory signals capable of effecting mRNA processing or gene expression.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein.

The present invention provides a tissue specific promoter isolated from rice wherein the promoter is responsible for conferring tissue-specific expression in the floral male organ tissues. The floral male organ tissues include the stamen, filament, anthers, pre-meiotic anthers, meiotic anthers, anthers containing uni-nucleate pollen and pollen. The present invention also provides a recombinant DNA expression cassette, a recombinant vector and host cell comprising one or mere genes of interest under the control of the tissue specific promoter disclosed in the present invention. The present invention

further provides a method of regulating expression of one or more genes in plants using the tissue specific promoter disclosed in the present invention.

The promoters of the present invention can be used further for manipulating various processes involved in anther development and can be exploited for biotechnological applications like generating male-sterile plants for hybrid seed production or transgene containment. The promoters of the present invention are isolated from rice plant and confer anther-specific expression. The present invention further provides a method for transformation of plant using the promoters as disclosed in the present invention, wherein the promoter in the transgenic plant induces expression of the operably linked reporter/target gene. The present invention further provides transgenic plants comprising the promoter in combination with any target gene, wherein the transgenic plants express the respective promoter:gene combination in different tissues of anther only.

The recombinant vectors as described in the present invention may comprise selection marker gene, reporter gene, enhancer element, poly (A) sequence, ribosomal binding sequence or transit peptide DNA sequence.

The present invention provides anther-specific promoters that are effectively functional in monocotyledonous plants. If one or more target genes causing cell ablation is linked to the promoter and introduced into plants, accordingly, transgenic male sterile monocotyledonous plants, such as rice, can be produced.

The present invention provides promoters having nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof.

The present invention also provides a recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof.

The present invention further provides a process for transformation of a plant with a vector comprising a promoter having the nucleotide sequence selected from the group

consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof, wherein the promoter is operably linked to a target gene or gene of interest. The transgenic plants comprising the promoter (and the downstream sequences) showed the activity of promoter (measured by expression of the downstream gene) only in the various tissues of anther. Further, the transgenic plants comprising the DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof shows anther-specific expression.

In an embodiment of the present invention, there is provided a recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof, wherein the promoter is operably linked to a target gene or gene of interest, such as a reporter gene, an antibiotic resistance gene or an agronomically important gene.

In another embodiment of the present invention, there is provided a recombinant DNA vector comprising the recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof, wherein the promoter is operably linked to a target gene or gene of interest, such as a reporter gene, an antibiotic resistance gene or an agronomically important gene.

In yet another embodiment of the present invention, there is provided a host cell comprising the recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof, wherein the promoter is operably linked to a target gene or gene of interest, such as a reporter gene, an antibiotic resistance gene or an agronomically important gene.

In still another embodiment of the present invention, there is provided a host cell comprising the recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof, wherein the promoter is operably linked to a target gene or gene of interest, such as a reporter gene, an antibiotic resistance gene or an agronomically important gene, wherein the host cell is selected from a group consisting of *E. coli*, yeast and plant cell.

An embodiment of the present invention provides a process of expressing an anther-specific promoter in a plant, said process comprising providing a recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof, wherein the promoter is operably linked to a target gene or gene of interest, such as a reporter gene, an antibiotic resistance gene or an agronomically important gene; incorporating the recombinant DNA expression cassette into a plant cell to produce a transgenic plant cell under conditions such that the target nucleotide sequence of interest is expressed in the transgenic plant cell; identifying said transgenic plant cell expressing said target nucleotide sequence specifically in anther; and regenerating the transgenic plant cell into a transgenic plant.

In another embodiment of the present invention, there is provided a process of expressing an anther-specific promoter in a plant, said process comprising providing a recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof, wherein the promoter is operably linked to a target gene or gene of interest, such as a reporter gene, an antibiotic resistance gene or an agronomically important gene; incorporating the recombinant DNA expression cassette into a plant cell to produce a transgenic plant cell under conditions such that the target nucleotide sequence of interest is expressed in the transgenic plant cell; identifying said transgenic plant cell expressing said target

nucleotide sequence specifically in anther; and regenerating the transgenic plant cell into a transgenic plant, wherein the transgenic plant is a monocot plant or a dicot plant.

In yet another embodiment of the present invention, there is provided a process of expressing an anther-specific promoter in a plant, said process comprising providing a recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof, wherein the promoter is operably linked to a target gene or gene of interest, such as a reporter gene, an antibiotic resistance gene or an agronomically important gene; incorporating the recombinant DNA expression cassette into a plant cell to produce a transgenic plant cell under conditions such that the target nucleotide sequence of interest is expressed in the transgenic plant cell; identifying said transgenic plant cell expressing said target nucleotide sequence specifically in anther; and regenerating the transgenic plant cell into a transgenic plant, wherein the transgenic plant is a food plant or horticultural plant.

In still another embodiment of the present invention, there is provided a process of expressing an anther-specific promoter in a plant, said process comprising providing a recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof, wherein the promoter is operably linked to a target gene or gene of interest, such as a reporter gene, an antibiotic resistance gene or an agronomically important gene; incorporating the recombinant DNA expression cassette into a plant cell to produce a transgenic plant cell under conditions such that the target nucleotide sequence of interest is expressed in the transgenic plant cell; identifying said transgenic plant cell expressing said target nucleotide sequence specifically in anther; and regenerating the transgenic plant cell into a transgenic plant, wherein the monocot is selected from a group consisting of rice, wheat, sorghum, pearl millet and maize.

In an embodiment of the present invention, there is provided a process of expressing an anther-specific promoter in a plant, said process comprising providing a recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof, wherein the promoter is operably linked to a target gene or gene of interest, such as a reporter gene, an antibiotic resistance gene or an agronomically important gene; incorporating the recombinant DNA expression cassette into a plant cell to produce a transgenic plant cell under conditions such that the target nucleotide sequence of interest is expressed in the transgenic plant cell; identifying said transgenic plant cell expressing said target nucleotide sequence specifically in anther; and regenerating the transgenic plant cell into a transgenic plant, wherein the dicot is selected from a group consisting of cotton, tomato, *Brassica*, tobacco, tomato, pea, soybean, peanut, chickpea, *Arabidopsis*, mulberry, *Petunia,* papaya and pigeon pea.

Another embodiment of the present invention provides a transgenic plant comprising a recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof.

Yet another embodiment of the present invention provides a transgenic progeny obtained from the transgenic plant comprising a recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof.

Still another embodiment of the present invention provides a transgenic seed obtained from the transgenic plant comprising a recombinant DNA expression cassette comprising a promoter capable of expressing one or more target gene or gene of interest in an anther-specific manner, wherein the nucleotide sequence of the promoter is

selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and complement thereof.

Very few anther-specific promoters are worked out in rice system. The anther specificity of the promoters as disclosed in the present invention can prove to be of great utility in driving downstream genes for generating male-sterile plants. Thus, the promoters of the present invention are of utmost importance in generation of hybrid rice plants or even other monocot crop plants e.g. wheat, barley and maize.

With an objective to characterize anther-specific promoter from Rice, promoters of three anther-specific genes, namely *OSIPK*, *OSbHLH* and *OSFbox*, were selected for functional characterization on the basis of differential expression profiles of these genes. The expression profile of these genes was studied using microarray data of two vegetative (mature leaf and 7-day-old seedling root), six panicle (P1-P6), premeiotic anther (PMA), meiotic anther (MA), single cell pollen stage anther (SCP), trinucleate pollen (TPA), mature pollen (MP) and five seed (S1-S5) stages as well as under three abiotic stress conditions (salt, cold and desiccation). Of the three genes, *OSIPK* showed maximum expression during mature pollen stage. *OSbHLH* was found to express in all the developmental stages of panicle and anthers, however, maximum transcript accumulation was observed in the meiotic anthers. Similarly, *OSFbox* also showed maximum expression during meiotic anther stage, with detectable levels during P3 and P4 stages of panicle development. All the three genes were found to be significantly down-regulated during the leaf, root and seed development, and also under stress conditions (Figure 1). Microarray-based expression profile of *OSbHLH* and *OSFbox* gene was verified by real-time PCR. The result showed good correlation between the two approaches confirming the expression during panicle development (Figure 2a). Expression pattern of these genes was also studied during different stages of anther development namely premeiotic (PMA), meiotic (MA), single cell pollen (SCP) and trinucleate pollen (TPA) by real-time PCR and maximum expression was observed in meiotic stage anthers (Figure 2b).

The *OSIPK*, *OSbHLH* and *OSFbox* promoter regions described herein were also searched for the presence of anther-/pollen-specific *cis* regulatory elements using PLACE database (http://www.dna.affrc.go.jp/PLACE/). Two pollen-specific elements

namely GTGANTG10 (GTGA; as set forth in SEQ ID NO: 29) and POLLEN1LeLAT52 (AGAAA; as set forth in SEQ ID NO: 30) were identified in the regulatory region of all the three genes. In the regulatory region of *OSFbox* gene, an extra QELEMENTZMZM13 (AGGTCA; as set forth in SEQ ID NO: 31) was also detected.

The regulatory region of *OSIPK* (1.568 kb), regulatory region of *OSbHLH* (1.927 kb) and regulatory region of *OSFbox* (1.959 kb) were PCR amplified from genomic DNA of *Oryza sativa* L. ssp. indica var IR64 using the primers as set forth in SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20 and SEQ ID NO: 21, respectively and cloned upstream to *GUS* or *GFP* reporter gene into Gateway® cloning binary vector pMDC-164-GUS and pMDC-110-GFP to obtain recombinant vector *OSIPK*::pMDC164/110, *OSbHLH*:: pMDC164/110 and *OSFbox*::pMDC164/110. The recombinant vectors comprising the *OSIPK, OSbHLH and OSFbox* promoters operably linked to *GUS/GFP* genes were mobilized into *Agrobacterium tumefaciens* strain AGL1 by triparental mating. Transformation of *indica* rice (*Oryza sativa* L. var. PB 1, IET10364) was performed.

GUS histochemical analysis of the various tissues/organs of *OSIPK*::pMDC164 transgenic lines revealed the expression to be localized in PMA, MA, SCP and TPA (Figure 4g-j). The pollen of dehisced anthers were found to have the maximum expression (Figure 4l). Pollen tubes of germinated pollen grains of transgenic lines also showed visible *GUS* expression, while no blue colour was detected in pollen tubes of wild-type anther (Figure 4m). Further histochemical characterization revealed that in developing florets the GUS activity was mainly confined to anthers, while no detectable expression was seen in glumes or ovary (Figure 4n). In case of SCP, the expression was localized in tapetum, connective tissue, microsporocytes, with weak expression also detectable in epidermal cells (Figure 4o). Transverse sections of dehisced anthers revealed maximum GUS activity in the pollen, however, weak expression was also detectable in cells of the anther wall (Figure 4p). In case of GFP expressing lines also, the *OSIPK* URR-driven expression was found in PMA, MA, SCP and TPA stage florets (Figure 4s-v). Similar to the *OSIPK*::GUS lines, the maximum *GFP* expression was observed in the pollen of dehisced anther (Figure 4w). Other organs namely root and

leaf of transgenic line harboring *OSIPK*::pMDC164/110 construct did not show any *GUS/GFP* expression. In the wild-type plant, *GUS/GFP* expression was not detectable in any of the tissues/organs tested.

In *OSbHLH*::pMDC164 transgenic rice plants, GUS histochemical staining was observed only in the anthers of PMA, MA and SCP stage florets (Figure 5c-e). Further, transverse sectioning of GUS stained P3 stage floret of *OSbHLH*::pMDC164 transgenic rice plants showed GUS activity only in the anthers, while no expression was detected in lemma, palea or ovary (Figure 5k). Within MA, maximum expression was observed in tapetum and microsporocytes, while weak expression was also detected in epidermal cells and connective tissue (Figure 5l). In transgenic rice plants harboring *OSbHLH*::pMDC110, *GFP* expression was observed in PMA, MA and SCP stage florets (Figure 5o-q). Like *OSbHLH*::GUS lines, maximum *GFP* expression was observed during the MA stage. Transgenic lines harboring *OSbHLH*::pMDC164/110 construct did not show any *GUS/GFP* expression in the other assayed organs/tissues namely root, leaf and TPA. Also, in wild-type plant, no *GUS/GFP* expression was observed in any of the tissues/organs analyzed.

In *OSFbox*::pMDC164 plants, the maximum GUS expression was observed in PMA and MA (Figure 6g, h). Further histochemical staining of sectioned GUS stained P2 stage floret showed maximum GUS activity only in the anthers (Figure 6k). In MA, a weak GUS activity was detected in microsporocytes, while very faint expression was detected in tapetum, epidermis and connective tissue (Figure 6l). In transgenic lines carrying *OSFbox*::pMDC110, *OSFbox* regulatory region drove *GFP* expression in PMA and MA. (Figure 6o, p). Maximum expression was observed during the MA stage as also observed in GUS expressing lines. A blue colored end product was not detected in root, leaf, SCP and TPA of transgenic lines carrying *OSFbox*::pMDC164/110 constructs, and in any of the organs/tissues evaluated for wild-type plants.

In order to find the relative strength of *OSIPK*, *OSbHLH* and *OSFbox* promoters in rice, different organs [root, leaf, premeiotic, meiotic, single cell pollen, trinucleate pollen containing anthers and dehisced anthers (only in case of *OSIPK*::pMDC164 rice transgenic plants)] of transgenic rice plants, were subjected to detailed fluorometric analysis. The activity of GUS (expressed in nmol of 4-MU/mg protein/h) for the wild-

type plants was deducted from the activity of transgenic plants to obtain the final values. In case of *OSIPK*::pMDC164 lines, the highest GUS activity was observed in dehisced anthers (49.01±2.36 to 126.90±2.66), followed by TPA (40.67±0.73 to 108.86±0.36). The PMA, MA and SCP gave almost similar fluorometric values, which were approximately 4-fold lower than that of the dehisced anther values (Figure 7a). In plants carrying *OSbHLH*::pMDC164 construct, the highest GUS activity was observed in the meiotic anther (3.74±0.08 to 8.73±0.12).A 2-fold lower activity was detected in premeiotic anther (1.81±0.09 to 4.73±0.08) followed by single cell pollen stage anthers (1.01±0.14 to 3.61±0.19). In TPA, though the histochemical staining did not show any *GUS* expression, yet the fluorometric analysis revealed some GUS activity (Figure 7b). In *OSFbox*::pMDC164 transgenic rice plants, the highest GUS activity was observed in MA (3.70±0.08 to 5.08±0.32) followed by PMA (3.08±0.10 to 4.47±0.18; Figure 7c). No significant GUS activity was observed in case of root and leaf of *OSIPK/OSbHLH/OSFbox*::pMDC164 transgenic plants.

The promoter disclosed herein is a significant advance in directing anther-specific expression. One of the most important applications of anther-specific promoter is in the generation of male-sterile plants for hybrid seed production.

Although the subject matter has been described in considerable detail with reference to certain preferred embodiments thereof, other embodiments are possible.

## EXAMPLES

The disclosure will now be illustrated with working examples, which is intended to illustrate the working of disclosure and not intended to take restrictively to imply any limitations on the scope of the present disclosure. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein.

**Example 1**

**Plant material and growth conditions**

*Oryza sativa indica* var. PB1 seeds were obtained from Indian Agricultural Research Institute, New Delhi and Indian Agricultural Research Institute Sub-station, Karnal, Haryana. The seeds were surface disinfected with 0.1% mercuric chloride ($HgCl_2$) for 10 minutes and, after thorough washing with reverse-osmosis (RO) water, were incubated overnight in RO water in culture room maintained at 28+2°C, 16 hours light (100-125 µmolm-2s-1)/8 hours dark conditions. Subsequent day, seeds were spread over a meshed float and grown hydroponically at 28±1°C in culture room with a daily photoperiodic cycle of 14 hours light and 10 hours dark. Cold, salt and desiccation stress treatments were given to the 1 week-old seedlings. As a control, 1week-old seedlings were maintained in water in 100 ml beaker for 3 hours. Root tissue was also harvested from 1 week-old seedlings. Rice panicle, seeds and anthers of various developmental stages and mature leaves were collected from field-grown rice (*O. sativa* ssp. *indica* var. IR64; obtained from Indian Agricultural Research Institute, New Delhi and Indian Agricultural Research Institute Sub-station, Karnal, Haryana) and were frozen in liquid nitrogen. Panicle stages include P1, 0–3 cm; P2, 3–5 cm; P3, 5–10 cm; P4, 10–15 cm; P5, 15–22 cm and P6, 22–30 cm (Arora, R., Agarwal, P., Ray, S., Singh, A.K., Singh, V.P., Tyagi, A.K. and Kapoor, S. (2007). MADS-box gene family in rice: genome-wide identification, organization and expression profiling during reproductive development and stress. BMC Genomics, 8, 242). Seeds of different developmental stages were tagged from the day of pollination (DAP) and collected. The seed stages were named as S1 (0-2 DAP), S2 (3-4 DAP), S3 (5-10 DAP), S4 (11-20 DAP), and S5 (21-29 DAP) (Agarwal, P., Arora, R., Ray, S., Singh, A.K., Singh, V.P., Takatsuji, H., Kapoor, S. and Tyagi, A.K. (2007). Genome-wide identification of C2H2 zinc-finger gene family in rice and their phylogeny and expression analysis. Plant Mol Biol 65, 467-485). Anthers were categorized into different developmental stages based on floret length and named as premeiotic, meiotic, single cell pollen and trinucleate pollen (Deveshwar, P., Bovill, W.D., Sharma, R., Able, J.A. and Kapoor, S. (2011). Analysis of anther transcriptomes to identify genes contributing to meiosis and male gametophyte development in rice. BMC Plant Biol 11, 78). Anthers were then dissected

under dissecting microscope Leica MZ 12.5 (Gmbh, Germany) and kept in a microcentrifuge tube (MCT) containing Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) and stored at -70°C.

**Example 2**

**Microarray and Quantitative Real Time PCR analysis**

In order to study the transcript abundance of *OSIPK, OSbHLH* and *OSFbox* genes, microarray and QPCR were performed. Total RNA was isolated using TRIzol method (Invitrogen Life Technologies, Carlsbad, CA). Quantitative estimation of RNA was carried out by using Nanodrop® ND-1000 Spectrophotometer. The cDNA was synthesized by using High capacity cDNA archive kit (Applied Biosystems). The cDNA sequence of *OSIPK* (LOC_Os12g12860) is as set forth in SEQ ID NO: 4. The cDNA sequence of *OSbHLH* (LOC_Os01g18870) is as set forth in SEQ ID NO: 5. The cDNA sequence of *OSFbox* (LOC_Os05g46050) is as set forth in SEQ ID NO: 6. The amino acid sequence of *OSIPK* is as set forth in SEQ ID NO: 7. The amino acid sequence of *OSbHLH* is as set forth in SEQ ID NO: 8. The amino acid sequence of *OSFbox* is as set forth in SEQ ID NO: 9. Figure 1 shows heat map of *OSIPK, OSbHLH* and *OSFbox* genes drawn using microarray data from leaf, root, seedling (SDL), six panicle developmental stages (P1-P6), premeiotic anther (PMA), meiotic anther (MA), single cell pollen (SCP), trinucleate pollen containing anther (TPA), mature pollen (MP), five stages of seed development (S1-S5) and three abiotic stress conditions (cold, desiccation and salt). Of the three genes, *OSIPK* showed maximum expression during mature pollen stage. *OSbHLH* was found to express in all the developmental stages of panicle and anthers, however, maximum transcript accumulation was observed in the meiotic anthers. Similarly, *OSFbox* also showed maximum expression during meiotic anther stage, with detectable levels during P3 and P4 stages of panicle development. All the three genes were found to be significantly down-regulated during the leaf, root and seed development, and also under stress conditions.

Microarray-based expression profile of *OSbHLH* and *OSFbox* gene was verified by Quantitative Real Time PCR. QPCR analysis was performed for *OSbHLH* and *OSFbox* by using the primers having the nucleotide sequence as set forth in SEQ ID NOs: 10-15

as provided in Table 1. For all the samples, *ACTIN* was used as an internal control. The final values obtained were further extrapolated to match the amplitude of profiles with those obtained from microarray analysis. Two biological replicates and three technical were taken for the each sample to be analyzed. Figure 2 shows validation of microarray based expression profiles for by QPCR. The result showed good correlation between the microarray and QPCR approaches confirming the expression during panicle development (Figure 2a). Expression pattern of these genes was also studied during different stages of anther development namely premeiotic (PMA), meiotic (MA), single cell pollen (SCP) and trinucleate pollen (TPA) by real-time PCR and maximum expression was observed in meiotic stage anthers (Figure 2b).

**Example 3**

**Construction of binary vectors for plant transformation**

Genomic DNA of Rice, IR64 was used as a template for amplification of regulatory regions of 1568 bp, 1927 bp and 1959 bp upstream to the translation initiation site of *OSIPK*, *OSbHLH* and *OSFbox* genes. The sequence of primers used for amplification of *OSIPK*, *OSbHLH* and *OSFbox* regulatory regions are as set forth in SEQ ID NOs: 16-21 as provided in Table 2. Amplified fragments were first cloned in TOPO® vector (pENTR vector). The cloning was confirmed by restriction digestion (*Not*I and *Bgl*II for *OSIPK*, *Apa*I for *OSbHLH* and *Hind*III for *OSFbox*) and by sequencing with primers having the nucleotide sequence as set forth in SEQ ID NOs: 22-26. The nucleotide sequence of *OSIPK* promoter is as set forth in SEQ ID NO: 1. The nucleotide sequence of *OSbHLH* promoter is as set forth in SEQ ID NO: 2. The nucleotide sequence of *OSFbox* promoter is as set forth in SEQ ID NO: 3. Recombination-mediated gateway cloning was used to clone *OSIPK*, *OSbHLH* and *OSFbox* upstream regulatory regions (URRs) in binary vectors pMDC-110-GFP and pMDC-164-GUS (Curtis, M.D. and Grossniklaus, U. (2003). A gateway cloning vector set for high-throughput functional analysis of genes in planta. Plant Physiol 133, 462-469). Amplified fragments were first cloned in TOPO® vector (pENTR vector). The cloning was confirmed by restriction digestion (*Not*I and *Bgl*II for *OSIPK*, *Apa*I for *OSbHLH* and *Hind*III for *OSFbox*) and automated DNA sequencing. After sequence verification, the regulatory regions were subcloned in the binary vectors pMDC164 (GUS) and pMDC110 (GFP) by

recombination-mediated cloning method to obtain recombinant vector *OSIPK*::pMDC164/110, *OSbHLH*:: pMDC164/110 and *OSFbox*::pMDC164/110. Figure 3 shows recombinant vector used to express *GUS/GFP* gene using *OSIPK, OSbHLH and OSFbox* promoter.

**Example 4**

***Agrobacterium* mediated transformation**

The constructed plasmids were mobilized into *Agrobacterium tumefaciens* strain AGL1 by triparental mating method. Various culture media, glasswares, plasticwares and tissue culture tools used during the experiments were sterilized by autoclaving at 121°C and 15 lb/sq. inch pressure for 15 minutes. Antibiotics and AAM medium (used in rice transformation) were filter-sterilized using disposable syringe filter of 0.2 μm pore size (Advanced Microdevices Pvt. Ltd., India).

Rice Transformation

Rice transformation was performed using protocol by Mohanty et al. (1999) [Mohanty, A., Sharma, N.P. and Tyagi, A.K. (1999). *Agrobacterium*-mediated high frequency transformation of an elite *indica* rice variety Pusa Basmati and transmission of the transgene to R2 progeny. Plant Sci 147, 125-135] with some modifications. Dehusked seeds of rice Pusa Basmati 1 (PB1) were obtained from Indian Agricultural Research Institute, New Delhi and Indian Agricultural Research Institute Sub-station, Karnal, Haryana and taken in an autoclaved flask and washed with 70% ethanol for 30 seconds with constant shaking. Ethanol was decanted and 100 ml of 0.1% $HgCl_2$ and approximately 3-4 drops of teepol were added to the flask. After 10 minutes of constant shaking, the seeds were rinsed with autoclaved MQ water five times. Finally, the seeds were soaked in autoclaved MQ water and kept overnight in culture room in dark for imbibition. Water from the overnight soaked seeds was removed and the seeds were decanted in a Petri plate with autoclaved tissue paper. The seeds were then inoculated on 2MS medium. The composition of the MS medium is as provided in Table 3. The MS medium further comprises 30 g/l sucrose, 2 mg/l 2,4-D and 4 g/l Phytagel. The final pH of the medium was adjusted to 5.8. The plates were incubated in dark in the culture room maintained at 28±2°C for callus induction.

On 21st day, embryogenic calli were subcultured. Unwanted roots and shoots were removed and the calli were transferred on 2MSCA plates comprising 2MS medium and 1 g/l casamino acid, pH 5.8. The plates were incubated in dark at 28±2°C in culture room. The same day, *Agrobacterium tumefaciens* AGL1 strain harboring various promoter constructs was inoculated in YEM medium with respective antibiotics and grown at 28°C and 200 rpm for 3 days.

Co-cultivation was carried out on 25th day after rice seed inoculation. A day prior to it (day 24), secondary culture was raised in 50 ml YEM medium using 1 ml of primary culture. Overnight raised *Agrobacterium* culture was pelleted at 4,000 rpm, for 15 minutes at 4°C. Supernatant was discarded and the pellet was resuspended in 30 ml filter-sterilized AAM medium. The composition of the AAM medium is as provided in Table 4. AAM medium further comprises 68.5 g/l sucrose, 36 g/l glucose, 500 mg/l casamino acids and 400 µM acetosyringone. The bacterial suspension was poured in a sterile flask and the calli were dropped into it one by one followed by incubation on shaker at 80 rpm for half an hour. After 30 minutes, the calli were decanted on autoclaved tissue paper placed in Petri plates. The calli were then transferred on 2MSAS medium [2 MSCA with 10 g/l glucose and 400 µM acetosyringone) and incubated in dark at 28±2°C. One plate with untransformed calli was kept as a control.

On day 28 post-seed inoculation or 3rd day after co-cultivation, the calli were transferred to a sterile flask and washed once with 100 ml autoclaved RO water. This was followed by wash with 100 ml of RO water containing 200 µl of 500 µg/ml cefotaxime at 80 rpm for 45 minutes at room temperature. The next two washes were given with water containing 200 µl of 500 µg/ml cefotaxime for 15 minutes each. The calli were finally rinsed with water containing 100 µl of 500 µg/ml cefotaxime for 15 minutes. The calli were then blotted on autoclaved tissue paper and plated on selection medium 2MSCACH (2MSCA with 250 mg/l cefotaxime and 50 mg/l hygromycin). The calli were subjected to three rounds of selection of 15 days each. Calli resistant to hygromycin were transferred to regeneration medium MSRNH (MS salts and vitamins, 30 g/l sucrose, 1 g/l casamino acids, 1 mg/l BAP, 250 mg/l cefotaxime, 50 mg/l hygromycin, 4 g/l Phytagel, pH 5.8) and kept in a culture room maintained at 28±2°C with a 16 hours/8 hours light/dark cycle. The light source used was fluorescent tube

(Philips Champion 40 W/54, Philips India Limited, India) providing light at a fluence rate of 100-125 $\mu molm^{-2}s^{-1}$. The calli were then subjected to 2-3 rounds of subculture on regeneration medium for a period of 15 to 20 days (under light) each till the shoots emerged from the calli. After the shoots attained a height of 4-5 cm, they were transferred to MSB (MS salts and vitamins, 20 g/l sucrose and 2 g/l Phytagel, pH 5.8) for root formation. After root proliferation, the plants were transferred to Rice Growth Medium or Yoshida's medium (Yoshida, S., Forno, D.A., Cook, J.H. and Gomes, K.A. (1976). Routine procedure for growing rice plants in culture solution. In: Laboratory Manual for Physiological Studies of Rice. Yoshida S, Forno DA, Cock JH and Gomez KA, (eds), The International Rice Research Institute, Manila, The Philippines, pp 61) for 10-15 days for hardening. The composition of Yoshida's medium is provided in Table 5.

The plants were then transferred to pots containing a 1:1 mixture of soil and organic manure in the greenhouse maintained at 28±2°C, 16 hours light/8 hours dark conditions, 70-75% relative humidity. Natural sunlight was supplemented with photosynthetically active radiation tube lights (Lumilux plus, India). Plants were watered regularly till the grains begin to mature. After the plants had completely dried, the seeds were harvested and stored in a cool and dry place.

**Example 5**

**Analysis of Transgenic Lines**

Confirmation of transgenics by PCR

Genomic DNA was isolated from hygromycin resistant rice plants by essentially following the method given by Dellaporta et al. (1983) [Dellaporta, S., Wood, J. and Hicks, J.B. (1983). A plant DNA minipreparation, version II. Plant Mol Biol Rep 1, 19-21]. Transgenic nature of rice plants carrying *OSIPK*::110/164, *OSbHLH*::110/164 and *OSFbox*::110/164 was confirmed by using forward URR-specific and reverse *GUS-/GFP*-specific primers having the nucleotide sequence as set forth in SEQ ID NOs: 24-28.

Histochemical analysis of β-glucuronidase activity

Histochemical assay for *GUS* gene expression in different organs of transgenic plant was carried out according to protocol given by Chaudhury et al. (1995) [Chaudhury, A., Maheshwari, S. and Tyagi, A. (1995). Transient expression of *gus* gene in intact seed embryos of *Indica* rice after electroporation-mediated gene delivery. Plant Cell Rep 14, 215-220]. The plant organs were dissected and incubated in GUS histochemical buffer (50 mM sodium phosphate pH 7.0, 50 mM EDTA pH 8.0, 0.5 mM $K_3Fe(CN)_6$, 0.5 mM $K_4Fe(CN)_6$, 0.1% Triton® X-100, 1 mM X-Gluc, 20% methanol) at 37°C for 16 to 24 hours. A blue coloured end product was produced as GUS enzyme cleaved the substrate, X-gluc. The chlorophyll was removed from the tissue with a solution of acetone:ethanol (1:3) and the same was observed under Leica DM 5000 B fluorescence microscope (Leica, Germany). The photographs of the stained region were taken by using a camera attached to the microscope.

*In vitro* pollen germination

Pollen germination assay was performed using protocol by Han et al. (2006) [Han, M.J., Jung, K.H., Yi, G., Lee, D.Y. and An, G. (2006). *Rice Immature Pollen 1* (*RIP1*) is a regulator of late pollen development. Plant Cell Physiol 47, 1457-1472]. Pollen grains from dehisced rice anthers were placed on a Petri plate at 35°C for 2 hours in a pollen germination medium (1 mM $CaCl_2$, 1 mM KCl, 0.8 mM $MgSO_4$, 1.6 mM $H_3BO_3$, 30 µM $CaSO_4$, 0.03% casein, 0.3% 2-(*N*-morpholino) ethanesulfonic acid, 10% sucrose and 12.5% polyethylene glycol). Germinated pollen grains were then stained in the GUS histochemical buffer at 37°C for 16 hours and observed under Leica DM 5000 B fluorescence microscope (Leica, Germany).

Histological analysis for cell/tissue specificity

In order to study the cell-/tissue-specific expression pattern under the control of *OSIPK*, *OSbHLH* and *OSFbox* promoter, microtome sections of GUS stained rice floret were prepared. GUS stained tissue was immersed in FAE fixative [(10% formaldehyde (Sigma, USA), 5% glacial acetic acid (Qualigens, India) and 50% ethanol (Merck, Germany)] and incubated at 4°C overnight. This was followed by dehydration of tissue through an ethanol series (30%, 50%, 70% and 80%). After this, the ethanol was replaced by tert-butanol (Qualigens, India) via an ethanol-butanol series (80%-20%,

70%-30%, 30%-70%, 20%-80%. 10%-90%). The tissue was embedded in paraplast wax. About 8-10 μm thick sections of the embedded tissue were cut using a microtome (Rattansons, India). The slides were deparaffinized, mounted in DPX mountant and covered with a coverslip. The slides were then observed under Leica DM 5000 B fluorescence microscope (Leica, Germany) in DIC (Differential interference contrast) and dark field.

GFP visualization in transgenic plants

Expression of *GFP* in various organs of rice transgenic plants was observed under Leica DM 5000 B fluorescence microscope (Leica, Germany).

Figure 4 shows GUS histochemical assay of root (a), leaf (b), floret and anther of PMA (c, g), MA (d, h), SCP (e, i), TPA (f, j) stage, dehisced anther (k), pollen (l) and *in vitro* germinated pollen (m) from wild-type and *OSIPK*::pMDC164 transgenic rice plants. The scale bar is equal to 0.05 mm; Tissue-specific expression pattern of *OSIPK* URR::reporter gene construct in rice. GUS stained P4 stage floret and anthers of transgenic rice plants harboring *OSIPK* URR (SCP, n, o; dehisced anther, p), were sectioned using microtome and observed under dark field microscope (An; anther, Aw; anther wall, E; epidermis, Gl; glume, Msp; microsporocytes, Ov; ovary, Po; pollen, T; tapetum). Red colour under dark field microscope depicts *GUS* expression. The scale bar is equal to 0.1 mm; *GFP* expression pattern in various tissues of transgenic rice plants. Expression of *GFP* was monitored in root (q), leaf (r), floret of PMA (s), MA (t), SCP (u) and TPA stage (v) and dehisced anther (w) of wild-type and *OSIPK*::pMDC110 transgenic plants. The scale bar is equal to 0.05 mm. GUS histochemical analysis of the various tissues/organs of *OSIPK*::pMDC164 transgenic lines revealed the expression to be localized in PMA, MA, SCP and TPA (Figure 4g-j). The pollen of dehisced anthers were found to have the maximum expression (Figure 4l). Pollen tubes of germinated pollen grains of transgenic lines also showed visible *GUS* expression, while no blue colour was detected in pollen tubes of wild-type anther (Figure 4m). Further histochemical characterization revealed that in developing florets the GUS activity was mainly confined to anthers, while no detectable expression was seen in glumes or ovary (Figure 4n). In case of SCP, the expression was localized in tapetum, connective tissue, microsporocytes, with weak expression also detectable in

epidermal cells (Figure 4o). Transverse sections of dehisced anthers revealed maximum GUS activity in the pollen, however, weak expression was also detectable in cells of the anther wall (Figure 4p). In case of GFP expressing lines also, the *OSIPK* URR-driven expression was found in PMA, MA, SCP and TPA stage florets (Figure 4s-v). Similar to the *OSIPK*::GUS lines, the maximum *GFP* expression was observed in the pollen of dehisced anther (Figure 4w). Other organs namely root and leaf of transgenic line harboring *OSIPK*::pMDC164/110 construct did not show any *GUS/GFP* expression. In the wild-type plant, *GUS/GFP* expression was not detectable in any of the tissues/organs tested.

Figure 5 shows spatio-temporal expression patterns of *OSbHLH*::*GUS/GFP* in transgenic rice. GUS histochemical assay of root (a), leaf (b), floret and anther of PMA (c, g), MA (d, h), SCP (e, i), TPA (f, j) stage from wild-type and *OSbHLH*::pMDC164 transgenic rice plants. The scale bar is equal to 0.05 mm; Transverse section of GUS stained floret of P3 stage of panicle (k) and MA (l) under dark field microscope are also shown (An, anther; E, epidermis; Gl, glume; Msp, microsporocytes; Ov, ovary; T, tapetum). The scale bar is equal to 0.1 mm; GFP-based analysis of *OSbHLH* URR activity in rice. *GFP* expression was analyzed in root (m), leaf (n), floret of PMA (o), MA (p), SCP (q) and TPA (r) of wild-type and *OSbHLH*::pMDC110 transgenic plants. The scale bar is equal to 0.05 mm. In *OSbHLH*::pMDC164 transgenic rice plants, GUS histochemical staining was observed only in the anthers of PMA, MA and SCP stage florets (Figure 5c-e). Further, transverse sectioning of GUS stained P3 stage floret of *OSbHLH*::pMDC164 transgenic rice plants showed GUS activity only in the anthers, while no expression was detected in lemma, palea or ovary (Figure 5k). Within MA, maximum expression was observed in tapetum and microsporocytes, while weak expression was also detected in epidermal cells and connective tissue (Figure 5l). In transgenic rice plants harboring *OSbHLH*::pMDC110, *GFP* expression was observed in PMA, MA and SCP stage florets (Figure 5o-q). Like *OSbHLH*::GUS lines, maximum *GFP* expression was observed during the MA stage. Transgenic lines harboring *OSbHLH*::pMDC164/110 construct did not show any *GUS/GFP* expression in the other assayed organs/tissues namely root, leaf and TPA. Also, in wild-type plant, no *GUS/GFP* expression was observed in any of the tissues/organs analyzed.

Figure 6 shows localization of *OSFbox* URR activity in transgenic rice. GUSs histochemical assay of root (a), leaf (b), floret and anther of PMA (c, g), MA (d, h), SCP (e, i), TPA (f, j) stage from wild-type and *OSFbox*::pMDC164 transgenic rice plants. The scale bar is equal to 0.05 mm; Transverse section of GUS stained floret of P3 stage of panicle (k) and MA (l) under dark field microscope are also shown (An, anther; E, epidermis; Gl, glume; Msp, microsporocytes; Ov, ovary; T, tapetum). The scale bar is equal to 0.1 mm; *GFP* expression profile in various tissues of transgenic rice plants. *GFP* expression was investigated in root (m), leaf (n), floret of PMA (o), MA (p), SCP (q) and TPA (r) of wild-type and *OSFbox*::pMDC110 transgenic plants. The scale bar is equal to 0.05 mm. In *OSFbox*::pMDC164 plants, the maximum GUS expression was observed in PMA and MA (Figure 6g, h). Further histochemical staining of sectioned GUS stained P2 stage floret showed maximum GUS activity only in the anthers (Figure 6k). In MA, a weak GUS activity was detected in microsporocytes, while very faint expression was detected in tapetum, epidermis and connective tissue (Figure 6l). In transgenic lines carrying *OSFbox*::pMDC110, *OSFbox* regulatory region drove *GFP* expression in PMA and MA. (Figure 6o, p). Maximum expression was observed during the MA stage as also observed in GUS expressing lines. A blue colored end product was not detected in root, leaf, SCP and TPA of transgenic lines carrying *OSFbox*::pMDC164/110 constructs, and in any of the organs/tissues evaluated for wild-type plants.

Fluorometric analysis of β-glucuronidase activity

The activity of GUS protein was measured following the protocol described by Jefferson et al. (1987) [Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6: 3901-3907] with few modifications (Chaudhury, A., Maheshwari, S.C. and Tyagi, A.K. (1993). Transient expression of electroporated gene in leaf protoplasts of *indica* rice and influence of template topology and vector sequences. Physiol Plant 89, 842-846). Frozen tissue (100 mg) was homogenized in GUS extraction buffer (50 mM sodium phosphate buffer pH 7.0, 10 mM disodium EDTA, 0.1% Triton® X-100, 0.1% N-lauryl sarcosine and 10 mM β-mercaptoethanol). The concentration of unknown protein sample was estimated using Bradford reagent as per manufacturer instructions.

Bradford's dye was prepared by dissolving 100 mg of Coomasie Blue G250 in 50 ml ethanol with constant stirring. To this solution, 100 ml of *ortho*-phosphoric acid was added and stirred for 45 minutes in dark. The volume of the solution was raised to 1 liter with RO water and filtered through Whatman filter paper (125 mm discs) followed by storage in a dark colored bottle. A stock of 1 mg/ml BSA stock solution was diluted to concentrations ranging from 2-20 µg. The final volume of dilution was made to 100 µl by using 0.15 M NaCl solution. Subsequently, 5 ml Bradford's dye was added and contents were mixed thoroughly. The absorbance was recorded after 5 minutes of incubation at 595 nm using a spectrophotometer (U-2810, Hitachi, Japan). A standard curve was plotted. The concentration of unknown protein sample was estimated by aliquoting 10 µl of protein sample and raising the volume to 100 µl by 0.15 M NaCl. Further, 1.5 ml of Bradford's dye was added to the unknown protein sample. These contents were mixed well and absorbance was recorded at 595 nm. The quantity of the unknown protein sample was calculated extrapolating the values using the standard curve.

Quantitative GUS assay was performed, by incubating 6 µg of protein in GUS assay buffer (2 mM MUG dissolved in GUS extraction buffer) for 15 to 17 hours. After desired incubation, 0.2 M $Na_2CO_3$ (stop buffer) was added to terminate the reaction. The relative fluorescence was measured in a silica quartz cuvette employing a DNA Quant$^{TM}$ 200 flourometer (Hoefer Pharmacia Biotech, USA). The specific activity of GUS was expressed as nmol of 4-MU (mg protein)$^{-1}$ h$^{-1}$ and GUS activity of the control wild-type plants was deducted to obtain the final values. Only positive values of GUS activity with the standard variation in replicates (at least three) are given. The results are provided in figure 7.

Figure 7 shows fluorometric analysis of GUS activity in anthers of transgenic rice plants containing (a) *OSIPK*::pMDC164 construct, (b) *OSbHLH*::pMDC164 construct and (c) *OSFbox*::pMDC164 construct. GUS activity was determined using protein extracts from roots, leaves, PMA, MA, SCP, TPA and dehisced anther (in case of *OSIPK*::pMDC164) of transgenic plants as well as wild-type plants. GUS activity was determined using protein extracts from roots, leaves, PMA, MA, SCP, TPA and dehisced anther (in case of *OSIPK*::pMDC164) of transgenic plants as well as wild-type

plants. In case of *OSIPK*::pMDC164 lines, the highest GUS activity was observed in dehisced anthers (49.01±2.36 to 126.90±2.66), followed by TPA (40.67±0.73 to 108.86±0.36). The PMA, MA and SCP gave almost similar fluorometric values, which were approximately 4-fold lower than that of the dehisced anther values (Figure 7a). In plants carrying *OSbHLH*::pMDC164 construct, the highest GUS activity was observed in the meiotic anther (3.74±0.08 to 8.73±0.12). A 2-fold lower activity was detected in premeiotic anther (1.81±0.09 to 4.73±0.08) followed by single cell pollen stage anthers (1.01±0.14 to 3.61±0.19). In TPA, though the histochemical staining did not show any *GUS* expression, yet the fluorometric analysis revealed some GUS activity (Figure 7b). In *OSFbox*::pMDC164 transgenic rice plants, the highest GUS activity was observed in MA (3.70±0.08 to 5.08±0.32) followed by PMA (3.08±0.10 to 4.47±0.18; Figure 7c). No significant GUS activity was observed in case of root and leaf of *OSIPK/OSbHLH/OSFbox*::pMDC164 transgenic plants.

Table 1: Nucleotide sequence of Forward (F) and reverse (R) primers for QPCR analysis

| SEQ ID NOs. | Primer sequence | Gene name |
|---|---|---|
| SEQ ID NO:10 | 5'-TCGGCGACTGCCACATCTA-3' | *OSbHLH* |
| SEQ ID NO:11 | 5'-GCACTTGCAAACACTGGAGATC-3' | *OSbHLH* |
| SEQ ID NO:12 | 5'-AGACCACGTCCTCGTCAACCT-3' | *OSFbox* |
| SEQ ID NO:13 | 5'-GTGGTCGAGGCGGATCAC-3' | *OSFbox* |
| SEQ ID NO:14 | 5'-CAGCCACACTGTCCCCATCTA-3' | *ACTIN* |
| SEQ ID NO. 15 | 5'-AGCAAGGTCGAGACGAAGGA-3' | *ACTIN* |

Table 2: Nucleotide sequences of Forward (F) and reverse (R) primers used for amplification of *OSIPK*, *OSbHLH* and *OSFbox* promoter

| SEQ ID NOs. | Primer sequence | Gene name |
|---|---|---|
| SEQ ID NO: 16 | 5'-CACCTTAGGTGTGTATTGGAGGT-3' | *OSIPK* |
| SEQ ID NO: 17 | 5'- CTCTTCCTCTTCTTCGATCTTC -3' | *OSIPK* |
| SEQ ID NO: 18 | 5'- CACCAATTTGCCTTCTTTTTCACA -3' | *OSbHLH* |
| SEQ ID NO: 19 | 5'- GCTGATGAGAGGAGAGGAGAGA -3' | *OSbHLH* |
| SEQ ID NO: 20 | 5'- CACCGACGTAAGTGCCATTG -3' | *OSFbox* |
| SEQ ID NO: 21 | 5'- GAGGAGGGGAGTGGAGGTT -3' | *OSFbox* |

Table: 3 Composition of the MS medium (Murashige and Skoog, 1962)

| Component | Concentration (mg/l) |
|---|---|
| **Major salts** | |
| $NH_4NO_3$ | 1650 |
| $KNO_3$ | 1900 |
| $CaCl_2.2H_2O$ | 440 |
| $MgSO_4.7H_2O$ | 370 |
| $KH_2PO_4$ | 170 |
| **Minor salts** | |
| KI | 0.83 |
| $H_3BO_3$ | 6.2 |
| $MnSO_4.4H_2O$ | 22.3 |
| $ZnSO_4.7H_2O$ | 8.6 |
| $Na_2MoO_4.2H_2O$ | 0.25 |
| $CuSO_4.5H_2O$ | 0.025 |
| $CoCl_2.6H_2O$ | 0.025 |
| **Iron source** | |
| $FeSO_4.7H_2O$ | 27.8 |
| $Na_2EDTA.2H_2O$ | 37.3 |
| **Organic constituents** | |
| Nicotinic acid | 0.5 |
| Pyridoxine-HCl | 0.5 |
| Thiamine-HCl | 0.1 |
| Myo-inositol | 100.0 |
| Glycine | 2.0 |
| The pH was adjusted to 5.8 using 1 M KOH solution | |

Table 4: Composition of AAM medium (Toriyama and Hanata, 1985)

| Component | Concentration (mg/l) |
|---|---|
| **Major salts** | |
| $CaCl_2.2H_2O$ | 440 |
| $MgSO_4.7H_2O$ | 370 |
| $KH_2PO_4$ | 170 |
| **Minor salts** | |
| $MnSO_4.4H_2O$ | 22.3 |
| $H_3BO_3$ | 6.2 |
| $ZnSO_4.7H_2O$ | 8.6 |
| KI | 0.83 |
| $Na_2MoO_4.2H_2O$ | 0.25 |
| $CuSO_4.5H_2O$ | 0.025 |

| | |
|---|---|
| $CoCl_2.6H_2O$ | 0.025 |
| **Iron source** | |
| $FeSO_4.7H_2O$ | 27.8 |
| $Na_2EDTA$ | 37.3 |
| **Organic constituents** | |
| Glycine | 75.0 |
| Myoinositol | 100.0 |
| Nicotinic acid | 0.5 |
| Pyridoxine-HCl | 0.1 |
| Thiamine-HCl | 0.5 |
| **Amino acid** | |
| L-Glutamine | 877 |
| L-Arginine | 228 |
| L-Aspartic acid | 266 |
| **Sucrose** | 68,500 |
| **Glucose** | 36,000 |
| **Casamino acids** | 500 |
| pH is adjusted to 5.2. The medium is filter-sterilized and kept at -20°C. | |

Table 5: Composition of Yoshida's medium (1976)

| Element | Component | Concentration of element (ppm) (mg/l) |
|---|---|---|
| N | $NH_4NO_3$ | 40 |
| P | $NaH_2PO_4.2H_2O$ | 10 |
| K | $K_2SO_4$ | 40 |
| Ca | $CaCl_2$ | 40 |
| Mg | $MgSO_4.7H_2O$ | 40 |
| Mn | $MnCl_2.4H_2O$ | 0.5 |
| Mo | $(NH4)_6Mo_7O_{24}.4H_2O$ | 0.05 |
| B | $H_3BO_3$ | 0.2 |
| Zn | $ZnSO_4.7H_2O$ | 0.01 |
| Cu | $CuSO_4.5H_2O$ | 0.01 |
| Fe | $FeCl_3.6H_2O$ | 2.0 |
| | Citric Acid (Monohydrate) | 5.2 |

Any publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure.

SEQ ID NO: 1 shows nucleotide sequence of *OSIPK* promoter

CACCTTAGGTGTGTATTGGAGGTTGGTCGGCTTCGATGGTTATGGAAATGCTCAGTTTTAGGCGAATGAT
TGATTTACAACTCGATTGCTCTTTAGCTGATATGTGTGAAATTTGATCGATATAACGATTGGCCACTGAT
CTTGAAGATTTTATTTTCGATTGGAAGAAGACAACGACTATATGTAAGTCGTTGGGAAGAAATAATGTTG
TGCAAATTCTCACATATCACAGATGGAAAATTTATGTCTTGGAGAAGGTATTTTGAGTAGTTTTGCTATG
ATTTTATGATTCGATGTCGTTCGTCCTACGACCTCTATAATAATTATCTATATCTTTTTCGAACCAAGGC
CAAAATGAATTCAATTAAATTTAAAAAATGCATGTGCCTATAATTCAAAGGTGTTAAGTATGTGTAAATA
AGGAGTAAATTGGATTCATGGTACATAAACGTGGCAGGTAAGTGCAATTTGGTGCAAAAACTTGAGAAGT
GAGCATCCAAGTGCAATAGCTTGACATGTGAGTGCAATTTGTGCAATAACTTAATAAGTGAGCGCACGGG
TGCAATTTTGATAAAGTAACTTAAGAAGTGATGCAACAACTTAATTATTTCGAGTATTTTTTTCTACACA
CATTCAGTTTTTAAATATTTAATTTGTCAATACACTTCCACATATTTGTATTTATGAGCATACTTATAAA
TTTGTAATTTTAGTAATTAGAAGTCAAATAAATTTTACAATGTAGAAATGTTTGTATTTCGGTTGAGATT
TGAGAATGTGAAGCAAATTGTTGGTGCAAATTGGATGAGCAACATAATTTCTTATTATAGATTGAATTCA
AGATTAATTATAAGATAATACCCACATGATTGCTATTTAATGTCATATCGACCTCGGAAAAAAACTCACC
TACCACTCGTTAACTAAAACAACACATACTTAACCAAGTTGCTAAACTAAAATACTAAATTGTTAAGTCC
TTACTACTAGAACGCACCTACTTACAAGGATGTTCAATTCTCAAGTATTCATCCCATGATAGTAATTTAC
TCTTAAAAAGAAAGTGTATGTGCATGGATCATTTGGAGGAACAAAAGGAAGGTTTTAACTATTTTGCTAG
AATGCTATTATTGACTCTCCCCTAGAGAATATAAATGGACAGTTTATTCTCCTGAAAGTCTAAACTTGCC
ATCATAGTTTAAGATCTAGTGTGAGCTGTAGGGAGACCCTTTAGAGGCCTCCAAAAATGGCAAACCCAAG
AACGAATGATGCGGGAGAGAAGCGCCTCAAAGCCAACCACCCCCCCTGCACCGCCATTTCCTTCCATCTC
TCTCTCTCCTCCTCCTCTCTTGCCCTAGAGAGAGAGAGAGAGGGAGGGGGCCGCAAGAGAGAGAGAGATC
GATCACCGGCCGCCCTCCGGAGCTCGCGCGCTCGTTCGTTCGTTCGTTCGATCATTCGTACGTCCACAAC
AATTTCGGCGACGCGACCGTCGCCGTACGTTTGCGGTGGCGCGGCAGCGTCGTCGGCGGTGAAGAAGAAG
AAGAAGAAGATCGAAGAAGAGGAAGAGG

SEQ ID NO: 2 shows nucleotide sequence of *OSbHLH* promoter

ACCAATTTGCCTTCTTTTTCACACTCTTTTTAGTTGCATCCCTGTTCTGAATCGTACATCCCTGTTCTGA
ATCGTACATGGCCCCTAATCTGATAAAGTATACTTTTCTAAAGGGTGCGTTCTCATCGGACTATATATTA
TATATATTATGAAAGAAACTGAGTTTGTCACCATTATTTGTGGGCACAAAGGGTTAAAATATACTCCCTC
CATTTTAGGTTATAAGATATTTTAACTTTGGTTGAAATCAAACTGCTTTAGTTTGACTGTGTTTATAGAC
AAATATAATAATATTTATATTATCAAATTAATTTTATTAAATTAATAATTAAATATATTTTTATAGTAAA
TTTATCTTGGATCGAAAATATTACTATTTTTTTATATAAATTTGATCAAAATTGATATAGCTTGACTTTG
ACCAAAGTCAAAATATCTTATAACAAGTTGGTTTGAAAAGTATTCTGAGAAATTTTAACAAAGAGTTATG
TTATTTGTATCTGTTTGATTTCATTTCACTGATCATTTAATTTGGTGGTTCCCTTGATGATGGTCATGCA
CCATGCATGCATGCCGTCGCAAGCACATCCAGATATGCTTTTCTTCTGTTCCCTTTCAGCTTGCCAAAGC
TTTTGACTTGGTTTTTGTTGGTCGGTGCGCTGTGTACCGCTCTTTTTCCTGTACGTGTTTAAGCATGTCA
TCTTCTGCCATATGCCACTCGTCGATGCAATACATGTTACTACTCTTTTCGGATGCAGAGACTACAGTAC
AGAATTAACCTGACCTACATGGTCAGGTTAATTAACAAACTAATGCCAAAGTTTAGAACAGATGAAATGG
CGGATGATAGATATGATGATCTATTTCTTAGAAGGTTAATTAAATATTACATAATATAAAGCTAACCGAT
GCCTTGGGGAAAGAAAATTTTAAAGCAATGTTGATGAAGGAAAGCATTTGAAGAAATTTGTTTTTTTTTT
GGAAACGTGGCATGAATAATTAGCTCAATAATCCTTAGTTTGGTTTTAGTTAAACCGAACGGTTAAGTAA
CTAACATACCTGGACATTGTTAACATGAACACGCCAGTTGGACGACGTACATATATATCAGGATTTCATG
AGGTTTATTTAACAGGGGAACTTATCTTCTGATATTCATATACCATGTTCCTACACTTATGACTACATTT
ACACCTAGTAAAAATAACCACAATAGTTATGACTGTACTACTTACTCTTTATATTGGTTGAAATAAGAGA
GGGAAAGAAAACTACTCACATACTGTATCATCTTTATTCTTGCTAGTTGGTTGTCACCTGAAGAAAGAAA
ATATATGCATGTGGACTATTTGCTGCACTCAAATTAATACAGTAAAAACTACATCGATCTAACCATCAAC
CAGACAGTAAAACTAATTAAACACTACAACACCCTTGATCTTATCCCAATTCCCTAATAGGTTCCTAACC
TCTGATCACATAACCAACCATGCCGGCCACTTCTCTGTCTCTCTCCCTCGTGCAGGCTTCAGAAAAAGCT

AGGCAGCAGCCGGTGTACAAGTGTACATCAAACCAACAGCAACTGGGGGGGCATGCATGGATGCCTTTCT
GAAACCTGCATGATCATGTACCACTTAAACTGTCAGTGTGTTGCAGATTTTGGTTTGCTTTGGAGAGAGG
AGGGTCAGTGGGGCCCTCCTGGGAGGAATCCCAGCAACAAACCACTCTTCTGATGAAAGGCCAGCCAGCT
GAGTGATCTGCATTGCAGCTAGTACTACAAGCGAGCATTTTGTACAAGTGTCATTTTCTCTCTGCCAATC
CACAAGAAACCAACTGCTTTCTCCTACCCAATATCACCCTTGCCCCTTTTATATACTCTTCCTCTCATCA
CCTTCTCGATCGGCCTCTCTCCTCTCCTCTCATCAGC

SEQ ID NO: 3 shows nucleotide sequence of *OSFbox* promoter

CACCGACGTAAGTGCCATTGAGTTGTAAAACGGCGACAGGTTTAATTATCTCGAACGATTTTGACTTAGG
TGATTCCAAACTACTAAAAATGCAAGGCGGAAAACATATGAAAAGCTACAAACCTCCGGAGAAAGCAGGG
AATGAGATTATTGGATTGCAGCTAAGCTGGCCGGGATATGCCGTGCACACGTCCCTGTCATCGATCGTTA
CGTGTTCCTCCCTGTGGAGCCTCGCCATTGGTCCAACTGTCCATCAACGCCTTTAATTTTTATACGACGT
GTGAACTTAGCTGATGGGAGGAATCCTATATACGGATGATAAAGCGATCGATATTAGTAGTAGCTTTCAC
AGGAGCCCACAAAAGTGGCTCGTCTCGTGCATAATAACTTATTTCAAGGTCGAGCTCTTGCAGTGGTCCT
CAAACGTATCGAAGGTTTTGTATATGGCGCAAAAGACCGATATGAAGAAAACTTACCCTTTAATTTATGG
CATACTCAAAATATCTCAGTAGAAGTGGCAACTTACAAGCATCCATCTGCTGATCTGCATATATATAGTA
GTCGTCGGCCGCCTACTATTTGACCTAGCTAGTTAATTCCATCAGCCTGAGCTTGTATTGACGTCTGATT
TATCAGCAAAGTTCACCCAAAAAAAAAATCAAATCACGCTACGCAGAACAGAATGGTGGTACATGTAAAG
ATAGATTCACTCACGCGGGTGCGCTTAGAATTGAAATTCAATGAGAGCAAGGGATTTGCCCGTTGGTCTT
CATCCTTTTTAGCGCGTGCTAGGGGATGCACTAACGATAGTAACACGTGTGTTATGTTGTAAGCATGTAT
GTTTCTTGTTTAATAGAAAAAAAAAGGTGCCATCTATATAACATTTGATTAAAAAAAACACCCGACAAAT
CGTGCAAAATTTATACTGTGGGACCATTTTAAGATTCATGCACGATATTTCAAACCCTGACCCTCCTGCC
TCCGCTCGTCATTCCCTGCACCGCCACCACTAGTTGTGAGCTCGCACACGCGATGCCTAGGGTCAACGGC
AACAGTGAGCTCCTCCCCTTCATTCCTCTCTCCCTTCACTTGCCTAAATCGGCGGTGCGAGTGTCCCCGT
CCCACCGTGGCAGCTCCCTACCATCGGATCCGCCACCACTAGCAGTCGCCTCCTAACCACAAGCTTTGGT
TTATCTCCATCGCCGACGCCAGCCCACTATCCTTCTCCTATCCACGTGGCTTCGTGCAGCCAGTAGCGTC
TCGTCGTCCACTTGTGTCCACTGCCTCCCACAACCATCCTTCTAAGTCCAGTGATCCCCCTCCCCTCTTG
AGTTAGGGTTTAGTGATCGGGGACCCTAAACCCTAACTCTACTTCCTTTGTCGAGCTGCCAGAGTTGAAG
GGGGTGTCGTCGGAGCCAAAGAAGAAGAGTTGAGGTCACCGGAGCACGAATTATGAAGCAAAATCCCAAG
ATCAATAATGTGCACGATTTTAATAAAAAAAAATTTGTCGCCGGCCAAATTTGCCAGCTCAGCTGCATCAA
CACAAAAGTGCCCTAGTAGTTTCAAAGTACAGGCCTGGCCTATTTCATTCCGTGCTTCCGGTATTCAACA
GGCCGACCGCCGACGCAGCCCGCACCGCCACTCTCCAGCGGACGGATGCACTGGCCTGGCCCATCAAGTG
CACGCTCTCATGGACCGTAACGGTCTCGCCAGTCGCCACGAAACGTGCGGCCTATACTAAATGGGCCGAA
TCGGTTCAGCCACGTGCGGCCCATGATATTGGGCCCAGAAAGTCAAGTAGCCCATCGCCCCATCCCCTCC
GCGGCTTCCAGAAGCAAAACGGAATCAAACTCCTATCATTTTAAGCCTTCGTCTTCCCCTTCCCCGTCGA
GTCGTCGTCGTCGTCGTCCTCCCGTCTCGTGCCATTCGCCGCAGAGTCCCCAACCTCCACTCCCCTCCT

SEQ ID NO: 4 shows cDNA sequence of *OSIPK* (LOC_Os12g12860)

ATGGGCAACTGCTGCGTGTCCCGGCCGTCCGGCGCCGACAAGCGGCGGCGCTGCGGCAGCTCCACCGCCC
CGCACACCCGCGGCGGCAGGCGGGTGATCGGCGCCGCCAACATGCGGTGCCTCTCCACGGTCTCCTCCGT
GTCGGACGCGGCGCGCGCCGTGATGTCGAACGAGCCGGCGACCGTGCTGGGCAACAGCGGCAGCAGCGGC
AACGGCGGCGTGATGGCGGCGGAGGAGATGCTGCGGCGGTACGAGATCGGGGAGGAGCTGGGTCGGGGGG
AGTTCGGGGTGACGCGGCGGTGCCGGGACGCGGTGACGGGGGAGCGGCTGGCGTGCAAGTCGATCAGCAA
GCGGAAGCTCCGGAGCAGCGTGGACGTGGAGGACGTGCGGCGGGAGGTGGCCATCATGCGGTCGCTGCCG
GCGCACGCCAACGTGGTGAGGCTCCGGGAGGCGTTCGAGGACGCCGACGCCGTCCACCTCGTCATGGAGG
TCTGCGAGGGCGGCGAGCTCTTCGACCGCATCGTCGCCCGCGGCCACTACACCGAGCGCGCCGCCGCCGC
CGTCATGCGCACCATCATGGACGTCGTCCAGCACTGTCACAAGAACGGCGTCATGCATCGGGACCTCAAA
CCTGAGAACTTTCTGTATGCCAATGCATCTGAGAACTCCCCTCTCAAGGTCATCGACTTCGGCCTCTCAG
TGTGCTTCAAACCTGGTGCAAGATTCAATGAGATTGTTGGATCTCCCTACTACATGGCACCAGAAGTCCT
GAAGAGAAACTATGGACAAGAAATAGACATATGGAGTGCAGGAGTCATACTGTACATCTTGCTATGTGGT
GTTCCTCCTTTCTGGGCCGAGACCGACGAAGGGATCGCACAGGCCATCATCCGGTCACATATCGACTTCC
AAAGAGAGCCTTGGCCAAAGGTGTCAGATAATGCCAAGGATCTTGTAAGGAGGATGCTTGATCCAAACCC
ATATACTCGGTTGACGGCTCAACAGGTTCTAGAGCATCCTTGGATACAGAATGCAAGTGCAGCTCCCAAC
ATTCCTCTGGGAGAAGCAGTCAGGTCCAGGCTGAAGCAATTCACTGTTATGAACAAGTTCAAGAAGAAGG
CCTTACTTGTGGTGGCGGAGTACTTACCAACCGAAGAGCTGGATGCAATCAGGGAGCTGTTCAATATGTT
GGACACCAAAAAGAAGGGGCACTTGACACTAGAAGAGCTCAGAAAGGGACTGCAAGTGATAGGGCACAAC
ATCCATGACACAGATGTCGATATGCTCATGGAAGCTGCAGACATAGATGGAAATGGCATACTGGATTGCA

AGGAGTTTGTGACAGTATCCATTCACCTGAAGAAAATCCGTAGCGATGAGCACCTTCCGAAGGTGTTCAG
CTTCTTCGACAAGAACGGCAGCGGGTACATAGAGATCGAAGAGTTGAAAGAAGCCCTCTCCCCAAGAGGT
GACCAGAAGTCGATCGACGACATATTTCTGGATGTTGACATAGACAAGGATGGGAAGATAAGCTACGAGG
AGTTTGAGTTAATGATGTCAGCCGGAATGGACTGGAGGAATGCATCCCGGCAATACTCGAGAGCAGTTTA
CAACACTCTCAGCCGCAAGATATTCAAGGAAGTGTCCCTGAAGCTTGATCACAGTGGTCCACTTGTTGCA
GCAGGAAAATAG

SEQ ID NO: 5 shows cDNA sequence of *OSbHLH* (LOC_Os01g18870)

ATGTATCACCCGCAGTGCGAGCTCCTGATGCCGCTTGAGAGCCTGGAGATGGACGTCGGCCAGTCGCACC
TCGCCGCCGCCGTCGCAGCAGCCATGCCGGGGGAGCTCAACTTCCACCTCCTCCACTCGCTCGACGCCGC
CGCGGCGGCTGCCTCCTCCACCGCCGCCTCGGCCTCCTCCCAGCCCACCGTCGACTACTTCTTCGGCGGC
GCCGACCAGCAGCCGCCGCCGCCGGCGGCGATGCAGTACGACCAGCTGGCGGCGCCGCACCACCACCAGA
CGGTGGCCATGCTGCGCGACTACTACGGCGGCCACTACCCGCCGGCGGCGGCGGCGGCGGCCACCGA
GGCGTACTTCCGCGGCGGGCCAAGGACGGCCGGGTCGTCGTCGCTCGTGTTCGGCCCGGCCGACGACGAG
TCGGCCTTCATGGTCGGACCCTTCGAGAGCTCCCCGACGCCGCGGTCCGGCGGCGGCAGGAAGCGTAGCC
GCGCCACCGCCGGCTTCCACGGCGGCGGGCCGGCCAACGGCGTCGAGAAGAAGGAGAAGCAGCGCCGCCT
GCGGCTCACCGAGAAGTACAACGCCCTCATGCTCCTCATCCCCAACCGCACCAAGGAGGATAGAGCGACG
GTGATCTCAGACGCGATCGAGTACATCCAGGAGCTAGGGAGGACGGTGGAGGAGCTGACGCTGCTGGTGG
AGAAGAAGCGGCGGCGGAGGGAGATGCAGGGGGACGTGGTGGACGCGGCGACGTCGTCGGTGGTGGCGGG
GATGGATCAGGCGGCGGAGAGCTCGGAGGGCGAGGTGATGGCGGCGGCGGCGATGGGCGCGGTGGCACCG
CCGCCGCGGCAGGCGCCGATCCGGAGCACGTACATCCAGCGGCGGAGCAAGGAGACGTTCGTGGACGTGC
GGATCGTGGAGGACGACGTGAACATCAAGCTCACCAAGCGCCGCCGCGACGGCTGTCTCGCCGCCGCGTC
GCGCGCGCTGGACGACCTCCGCCTCGACCTCGTCCACCTCTCCGGCGGCAAGATCGGCGACTGCCACATC
TACATGTTCAACACCAAGATTCATTCGGGATCTCCAGTGTTTGCAAGTGCAGTGGCCAGCAGGCTGATTG
AAGTGGTGGATGAGTACTAA

SEQ ID NO: 6 shows cDNA sequence of *OSFbox* (LOC_Os05g46050)

ATGGATGGCCATCAGTTCGGGCCATGGAACCTCTTGCCCGACGACATCCTGGAGTTGCTGGTCGGCCGCA
ACCTGTGCGAGATCGACCGCCTCCACGCGCGCCGCGTCTGCCACTCCTGGCGCGCGGCGTTCGCGAGGAT
CGAGCCCCCGCCGCCGCCTCCACCGCTGCCGTTGCTCCTCCTCCCGGAAGCCGACGACAACGAGCACGGT
CTTGCCTTCTCCTGCGTCCTCAGCGGCTGGGACACCCACCCCTTCTTCCTCCCGCGCGCCGCGCGGCACC
GCGCCCGCTGCTTCGGCTCGTGCGATGGCGTCTGGCTCTTCCTCGCCATGGAGGACGGCCTGCAAGGCGA
CCGAGCTCGAGACCACGTCCTCGTCAACCTCCACAGCTTCCAGTTCCTCGACCTCCCCAACGTGATCCGC
CTCGACCACACGTTCCCGCAATTGATGAAGGACATCGAGATCGCGATCGTCGCCGTCACGCTCTCGCGCC
AGCCAACCCAGCAAGGATGCGTCGCCGCCGGCATCATCGAGCTCCCTCCCTTCCCCATCGGAGTGCGGCC
GTTCGCGTTCTGGCGCATGGGGGATCGGGTGATCTTGCCTTTCTACGAAGACGTGTTCGGGGATCAAGCA
GTAGAGGACGTCATATACCACAACGGCTACTTCCTCTTCCTCACCCAGGACGAACACATTCGTGTGTGCC
AAGAGCCGGTCTTCCACGACACCAACGTGGACGTGGACTCGATCCTGTTGCGATTCGAGCCGCGCGTCGA
CGACGGCGACGCCGTCCTTGCTCGCTACCTCGTGCTGTGCCGGGGGAAGGTGCTCATGGTCGTCAGGTTG
GGCTGTCCACATCGCCGCTCGCCGACGTCGGCGTTCCGGGTGTTCGAGAGAGTGGACTATCTGGTGGTGA
ACGCCGGCGTGGTCGAGGTGCTTGAGCACACCTGGAGCGAGATTGACGAGCTCGGCGGCAGGATGCTGTT
CCTCGGGCGAGGCTGCTCCAGATCCTACGAGGAGGCCGATGGGTATCCCGGCATGGAGGGCGTCTACTTC
TTGGATGACCGGAGCTTCCGTGACCCGATCTTCCACGACCCGGACATGGTGTTCGATCACACCTACCATT
GCTGCGACAATGGCAGATGGTAG

SEQ ID NO: 7 shows amino acid sequence of OSIPK

MGNCCVSRPSGADKRRRCGSSTAPHTRGGRRVIGAANMRCLSTVSSVSDAARAVMSNEPATVLGNSGSSG
NGGVMAAEEMLRRYEIGEELGRGEFGVTRRCRDAVTGERLACKSISKRKLRSSVDVEDVRREVAIMRSLP
AHANVVRLREAFEDADAVHLVMEVCEGGELFDRIVARGHYTERAAAAVMRTIMDVVQHCHKNGVMHRDLK
PENFLYANASENSPLKVIDFGLSVCFKPGARFNEIVGSPYYMAPEVLKRNYGQEIDIWSAGVILYILLCG
VPPFWAETDEGIAQAIIRSHIDFQREPWPKVSDNAKDLVRRMLDPNPYTRLTAQQVLEHPWIQNASAAPN
IPLGEAVRSRLKQFTVMNKFKKKALLVVAEYLPTEELDAIRELFNMLDTKKKGHLTLEELRKGLQVIGHN
IHDTDVDMLMEAADIDGNGILDCKEFVTVSIHLKKIRSDEHLPKVFSFFDKNGSGYIEIEELKEALSPRG
DQKSIDDIFLDVDIDKDGKISYEEFELMMSAGMDWRNASRQYSRAVYNTLSRKIFKEVSLKLDHSGPLVA
AGK

SEQ ID NO: 8 shows amino acid sequence of OSbHLH

MYHPQCELLMPLESLEMDVGQSHLAAAVAAAMPGELNFHLLHSLDAAAAAASSTAASASSQPTVDYFFGG
ADQQPPPPAAMQYDQLAAPHHHQTVAMLRDYYGGHYPPAAAAAAATEAYFRGGPRTAGSSSLVFGPADDE
SAFMVGPFESSPTPRSGGGRKRSRATAGFHGGGPANGVEKKEKQRRLRLTEKYNALMLLIPNRTKEDRAT
VISDAIEYIQELGRTVEELTLLVEKKRRRREMQGDVVDAATSSVVAGMDQAAESSEGEVMAAAAMGAVAP
PPRQAPIRSTYIQRRSKETFVDVRIVEDDVNIKLTKRRRDGCLAAASRALDDLRLDLVHLSGGKIGDCHI
YMFNTKIHSGSPVFASAVASRLIEVVDEY

SEQ ID NO: 9 shows amino acid sequence of OSFbox

MDGHQFGPWNLLPDDILELLVGRNLCEIDRLHARRVCHSWRAAFARIEPPPPPPPLPLLLLPEADDNEHG
LAFSCVLSGWDTHPFFLPRAARHRARCFGSCDGVWLFLAMEDGLQGDRARDHVLVNLHSFQFLDLPNVIR
LDHTFPQLMKDIEIAIVAVTLSRQPTQQGCVAAGIIELPPFPIGVRPFAFWRMGDRVILPFYEDVFGDQA
VEDVIYHNGYFLFLTQDEHIRVCQEPVFHDTNVDVDSILLRFEPRVDDGDAVLARYLVLCRGKVLMVVRL
GCPHRRSPTSAFRVFERVDYLVVNAGVVEVLEHTWSEIDELGGRMLFLGRGCSRSYEEADGYPGMEGVYF
LDDRSFRDPIFHDPDMVFDHTYHCCDNGRW

SEQ ID NO: 10 shows RT Forward Primer of *OSbHLH*

TCGGCGACTGCCACATCTA

SEQ ID NO: 11 shows RT Reverse Primer of *OSbHLH*

GCACTTGCAAACACTGGAGATC

SEQ ID NO: 12 shows RT Forward Primer of *OSFbox*

AGACCACGTCCTCGTCAACCT

SEQ ID NO: 13 shows RT Reverse Primer of *OSFbox*

GTGGTCGAGGCGGATCAC

SEQ ID NO: 14 shows RT Forward Primer of *ACTIN*

CAGCCACACTGTCCCCATCTA

SEQ ID NO: 15 shows RT Reverse Primer of *ACTIN*

AGCAAGGTCGAGACGAAGGA

SEQ ID NO: 16 shows Forward Primer of *OSIPK* promoter

CACCTTAGGTGTGTATTGGAGGT

SEQ ID NO: 17 shows Reverse Primer of *OSIPK* promoter

CTCTTCCTCTTCTTCGATCTTC

SEQ ID NO: 18 shows Forward Primer of *OSbHLH* promoter

CACCAATTTGCCTTCTTTTTCACA

SEQ ID NO: 19 shows Reverse Primer of *OSbHLH* promoter

GCTGATGAGAGGAGAGGAGAGA

SEQ ID NO: 20 shows Forward Primer of *OSFbox* promoter

CACCGACGTAAGTGCCATTG

SEQ ID NO: 21 shows Reverse Primer of *OSFbox* promoter

GAGGAGGGGAGTGGAGGTT

SEQ ID NO: 22 shows M13 Forward Primer

TGTAAAACGACGGCCAGT

SEQ ID NO: 23 shows M13 Reverse Primer

CAGGAAACAGCTATGACC

SEQ ID NO: 24 shows Primer of *OSIPK*

TCGTACGTCCACAACAATTTCG

SEQ ID NO: 25 shows Primer of *OSbHLH*

TTCTCTCTGCCAATCCACAAG

SEQ ID NO: 26 shows Primer of *OSFbox*

CTCCTATCATTTTAAGCCTTCGTC

SEQ ID NO: 27 shows *GUS* Reverse Primer

TCTTGTAACGCGCTTTCCCACC

SEQ ID NO: 28 shows *GFP* Reverse Primer

TGTTGCATCACCTTCACCCTCTCC

SEQ ID NO: 29 shows pollen-specific element GTGANTG10

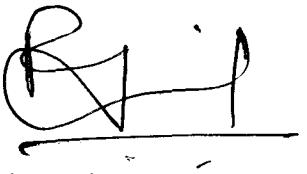
GTGA

SEQ ID NO: 30 shows pollen-specific element POLLEN1LeLAT52

AGAAA

SEQ ID NO: 31 shows extra element QELEMENTZMZM13

AGGTCA



Date 11 April, 2012

Rajani Jaiswal

IN/PA-1463

Agent for the Applicant

The Controller of Patents,

The Patent Office, at Delhi