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Spatio-temporal regulation of the *OsHFP* gene promoter establishes the involvement of this protein in rice anther development

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ABSTRACT

Anther development leading to pollen maturation, anther dehiscence and pollen dispersal depends upon the precise timing of programmed cell death (PCD) in specified anther tissues. The PCD necessitates a properly tuned transcriptional regulation of some crucial genes. However, the detailed genetic regulation of this PCD in rice anther is yet to be deciphered. Recently, we have established that the OsHFP, a structurally novel hemopexin fold protein of rice is a flower-specific heme binding protein, and plays a role in chlorophyll degradation. Here, we report the spatio-temporal transcriptional regulation of the *OsHFP* gene, which is proposed to be involved in anther PCD. The OsHFP was immunodetected in rice anthers, and OsHFP-related proteins were also found to be present in anthers of other monocot (lily) and dicot (tobacco) plant species. Unique *cis*-acting elements, possibly involved in the activation and anther-specificity of the *OsHFP* promoter were identified based upon *in silico* prediction and *in planta* expression profiling of the reporter gene driven by the *OsHFP* promoter (2051 bp) and its two deleted versions (1057 bp and 437 bp). The temporal regulation of the *OsHFP* promoter in different developmental stages of tobacco anther implies the physiological function of this protein in anther PCD.

1. Introduction

Among the different floral organs of a complete flower, anther is the site for male gametogenesis. Like any other biological process, anther development involves coordinated and complex interactions of several gene products that have distinct and overlapping functions in plant system. In tobacco, around 25,000 different genes including 10,000 unique transcripts have been reported to be expressed during anther development [1,2], indicating the intricacy of genetic regulation involved in the process. Successful anther development requires the formation and eventual disintegration of specialized tissue layers through perfect spatio-temporal regulation of various genes including some crucial ones. The developmental events in tobacco anther have been exhaustively studied and broadly divided in two phases; in the first phase, different specified tissue layers required for pollen formation (i.e., epidermis, endothecium, connective layer, stomium, etc.) differentiate, whereas in the second phase theses tissue layers degenerate through programmed cell death (PCD) leading to pollen maturation, anther dehiscence and pollen dispersal [3]. Both the phases are equally important for successful reproduction in angiosperms as the inability of the functional pollen to be dispersed through anther dehiscence is analogous to male-sterility (inability to produce functional pollen). However, the detailed genetic control underlying the coordinated anther dehiscence program in rice (*Oryza sativa*) is yet to be characterized. The first reported gene related to the anther dehiscence program in rice is *ANTHER INDEHISCENCE1* (*AID1*) encoding a single MYB DNA-binding domain protein [4].

In our recent study, we have reported the identification and preliminary characterization of a heme binding and structurally novel hemopexin fold protein OsHFP from rice [5]. The single copy *OsHFP* gene has been documented to be expressed specifically in flower buds, and the OsHFP plays a role in chlorophyll degradation. On the basis of the biochemical and physiological properties, we also anticipated a certain but yet to be characterized role of OsHFP in anther development of rice. In the present study, we further extend our investigation on the physiological role of OsHFP during anther development through characterizing its promoter region. Spatio-temporal regulation of the *OsHFP* promoter, as established through functional validation by transgenesis, deletion analysis and *in silico* characterization implies the involvement of OsHFP in PCD during the anther dehiscence of rice. To the best of our knowledge, this is the first report that highlights the involvement of a novel hemopexin fold protein in anther PCD.

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2. Materials and methods

2.1. Bioinformatics analysis

Amino acid sequences of the hemopexin fold proteins of plant origin were obtained from the NCBI database after BLAST search using OsHFP as the query sequence. The GenBank accession numbers of the proteins are – *O. sativa* hemopexin fold protein (OsHFP): JX233809, *Vigna radiata* seed albumin (VrAlb): CAA50008, *Phaseolus vulgaris* albumin-2 (PvAlb-2): ADR30065, *Medicago truncatula* albumin-2 (MtAlb-2): XP_003627739, *M. truncatula* hemopexin (MtHpx): ABE87586, *M. truncatula* pierisin (MtPierisin): AET02245, *Pisum sativum* albumin-2 (PsAlb-2): P08688, *P. sativum* anther-specific protein (PsASP/END1): AAM12036. The PDB IDs of the other three OsHFP-related proteins are: *Vigna unguiculata* hemopexin fold protein (VuHFP/Cp4): 3OYO, *Lathyrus sativus* seed albumin (LS-24): 3LP9, *Cicer arietinum* albumin (CaAlb): 3V6N. Multiple sequence alignment was carried out in Clustal W [6] and phylogenetic analysis was performed in the Phylogeny.fr web service [7]. Frequency distribution of the different *cis*-regulatory elements present in the *OsHFP* promoter region was analyzed in the PLACE database [8].

2.2. Immunolocalization of OsHFP and related proteins in anthers

Immunolocalization of OsHFP and its related proteins in the anther tissues of rice, lily and tobacco was carried out as described elsewhere [9] with minor modifications. Anti-OsHFP primary antibody [5] and FITC conjugated anti-rabbit secondary antibody (G Bioscience) were used in optimized dilutions. Samples incubated in pre-immune sera in place of primary antibody were used as −ve control. Anther cross-sections were finally imaged in Fluo View FV1000 confocal microscope (Olympus).

2.3. Preparation of recombinant plasmids for plant transformation

Detailed description on the preparation of recombinant plasmids for plant transformation is given in Supplementary information (Si. 1). Schematic diagrams of the recombinant plasmids are presented in Supplementary Fig. Sf1.

2.4. Plant transformation and analysis of transgenic lines

Rice and tobacco transformation was carried out as described previously [10]. Detailed description is given in Supplementary information (Si. 2).

2.5. Histochemical GUS assay

Histochemical GUS assay was carried out taking tissues from different parts of the control and transgenic plant lines as described previously [11]. Plants transformed with the appropriate *Agrobacterium* strain harboring the blank pCAMBIA1391Z plasmid (Cambia, Australia) were used as control.

3. Results and discussion

3.1. The OsHFP is a novel anther-specific protein in rice and OsHFP-related proteins exist in anthers of other plant species

Multiple amino acid sequence alignment was carried out taking OsHFP-related plant protein sequences to address the distinctive-

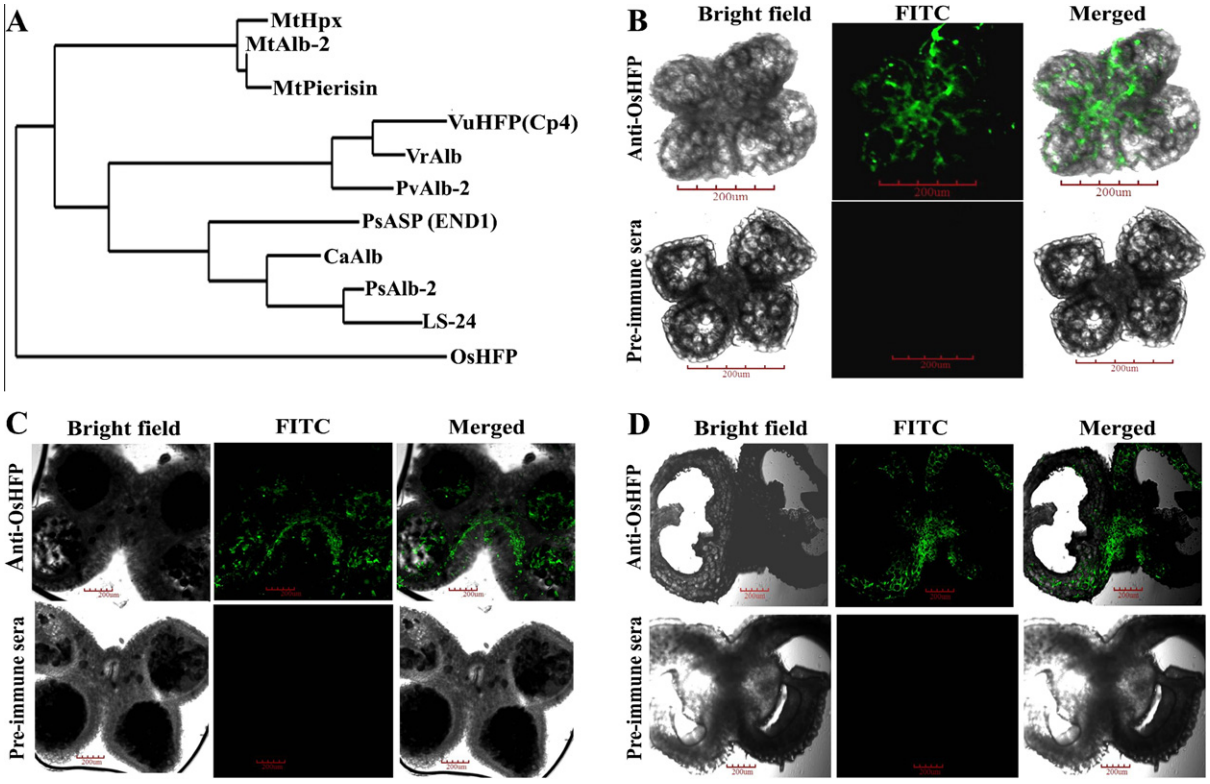

Fig. 1. The novel OsHFP is an anther-specific protein of rice. (A) Multiple amino acid sequence alignment based phylogram showing the OsHFP as a unique protein in respect to other related plant proteins. (B) Fluorescence microscopy showing immunolocalization of OsHFP using anti-OsHFP antibody in rice anther tissues, where OsHFP is predominantly localized in the connective tissues. (C) Immunolocalization of OsHFP-related protein(s) in the anther tissues of lily using anti-OsHFP antibody. (D) Immunolocalization of OsHFP-related protein(s) in the anther tissues of tobacco using anti-OsHFP antibody. The pre-immune sera samples represent −ve control in (B), (C) and (D).

ness of OsHFP. The analysis revealed a considerably high sequence similarity with a low level of sequence identity among these proteins (Supplementary Fig. Sf2). The phylogram, generated on the basis of the sequence alignment showed that the OsHFP is quite unique from all the other proteins taken under consideration (Fig. 1A). The findings revealed that most of the OsHFP-related proteins belong to the class seed-albumin. Among them, crystal structure of the LS-24 protein from *L. sativus* has been deciphered, and a possible biological role of LS-24 in plant stress response on the basis of oligomeric state-dependent hemin/spermine binding property has been postulated [12]. The OsHFP-related protein PA2 (PsAlb-2) from pea has been documented to be involved in polyamine biosynthetic pathway [13]. Interestingly, the hemopexin fold bearing protein VuHFP (Cp4) from *V. unguiculata* is capable of binding spermine but not hemin [14]. These observations are very significant, indicating that the heme binding property of plant hemopexin fold proteins have significant physiological implication.

Apart from the seed-albumin class proteins, the OsHFP showed significant sequence similarity (overall identity 26%) with the C-terminal region of MtHpx, the hemopexin protein from *M. truncatula*. Interestingly, the OsHFP was found to share sequence similarity (overall identity 23%) with the *M. truncatula* pierisin protein, a type of protein that acts as an inducer of apoptosis in animal system [15]. Furthermore, the biologically active heme molecule has been proposed to play important role in PCD of plants [16–18]. Naturally, the heme binding protein OsHFP could be speculated to be involved in plant PCD, in a similar or different manner.

The OsHFP shared considerable sequence similarity (overall identity 36.8%) with the anther-specific protein PsASP (PsEND1) from pea (*P. sativum*) [19]. This observation, along with the flower bud specific expression of OsHFP [5] indicated the possibility of the protein to be localized in rice anther. Immunolocalization indeed revealed the presence of OsHFP in the anther tissues of rice, predominantly in the connective tissues (Fig. 1B). Existence of OsHFP-related protein(s) in the anther of other plant species was also investigated, taking lily (*Lilium* spp.) as a monocot and tobacco (*Nicotiana tabacum*) as a dicot representative. Immunological cross-reactivity revealed the presence of OsHFP-related proteins in both the systems, particularly in the connective tissues, epidermis and endothecium of lily (Fig. 1C) and tobacco (Fig. 1D) anthers. The results indicate that OsHFP-related proteins might be evolutionary conserved in playing a crucial role in anther development of both monocot and dicot plants.

3.2. The promoter region of the OsHFP gene drives reporter gene expression in anther tissues

The OsHFP has been documented to play a role in chlorophyll degradation [5], nullifying the feasibility of over-expression based gain-of-function analysis of OsHFP in stable transgenic plants. Hence, in order to establish the physiological role of the OsHFP in anther development, the promoter region of the gene was functionally characterized in details as the spatio-temporal regulation of a gene reflects its possible biological function. The ~2 kb putative promoter region (annotated hereafter as FLP version), representing the −2051 to +3 part of the *OsHFP* gene (where +1 represents the translation start site) was functionally analyzed through transgenesis in rice and tobacco systems. Selected independent T_1 transgenic lines of rice and tobacco were verified through PCR (Fig. 2A and C, respectively) followed by *gusA* gene expression analyses. The FLP version of the *OsHFP* promoter was found to drive GUS expression in flower bud and anther tissues of the transgenic rice lines (Fig. 2B). The FLP promoter was further found to be active in transgenic tobacco lines showing GUS expression

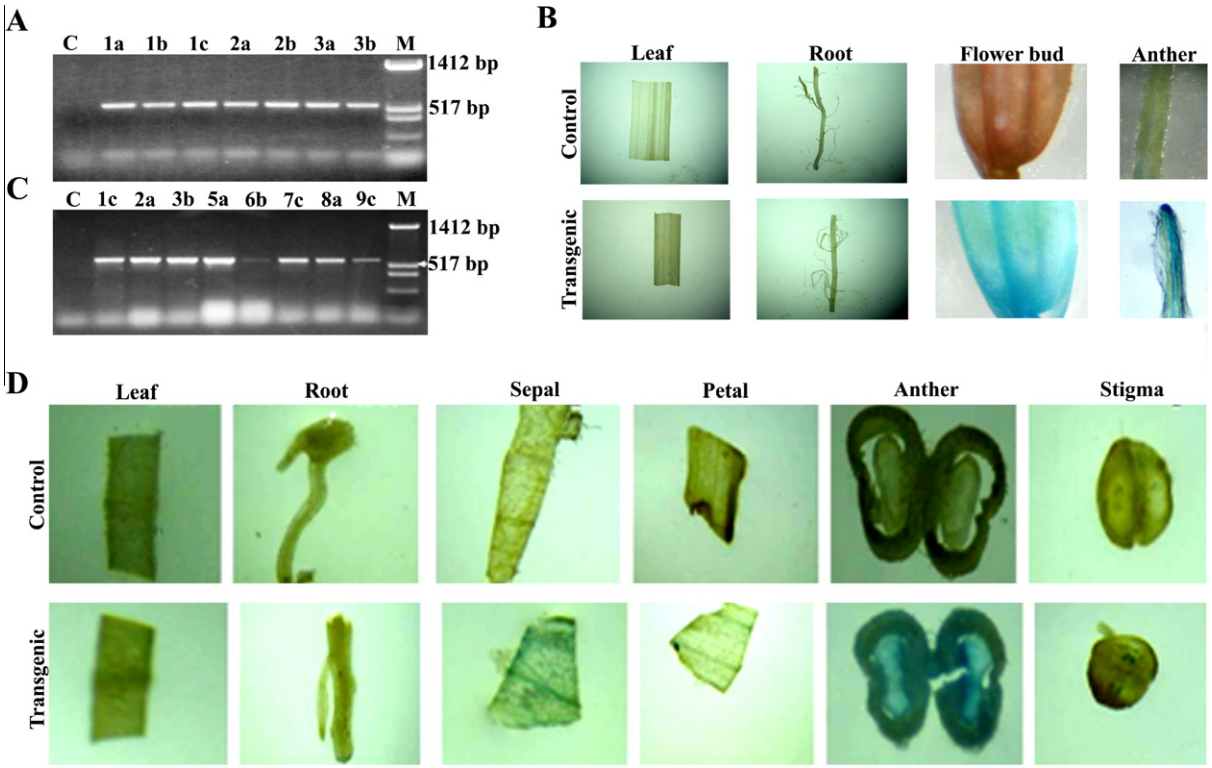

Fig. 2. The ~2 kb (FLP version) *OsHFP* promoter activity in transgenic rice and tobacco lines. (A) PCR based verification of FLP transgenic rice lines (T_1 generation) using *gusA* gene-specific primers. (B) Expression profiling of FLP promoter in transgenic rice line showing predominant GUS expression in the flower bud and anther tissues. (C) PCR based verification of FLP transgenic tobacco lines (T_1 generation) by *gusA* gene-specific primers. (D) Expression profiling of FLP promoter in transgenic tobacco line showing predominant GUS expression in connective tissues of anther. In both (A) and (C), transgenic lines contain the *gusA* gene-specific 558 bp amplicon. Lanes C and M represent −ve control and *Hin*fI digested pUC18 plasmid as standard molecular weight marker, respectively.

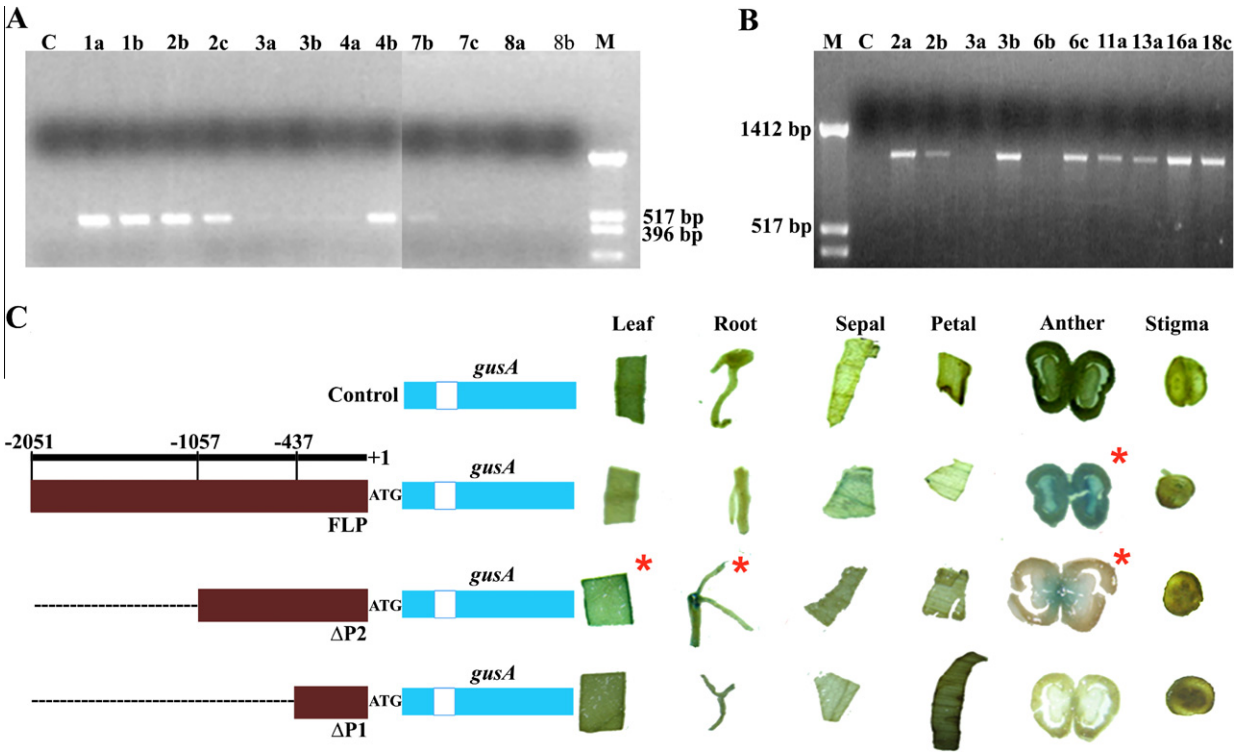

Fig. 3. Functional analysis of the deleted versions of the *OsHFP* promoter in transgenic tobacco lines. PCR based verification of ΔP1 and ΔP2 transgenic tobacco lines (T_1 generation) showing the presence of ΔP1 fragment-specific 460 bp amplicon (A) and ΔP2 fragment-specific 1080 bp amplicon (B). Lanes C and M represent −ve control and *Hin*fI digested pUC18 plasmid as standard molecular weight marker, respectively. (C) Summarized representation of the reporter gene expression profiles in case of FLP, ΔP2 and ΔP1 transgenic tobacco lines along with the control, as revealed by histochemical GUS assay. Detectable GUS expression is indicated by red asterisk mark. White box in the schematic diagrams of the corresponding promoter fragments indicates the presence of catalase intron within the coding DNA sequence of the *gusA* gene. The FLP promoter is found to drive anther-specific expression (2nd panel from the top) whereas the ΔP2 promoter is found to be active in anther, leaf and roots tissues of transgenic tobacco lines (3rd panel from the top). The ΔP1 promoter fails to be active in transgenic tobacco lines (bottom panel).

predominantly in the anther tissues and negligibly in sepals and petals (Fig. 2D). However, GUS expression was not detected in leaves, roots and female reproductive part (i.e., stigma). The results indicate that the *OsHFP* promoter is regulated in rice and tobacco in a similar manner. *In silico* analysis of the FLP version of *OsHFP* promoter revealed the presence of 11 common *cis*-regulatory elements (Supplementary Table St1) found in the promoter regions of male gamete- and tapetum-specific genes of rice [20]. However, the OsHFP was found to be absent in the tapetum cells of rice anthers as revealed by immunolocalization (Fig. 1B). In agreement to this observation, the *OsHFP* promoter was active primarily in the connective tissues but not in the tapetum cells of the anthers, as revealed in the transgenic tobacco lines (Fig. 2D). Interestingly, the *OsHFP* promoter does not contain any of the specific elements present in the promoter region of male gamete- and tapetum-specific genes of rice [20], which might result in the difference in spatial regulation of the *OsHFP* promoter.

3.3. Functional characterization of the OsHFP promoter through deletion reveals the presence of unique cis-regulatory elements

Analysis of the distribution of these 11 common regulatory elements in the FLP version of the *OsHFP* promoter suggested two possible deletions of the same. The abundance of the 11 common regulatory elements in the last 1057 bp part of the *OsHFP* promoter is ~50% of the same in the FLP promoter version (considering both + and − strand). Similarly, the last 437 bp part of the FLP promoter version was found to contain all the 11 common representative elements at least once in the + strand. These two deleted versions of the FLP promoter were annotated as ΔP2 (representing −1057 to +3 part of the *OsHFP* gene) and ΔP1 (representing the −437 to +3 part of the *OsHFP* gene) promoters. Frequency distribution of the 11 common regulatory elements in the FLP, ΔP2 and ΔP1 promoters is represented in Supplementary Fig. Sf3.

Further functional characterization of the *OsHFP* promoter was carried out *in planta* through analysis of the reporter gene expression driven by the two deleted versions of the promoter. Independent transgenic tobacco lines were verified (Fig. 3A and B), and analyzed through histochemical GUS assay. A comparative GUS expression profiling of the FLP, ΔP2 and ΔP1 versions of the *OsHFP* promoter is summarized in Fig. 3C. The ΔP1 promoter was found to be inactive in the transgenic tobacco plants as GUS expression was not observed in different plant parts (Fig. 3C, bottom panel). On the other hand, the ΔP2 promoter was found to be active but not anther-specific, as GUS expression was observed in the anther, leaf and root tissues of the concerned transgenic tobacco lines (Fig. 3C, 3rd panel from the top). Thus, only the FLP promoter version was able to drive the reporter gene expression selectively in anther (Fig. 3C, 2nd panel from top).

Occurrence of different *cis*-acting regulatory elements was further analyzed on the basis of this differential regulation of the FLP, ΔP2 and ΔP1 versions of the *OsHFP* promoter. Comparative analysis between the regulatory elements present in the FLP and ΔP2 promoters revealed the presence of 26 unique regulatory elements (Supplementary Table St2, Supplementary Fig. Sf4A) in the FLP version, i.e. the elements present in the FLP version but not in the ΔP2 version. In a similar manner, 27 unique regulatory elements (Supplementary Table St3, Supplementary Fig. Sf4B) were identified in the ΔP2 version, i.e. the elements present in the ΔP2 version but not in the ΔP1 version. On the basis of the tobacco transgenesis data, the 26 unique elements present in the FLP version might play a role in regulating the tissue-specificity of the *OsHFP* promoter.

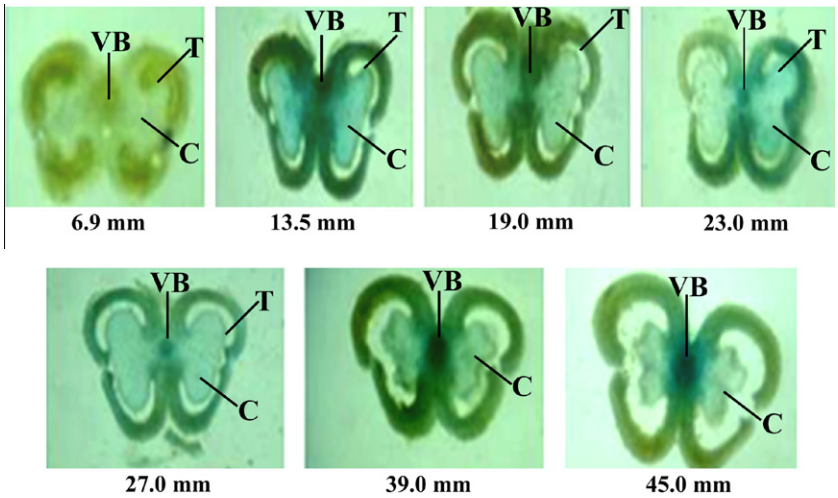

Fig. 4. Temporal regulation of the *OsHFP* promoter in transgenic tobacco lines. Histochemical GUS assay of the anthers from transgenic tobacco lines reveals developmental stage-specific regulation of the FLP version (~2 kb) of the *OsHFP* promoter. Lengths of the flower buds, from which anthers were taken for analysis, are mentioned at the bottom of each individual image. The promoter is found to be predominantly active in the connective tissues at late developmental stages of anther. C, T and VB represent connective tissue, tapetum, and vascular bundle, respectively.

Among them, the CARGCW8GAT is quite important. The CARGCW8GAT is a variant of the CArG element involved in binding with the MADS domain protein Agl15 (AGAMOUS-like 15) [21]. Interestingly, promoter region of the anther-specific gene PsEND1 contains three putative CArG elements [19], and the authors reported that deletion of one of these three putative CArG elements resulted in loss of the anther-specific activity of this promoter. From this point of view, presence of a CArG variant as an element responsible for anther-specificity in the *OsHFP* promoter is quite significant.

In a similar way, the unique 27 elements present in the ΔP2 version might be essential for the activation of the *OsHFP* promoter. These include regulatory elements responsive to calcium, sugar, phytohormone (gibberellin), elicitors (pathogen), salicylic acid, a few transcription factors and dehydration. Involvement of calcium in rice anther development is prominent from a comparative study on the distribution of the same in the fertile and sterile anthers of a photo-period sensitive genic male-sterile line [22]. Large vacuoles containing calcium oxalate appear before the anther dehiscence in tobacco [23]. Furthermore, role of calcium in the structural stability of the OsHFP has already been highlighted in our earlier study [5]. In this context, possible activation of the *OsHFP* promoter through calcium responsive elements is physiologically significant.

3.4. The OsHFP promoter is specifically active in anther tissues undergoing PCD

Anther-specific expression by the FLP version of the *OsHFP* promoter was found not to be constitutive throughout the different developmental stages of the anthers as revealed from the detailed analysis of transgenic tobacco lines. Histochemical GUS assay revealed that the FLP promoter is active in the transgenic tobacco anthers collected from the flower buds of length ⩾ 13.5 mm, whereas no GUS expression was observed in the anthers collected from the flower buds of length 6.9 mm (Fig. 4). During different developmental stages, GUS expression was observed in the connective tissues, epidermis and vascular bundle of the anther but not in the tapetum cell layers. As per the tobacco anther development program [24], the *OsHFP* promoter was found not to be switched-on upto the developmental stage −1. Activation of the *OsHFP* promoter was observed at a specific developmental stage after the microspore separation and in the subsequent developmental stages (Fig. 4). The developmental stages, when *OsHFP* promoter is active, are characterized by gradual degradation of tapetum and connective tissues through PCD, pollen maturation and finally anther dehiscence to release the functional pollens. During the last stage of anther development (45 mm flower bud, Fig. 4), when degradation of connective tissue is almost complete, the reporter gene expression was found to be localized in the vascular bundle of the anther. It is important to mention here that the spatio-temporal regulation of *OsHFP* promoter is significantly similar to that of PCD in tobacco anther tissues. Thus, the transcriptional regulation of the gene advocates a role of OsHFP in PCD during the second phase of anther development.

Taken together, the present study establishes the involvement of a novel hemopexin fold protein OsHFP in rice anther development. The *OsHFP* promoter, characterized in the present study, is found to be active during the second phase of anther development (that leads to anther dehiscence) but not in the first phase. The temporally regulated transcriptional activation of the promoter in heterologous plant system, i.e. transgenic tobacco lines indicates the *OsHFP* gene to have a certain physiological role during the late developmental events in rice anther. Furthermore, the promoter drives reporter gene expression specifically in the anther tissues undergoing PCD. The spatio-temporal regulation of the *OsHFP* promoter during anther development, supplemented by several propositions and observations including the involvement of heme in regulating plant PCD, the heme binding property of OsHFP and the capacity of OsHFP to degrade chlorophyll (the most important visible marker of plant PCD) strongly suggest the involvement of OsHFP in PCD during anther dehiscence of rice. However, the precise molecular mechanism of OsHFP in anther PCD is yet to be established. In addition, the present study characterized and established the activity of the novel anther-specific *OsHFP* promoter in rice (monocot) as well as in tobacco (dicot) systems. Our findings promise that the *OsHFP* promoter would be a useful genetic element in developing transgenic male-sterile lines in different crops for hybrid seed production.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.08.088.

References

[1] J.C. Kamalay, R.B. Goldberg, Regulation of structural gene expression in tobacco, Cell 19 (1980) 935–946.
[2] J.C. Kamalay, R.B. Goldberg, Organ-specific nuclear RNAs in tobacco, Proc. Natl. Acad. Sci. USA 81 (1984) 2801–2805.
[3] R.B. Goldberg, T.P. Beals, P.M. Sanders, Anther development: basic principles and practical applications, Plant Cell 5 (1993) 1217–1229.
[4] Q.-H. Zhu, K. Ramm, R. Shivakkumar, E.S. Dennis, N.M. Upadhyaya, The *ANTHER INDEHISCENCE1* gene encoding a single MYB domain protein is involved in anther development in rice, Plant Physiol. 135 (2004) 1514–1525.
[5] T. Chattopadhyay, S. Bhattacharyya, A.K. Das, M.K. Maiti, A structurally novel hemopexin fold protein of rice plays role in chlorophyll degradation, Biochem. Biophys. Res. Commun. 420 (2012) 862–868.
[6] J.D. Thompson, D.G. Higgins, T.J. Gibson, Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (1994) 4673–4680.
[7] A. Dereeper, V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J.-F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J.-M. Claverie, O. Gascuel, Phylogeny.fr: robust phylogenetic analysis for the non-specialist, Nucleic Acids Res. 36 (2008) W465–W469.
[8] K. Higo, Y. Ugawa, M. Iwamoto, T. Korenaga, Plant cis-acting regulatory DNA elements (PLACE) database, Nucleic Acids Res. 27 (1999) (1999) 297–300.
[9] Y. Lin, D.F. Seals, S.K. Randall, Z. Yang, Dynamic localization of Rop GTPases to the tonoplast during vacuole development, Plant Physiol. 125 (2001) 241–251.
[10] J. Banerjee, M.K. Maiti, Functional role of rice germin-like protein1 in regulation of plant height and disease resistance, Biochem. Biophys. Res. Commun. 394 (2010) 178–183.
[11] T. Chattopadhyay, S. Roy, A. Mitra, M.K. Maiti, Development of a transgenic hairy root system in jute (*Corchorus capsularis* L.) with *gusA* reporter gene through *Agrobacterium rhizogenes* mediated co-transformation, Plant Cell Rep. 30 (2011) 485–493.
[12] V. Gaur, I.A. Qureshi, A. Singh, V. Chanana, D.M. Salunke, Crystal structure and functional insights of hemopexin fold protein from grass pea, Plant Physiol. 152 (2010) 1842–1850.
[13] H. Vigeolas, C. Chinoy, E. Zuther, B. Blessington, P. Geigenberger, C. Domoney, Combined metabolomic and genetic approaches reveal a link between the polyamine pathway and albumin 2 in developing pea seeds, Plant Physiol. 146 (2008) 74–82.
[14] V. Gaur, V. Chanana, A. Jain, D.M. Salunke, The structure of a haemopexin-fold protein from cow pea (*Vigna unguiculata*) suggests functional diversity of haemopexins in plants, Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 67 (2011) 193–200.
[15] M. Watanabe, T. Kono, Y. Matsushima-Hibiya, T. Kanazawa, N. Nishisaka, T. Kishimoto, K. Koyama, T. Sugimura, K. Wakabayashi, Molecular cloning of an apoptosis-inducing protein, pierisin, from cabbage butterfly: possible involvement of ADP-ribosylation in its activity, Proc. Natl. Acad. Sci. USA 96 (1999) 10608–10613.
[16] B. Buckner, D. Janick-Buckner, J. Gray, G.S. Johal, Cell-death mechanisms in maize, Trends Plant Sci. 3 (1998) 218–223.
[17] G. Hu, N. Yalpani, S.P. Briggs, G.S. Johal, A porphyrin pathway impairment is responsible for the phenotype of a dominant disease lesion mimic mutant of maize, Plant Cell 10 (1998) 1095–1105.
[18] A. Molina, S. Volrath, D. Guyer, K. Maleck, J. Ryals, E. Ward, Inhibition of protoporphyrinogen oxidase expression in *Arabidopsis* causes a lesion-mimic phenotype that induces systemic acquired resistance, Plant J. 17 (1999) 667–678.
[19] M.D. Gómez, J.-P. Beltrán, L.A. Cañas, The pea *END1* promoter drives anther-specific gene expression in different plant species, Planta 219 (2004) 967–981.
[20] T. Hobo, K. Suwabe, K. Aya, G. Suzuki, K. Yano, T. Ishimizu, M. Fujita, S. Kikuchi, K. Hamada, M. Miyano, T. Fujioka, F. Kaneko, T. Kazama, Y. Mizuta, H. Takahashi, K. Shiono, M. Nakazono, N. Tsutsumi, Y. Nagamura, N. Kurata, M. Watanabe, M. Matsuoka, Various spatiotemporal expression profiles of anther-expressed genes in rice, Plant Cell Physiol. 49 (2008) 1417–1428.
[21] W. Tang, S.E. Perry, Binding site selection for the plant MADS domain protein AGL15: an *in vitro* and *in vivo* study, J. Biol. Chem. 278 (2003) 28154–28159.
[22] H.Q. Tian, A. Kuang, M.E. Musgrave, S.D. Russell, Calcium distribution in fertile and sterile anthers of a photoperiod-sensitive genic male-sterile rice, Planta 204 (1998) 183–192.
[23] T.P. Beals, R.B. Goldberg, A novel cell ablation strategy blocks tobacco anther dehiscence, Plant Cell 9 (1997) 1527–1545.
[24] A.M. Koltunow, J. Truettner, K.H. Cox, M. Wallroth, R.B. Goldberg, Different temporal and spatial gene expression patterns occur during anther development, Plant Cell 2 (1990) 1201–1224.