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The rice *WUSCHEL*-related homeobox genes are involved in reproductive organ development, hormone signaling and abiotic stress response

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ABSTRACT

The *WUSCHEL*-related homeobox (*WOX*) genes are important transcription regulators participated in plant development processes. Rice (*Oryza sativa* L.) genome encodes at least 13 *WOX* members. In this study, a systematic microarray-based gene expression profiling of eleven *WOX* genes was performed for the whole life cycle of rice at 16 different tissues/organs of MH63 (rice *indica* cultivar), which included eight reproductive organs and eight vegetative tissues. The results demonstrated that four genes (*OsWUS*, *OsNS1/OsNS2*, *OsWOX3* and *OsWOX9A*) were specifically expressed in panicle and endosperm development, and six genes (*OsWOX5*, *OsWOX9B*, *OsWOX9D*, *OsWOX11*, *OsWOX12A* and *OsWOX12B*) were preferentially expressed in seeds (72 h after imbibitions) during root emergence or growth. In situ hybridization analysis revealed differential transcript levels of *OsWOX4*, *OsWOX5*, *OsWOX9A* and *OsWOX12B* during panicle development and embryogenesis. Results of qRT-PCR showed that expression of four rice *WOX* genes (*OsWOX5*, *OsWOX11*, *OsWOX12B* and *OsWOX12A*) was up- or down-regulated by plant hormones (auxin, cytokinin and gibberellin). More interestingly, most *WOX* genes were responsive to abiotic stress stimuli of drought, salt and cold. The molecular studies presented here will further provide insight in understanding the functions of rice *WOX* gene family in rice development, hormone signaling, and abiotic stress response.

1. Introduction

The *WOX* (*WUSCHEL* related homeobox) gene family is one of plant homeobox (HB) transcription factor families, which is characterized by the presence of a short stretch of amino acid residues with the helix–loop–helix–turn–helix structure. It is distinguished by the phylogenetic relatedness of its homeodomains from other HB transcription factor (Gehring et al., 1990). Compared with the animal HOX homeodomain, homology modeling of the plant WOX homeodomain reveals two extended loops between helices 1 and 2 within a generally highly conserved structure (Haecker et al., 2004). In addition to homeodomain, some WOX proteins contain the distinct WUS-box motif that locates carboxy-terminal to the homeodomain (van der Graaff et al., 2009). In *Arabidopsis*, the WUS-box motif is shown to be essential for WUS function in the shoot stem-cell population maintenance or differentiation, lateral organ formation, floral patterning, and embryogenesis (Haecker et al., 2004; Kamiya et al., 2003; Skylar et al., 2010).

Analysis of *WOX* gene expression, function and evolution in a variety of plant species indicates that they play important roles in regulating key development of plant tissues and organs by determining cell fate, such as shoot/root apical meristem (SAM/RAM) cell maintenance or differentiation, lateral organ formation, and embryogenesis (Chandler et al., 2008; Hedman et al., 2013; Nardmann and Werr, 2006; Ten Hove and Heidstra, 2008; Zhang et al., 2010). For instance, *Arabidopsis WUSCHEL* (*WUS*) is the founding member of this family, which is found to be specifically required in maintaining central meristem identity of shoot and floral meristem structural and functional integrity (Haecker et al., 2004; Laux et al., 1996; Mayer et al., 1998). *AtWOX5* performs a similar function in the root apical meristem and is specifically expressed in the cells of quiescent center (QC) of root (Gonzali et al., 2005; Sarkar et al., 2007). *PRESSED FLOWER1* (*PRS1/AtWOX3*) is involved in the development of lateral and marginal regions of leaves and leaf orthologs of the flower by recruiting founder cells from all meristem (Matsumoto and Okada, 2001; Shimizu et al., 2009). While another member of Arabidopsis *WOX* genes, *PRETTY FEW SEEDS2/WOX6* affects either ovules patterning by regulating cell proliferation of the maternal integuments or differentiation of megaspore mother cell (MMC) (Park et al., 2005). *STIMPY1/WOX9* is required for maintaining cell division and preventing premature differentiation in vegetative shoot apical meristem (SAM) and early embryogenesis in *Arabidopsis* (Skylar et al., 2010; Wu et al., 2007, 2005). *Arabidopsis WOX2* and *STIMPY-LIKE1/WOX8* (*STPL1*) are important cell fate regulators of early pre-embryo

Abbreviations: *WOX* gene, *WUSCHEL* homeobox gene; SAM/RAM, shoot/root apical meristem; PAT, polar auxin transport; DAP, day after pollination.

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and cotyledon boundary formation (Breuninger et al., 2008; Haecker et al., 2004; Lie et al., 2012; Wu et al., 2007). In *Arabidopsis* and tomato, accumulation of *WOX4* mRNA is found in the developing vascular bundle of root and shoot lateral organs, which promotes differentiation and/or maintains the vascular procambium (Ji et al., 2010). *WUS* homolog *ZmWUS1* is expressed in a few cells underlying the emerging coleoptile and in the seedling SAM after germination, while *ZmWUS2* is activated in the P1 leaf primordium in *Maize* (Nardmann and Werr, 2006). *PaWOX2* is found to be regulated by auxin polar transport (PAT), and *PaWOX8* and *PaWOX9* are involved in zygote and embryo development in conifers (Palovaara and Hakman, 2009; Palovaara et al., 2010). Recent results also show that *WOX* genes have redundant functions in plant development. For example, *WOX4* acts redundantly with *WOX14* in regulating vascular cell division (Etchells et al., 2013). *WOX11* acts redundantly with its homolog *WOX12* to function in the first-step cell fate transition during de novo root organogenesis (Liu et al., 2014). In green algae, bryophytes, lycophytes, fern and gymnosperm, many *WOX* complete genome sequences provide an unprecedented opportunity to explore the diversity among this class of protein in various organisms and to study their roles in regulating critical developmental processes at the whole genome level.

Based on the above-mentioned observations that *WOX* genes play important roles in regulating plant development in a variety of species, presumably rice *WOX* gene function would be also important for programming rice development and growth regulation. By far at least 13 *WOX* genes are found in rice (*Oryza sativa* L.) genome (Zhang et al., 2010). Among them, *WOX5*, also named *QHB*/*OsWOX9*, which is a homolog of *AtWOX5*, is involved in specification and maintenance of QC cell in root apical meristem (Cho et al., 2013; Kamiya et al., 2003). *WOX11* is shown to be induced by exogenous auxin or cytokinin and involved in activation of crown root growth and development (Zhao et al., 2009). *OsWUS* is expressed at the abaxial face of the auxiliary bud, not expressed in the organizing center (OC) of the vegetative SAM (Nardmann and Werr, 2006). *OsWOX3* (or *OsNS2*), which is homolog of *AtWOX3* (or *PRS*), is expressed in the leaf and floral organ primordia and its expression is shown to be required for leaf development (Cho et al., 2013; Dai et al., 2007; Ishiwata et al., 2013). Recently, *WUSCHEL-related homeobox4* (*WOX4*), which is associated with cytokinin action, is involved in the maintenance of vegetative and reproductive meristem in rice (Ohmori et al., 2013). *DAWAR TILLER*, a *WUSCHEL*-related homeobox transcription factor is required for rice tiller growth (Wang et al., 2014). However, the functions of the other rice *WOX* genes are not clear.

Because plant growth and development are also controlled by external conditions such as abiotic stress and hormones that work through impacting the expression of development-related genes, it would be interesting to know whether *WOX* gene expression is regulated by abiotic stress and plant hormones. In the present study, an attempt was made to gain insight into the expression pattern of rice *WOX* genes during seed germination, panicle development, and embryogenesis, responsiveness to plant hormones and abiotic stresses. Utilizing a microarray-based gene expression data, expression profiles of eleven *WOX* genes were compiled from tissues of the seed, vegetative organs, panicle and endosperm, together with seedlings subjected to plant hormones (GA, NAA and 6-BA) and abiotic stresses including cold, NaCl and drought. These results will be greatly useful for further exploring the developmental and regulatory function aimed at understanding the roles of rice *WOX* genes.

2. Results

2.1. Expression profiles of rice WOX genes during the entire life cycle

To gain insight into the developmental windows during which rice *WOX* genes are expressed, spatial and temporal expression profiles of these genes were analyzed in different tissues/organs. For this purpose, affymetrix microarray data were collected from Rice Expression Profiles database (http://crep.ncpgr.cn), in which the expression profiles of whole rice genome were available for various tissues/organs collected during the entire life cycle of rice. In this study, a total of 16 representative tissues/organs, including eight from vegetative tissues and eight from reproductive organs, were selected for expression level analysis of *WOX* genes. Eleven *WOX* genes had corresponding probe sets on the Affymetrix Genechips.

Based on the normalized hybridization signals in 16 tissues/organs including the seed (72 h after imbibitions), radicle (48 h after emergence), root, leaf, flag leaf, panicle and endosperm, almost all of the studied *WOX* genes showed tissue-/organ-specific expression patterns (Fig. 1). According to the analysis, expression patterns of the eleven genes could be classified into two types: reproductive organ-preferential and vegetative organ-preferential. *OsWUS*, *OsNS1*/*OsNS2*, *OsWOX3*, *OsWOX4* and *OsWOX9A* predominantly expressed in reproductive tissues such as young panicles at stages 3–5 and heading stage, spikelet at 3 days after pollination, and endosperm (7, 14 and 21 days after pollination, DAP) (Fig. 1). In reproductive tissues/organs, *WOX* genes had different expression levels. For example, *OsWOX3* was mainly expressed in the spikelet (3 DAP) and endosperm (7 and 14 DAP). Expression pattern of *OsWUS* was similar to that of *OsWOX4*, they had higher transcription level in panicle at stages 4 and 5, and heading time. *OsNS1*/*OsNS2* and *OsWOX9A* were expressed during reproductive organ formation processes except 21-day endosperm after pollination. Additionally, *OsWOX4* accumulated more mRNA in flag leaf and *OsWOX9A* was highly expressed in the seed (72 h after imbibitions) (Fig. 1). Six genes (*OsWOX9D*, *OsWOX12B*, *OsWOX12A*, *OsWOX5*, *OsWOX11* and *OsWOX9B*) seemed to be preferentially expressed in the seed at 72 h after imbibitions, and radicle (48 h after emergence) and root (seedling with two tillers) in comparison with panicle and endosperm (Fig. 1). The results indicated that expression of *WOX* genes in rice were specific to tissues/organs.

2.2. Rice WOX genes involved in floral organ development

To further validate the microarray data, panicle at different developmental stages were selected and detail tissue expression levels of ten genes were tested by qRT-PCR. The results showed that *OsWUS*, *OsNS1*/*OsNS2*, *OsWOX4*, *OsWOX5* and *OsWOX9B* were found to be expressed highly in young panicle at early stage (3–5 and 4–5 cm), while transcripts for *OsWOX3*, *OsWOX9A* and *OsWOX11* showed maximum accumulation in spikelet (3 DAP), stamen at one day before flowering (Fig. 2). *OsWOX4*, *OsWOX5*, *OsWOX9A*, and *OsWOX12B* were further confirmed by in situ hybridization. The transcript accumulation patterns for all four genes observed were similar to those observed from microarray analysis (Figs. 1 and 3). *OsWOX4*, *OsWOX9A* and *OsWOX12B* mRNA had more accumulation in floral primordium (Fig. 3A, G, J) and in gynoecium primordium (gp) and developing stamen (st) (Fig. 3B, H and K). *OsWOX5* transcripts were only observed in floral primordium (fp) (Fig. 3D). In mature flower, only *OsWOX9A* had weaker mRNA signal in anther (Fig. 3I). These results suggested that WOX family proteins played different roles in the panicle formation process and might have redundancy functions.

2.3. Expression pattern of rice WOX genes in embryogenesis

In order to know the functions of *WOX* family genes in rice embryogenesis, four *WOX* gene (*OsWOX4*, *OsWOX5*, *OsWOX9A* and *OsWOX12B*) transcripts during embryogenesis were detected by in situ hybridization. *OsWOX4* and *OsWOX12B* mRNA had similar distribution, and were transcribed specifically in leaf primordium (lp) and margin of root primordium (rp) in mature embryo (7-DAP) (Fig. 4C, L). Weaker expressions of *OsWOX4* and Os*WOX12B* mRNA were detected in 1-DAP and 3-DAP embryos (Fig. 4A, B, J and K). *OsWOX5* was expressed in early embryo development, such as in embryo proper of 1-DAP embryo (Fig. 4D) and in