Wall associated kinases from plants – an overview

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ABSTRACT

Wall Associated kinases (WAKs) represent a unique class of receptor-like kinase genes that span the plasma membrane and allow cells to recognize and respond to their extracellular environment. 26 WAK/WAK-like genes were identified from the *Arabidopsis* genome. Functional studies of the different WAK members in *Arabidopsis* demonstrated that they are involved in various functions in plants, including pathogen resistance, heavy-metal tolerance and plant development. 125 genes from rice (subsp. *Japonica*) belonging to wall associated kinase gene family were identified by reiterative database searches. We isolated a new member of WAKs in rice, designated as *OsiWAK1*, the silencing of which led to impaired root development and sterility due to anther indehiscence. In the current review, we discuss about the isolation and identification of WAK members from various plant species, different domains found in the WAK proteins that make them unique and the various roles played by WAKs in the plant growth and development. [Physiol. Mol. Biol. Plants 2008; 14(1&2) : 109-118] E-mail : akg54@yahoo.com

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The plant cell wall is composed of a complex network of cellulose microfibrils, polysaccharides, and glycoproteins (Carpita and Gibeaut, 1993). Cell wall components are made and secreted by their enclosed or adjacent cells and in return define the cell's immediate environment and shape. It is proposed that components of the cell wall interacts with the plasma membrane and the cytoplasm and the inter-connections between them are pivotal in influencing cell shape, cell size, cell differentiation, and cell death (Carpita *et al.*, 1996; Braam, 1999; Kohorn, 2000). The cell wall not only defines the structural and morphological identity of the plant cell but also mediates how plant cells interact with each other to maintain organismal integrity and interact with the environment to survive (Cosgrove, 1997).

The cell wall contains numerous potential signaling molecules, including small peptides, proteins, and oligosaccharides, which may interact with receptor molecules on the cell surface to elicit cellular responses (McCarty and Chory, 2000; Torii, 2004). The connectivity of the cell wall components also plays an important role in growth and development. The relaxation of the cell wall

can have a direct and profound effect on plant morphogenesis (Fleming *et al.*, 1997). Changes of cell wall architecture are sensed through direct physical links between the cell wall and the plasma membrane, and these links have been proposed to play important roles in plant cell communication and plant development. Together with the cytoskeletal network and the plasma membrane, the cell wall forms a molecular continuum that is thought to be essential in determining the direction of cell expansion and, ultimately, the cell's final shape (Wyatt and Carpita, 1993; Roberts, 1994; Wymer and Lloyd, 1996; Kohorn, 2000).

In animal cells, a class of plasma membrane receptors called integrins mediates the attachment of extra cellular matrix (ECM) to the plasma membrane *via* interactions with adhesive glycoproteins, such as fibronectin, vitronectin and collagen (Hay, 1981; Hynes, 1981; 1987). In plant cells, proteins immunologically related to vitronectin and fibronectin have been identified in salt-adapted cell walls (Zhu *et al.*, 1993). Associations between the ECM and the plasma membrane have also been observed (Pont-Lezica *et al.*, 1993). Nevertheless, the nature of the molecular connections between the cell wall and the cytoplasm is not very clear, and exactly how the molecular continuum functions in cell expansion is largely unknown. Only a few proteins have been identified that have the potential to link these two compartments (Baluska *et al.*, 2003; Kohorn, 2000;

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Kohorn, 2001), which include cellulose synthases (Pear *et al.*, 1996), arabinogalactan proteins (AGP's) that are reversibly attached to the plasma membrane *via* a glycosyl phosphatidyl inositol anchor (Oxley and Bacic, 1999; Svetek *et al.*, 1999), a glycolytic enzyme with transmembrane domain (Nicol *et al.*, 1998), Wall associated kinases (WAKs, Wagner and Kohorn, 2001), the proline rich extension-like receptor kinases (PERKs, Nakhamchik *et al.*, 2004), the formins (Deeks *et al.*, 2002) and COBRA (Schindelman *et al.*, 2001). Apart from the above proteins, several other cell surface receptors generally termed as Receptor Like Kinases (RLKs) are also located in the plasma membrane. RLK's are characterized by the presence of an N-terminal signal sequence, an extracellular domain that varies in structure, a single membrane-spanning region, and a cytoplasmic protein kinase catalytic domain (Shiu and Bleecker, 2001) and play a vital role in cell signaling in both plants and animals (McCarty and Chory, 2000; Gomez-Gomez and Boller, 2002; Clouse, 2002; Schafer and Schmulling, 2002; Scheer and Ryan, 2002; Stracke *et al.*, 2002; Rojo *et al.*, 2002; Kemp and Doughty, 2003; Torii, 2004). Here an attempt has been made to review the current status of work done on Wall Associated Kinases, a unique group of RLK's from plants.

Wall Associated Kinases (WAKs)

A small subset of RLKs has been identified as Wall Associated Kinases (WAKs) as they are found to be tightly bound to the cell wall (He *et al.*, 1996). *Arabidopsis* has five isoforms of WAK genes, WAK1-WAK5, that are clustered (30 kb gene cluster) on the chromosome 1 (He *et al.*, 1999). The existence of WAKs is not unique to *Arabidopsis* since protein gel blot analysis using the WAK1 antibody as probe revealed immunologically related proteins in Pea (*Pisum sativum*), Tobacco (*Nicotiana tabacum*) and maize (*Zea mays*) (He *et al.*, 1996; Gens *et al.*, 2000).

Structure of WAKs

All the five isoforms of *Arabidopsis* WAK proteins are predicted to have a similar organization, with a highly conserved Serine/Threonine protein Kinase domain (86 % similarity) on the cytoplasmic side of the plasmamembrane and an extracellular domain, the sequence of which varies among the five genes. This transmembrane structure suggests that WAKs serve a signaling function between the cell wall compartment and the cytoplasm, analogous to one of the RLK, CLAVATA1, which has been hypothesized to function as a sensor of extracellular signals to regulate meristem size (Trotochaud *et al.*, 1999; Clark *et al.*, 1997). The

aminoacid sequences of all five WAKs extracellular domains share only 40 % to 64 % identity. All WAK proteins have epidermal growth factor (EGF) like repeats located at the amino-terminal side adjacent to the transmembrane domains. Presence of two types of EGF repeats namely EGF-2 and EGF-Ca²⁺ were reported in *Arabidopsis* WAKs. The positions of the six cysteins found in the EGF repeats are conserved in all the five WAKs, although the aminoacid sequences between the cysteins vary. The EGF fold is generally found in peptide hormones, and also in variety of other metazoan proteins that are usually cell surface or secreted (Zushi *et al.*, 1989) except for a vacuolar sorting receptor BP-80 of angiosperms, which is cytosolic (Cao *et al.*, 2000). EGF-like domains in some proteins from animals were shown to be involved in protein-protein interactions (Appella *et al.*, 1987; Kuroda and Tanizawa, 1999; Stenberg *et al.*, 1999). It is not known whether the EGF-like domains in WAK proteins are involved in protein-protein interactions.

WAK isoforms also contain in their extracellular domains limited aminoacid identities to proteins, which are exclusively found in the ECM of mammalian cells (Figure 1). For example WAK2 show limited identities to collagen type IV and neurexin sequences whereas, WAK4 show identity with type VI collagen and tenascin sequences. The significance of these similarities is not clear but it is proposed that this region of identity may describe a domain that enables the extracellular domains of WAK protein to function in a carbohydrate rich environment and may be involved in protein-protein interactions and oligomerization (He *et al.*, 1999).

Fig. 1. Comparative cartoon of the five WAK proteins from *Arabidopsis*. The transmembrane region separates the extracellular domain (in the ECM) from the serine/threonine kinase domain (in the cytoplasm). Domains that share sequence identities with other proteins in the database are indicated.

Another unique feature of all WAK1-5 is the presence of Asp/Asn hydroxylation site (Cx[DN]x(4)[FY]xCxC; Prosite PS00010) overlapping with calcium binding EGF domains. Many animal coagulation proteases with calcium-binding EGF domains also have an associated Asp/Asn hydroxylation site (Sunnerhagen *et al.*, 1992). It was initially thought that hydroxylation of the conserved Asp/Asn residue was required for calcium binding; however, both hydroxylated and nonhydroxylated forms of coagulation proteases have equal affinities for calcium at physiological concentrations (Sunnerhagen *et al.*, 1993). As such, the role of this posttranslational modification is presently unclear. It is speculated that the hydroxyl group may be involved in hydrogen bonding in protein-protein interactions mediated by the EGF-like domain (Stenflo *et al.*, 2000).

WAK-Cell Wall Interactions

The linkage of WAKs to the cell wall was first demonstrated by immunolocalization using antibodies against the conserved kinase domain (He *et al.*, 1996). WAK epitopes were found to be tightly associated with cell-wall fragments; that they could not be released by detergents that solubilized membranes, but were released by boiling the walls in 4 % SDS and 50 mM dithiothreitol (a strong thiol reductant) or by treatments with protoplasting enzymes that digested the cell wall or more precisely by a pectinase treatment (Wagner and Kohorn, 2001). This suggests that WAK1 might be covalently bound to pectin. Pectins are complex oligopolysaccharides that form a hydrophilic gel-like matrix between the cellulose microfibrils, and can be concentrated in different regions of the cell wall (Carpita and Gibeau, 1993; Cosgrove, 2001). The interaction between WAK1 and pectins was confirmed by using anti-WAK1 and anti-pectin JIM5 and JIM7 antibodies. Both the antibodies recognized the same 68 kDa protein band in western blots of the cell wall proteins extracted from pectinase-treated cell walls (Wagner and Kohorn, 2001). This pectin-kinase hybrid is the first of its kind to be identified, and it seems ideally situated for reporting to the cytoplasm on the state of the cell wall. Various enzyme-linked immunosorbent assays (ELISA's) showed that the extracellular domain of WAK1 is bound in a calcium-induced conformation to polygalacturonic acid, oligogalacturonides and pectins extracted from cell walls. And this interaction was prevented by methyl esterification, calcium chelators and pectin depolymerization (Decreux and Messiaen, 2005; Decreux *et al.*, 2006). Although, it has been shown that oligogalacturonides bind to cell walls (Mathieu *et al.*,

1998) and plasmamembranes (Reymond *et al.*, 1995), no direct link has been made between this binding, a putative oligogalacturonide receptor and biological response induced by pectin oligomers. It is shown that the interaction of pectin polyanion with the cell wall or plasmalemma located basic proteins like extensin could induce conformational changes in the pectin polymers that affect their gelling and swelling behavior in presence of the calcium (Mac Dougall *et al.*, 2001). In the similar fashion, the binding of pectins to WAK1 in the presence of calcium could result in *in muro* disturbances of the pectin network, which in turn could generate signals within the cell wall. These results show that WAK1 might belong to one of the perception mechanisms used by the plant cell to sense directly or indirectly the pectins deposited in the cell wall and to respond efficiently to environmental cues that alter pectin content, structure and biological activity. Recent studies indicated that WAK1 is crosslinked in endomembranes, and its transport to the cell surface requires correct cell-wall synthesis (Kohorn *et al.*, 2006 b).

WAK-ligands and substrates

Identification of endogenous WAK ligands and the conditions, which activate or inactivate WAKs and the phosphorylation targets of WAK action could be useful in understanding how WAKs might interact with the cells growth machinery. Two-hybrid and *in vitro* binding experiments using a recombinant subdomain (amino acids 178–334) of the extracellular region of WAK1 allowed the identification of a secreted glycine-rich protein (*At*GRP3) that specifically interacts with WAK1. Moreover, exogenous GRP3 induced the transcription of WAK1, *At*GRP3 and PR1 genes in *Arabidopsis*. It was also shown that the WAK1–GRP3 complex interacts with the intracellular kinase-associated protein phosphatase (KAPP) to form a 500 kDa complex (Park *et al.*, 2001), which is similar to the complexes involved in the regulation of meristem identity (Trotochaud *et al.*, 1999). The putative signaling role of this WAK1–GRP3–KAPP complex is largely unknown. One of the possible targets of this complex could be the chloroplast oxygen-evolving enhancer protein 2 (OEE2), which becomes phosphorylated in a WAK1/GRP3-dependent manner in *Arabidopsis* (Yang *et al.*, 2003). The physiological significance of this interaction is unknown.

Expression profile of WAKs

From northern blots, the reverse transcription – polymerase chain reaction (RT-PCR), *in situ* hybridizations and experiments using promoter-reporter gene constructs, the WAK genes have been shown to be

selectively and differentially expressed in *Arabidopsis* (He *et al.*, 1999; Wagner and Kohorn, 2001). The WAK1 and WAK2 promoters were shown to direct partially overlapping expression in growing tissues, such as young seedlings, meristems and young leaves. The WAK3 promoter was found to be active in irregular 'splotches' in leaves and stems, suggestive of lesions, whereas the WAK4 and WAK5 promoters were not strong enough to give good signals with the β -glucuronidase gene as the reporter. But RT-PCR analysis indicated that WAK4 and WAK5 are expressed in fruits and roots, respectively (He *et al.*, 1999).

Of all the isoforms, WAK1 is the most studied receptor kinase. WAK1 was shown to be induced by *Pseudomonas syringae* infection, exogenous application of salicylic acid (SA) and its analog, 2,2 dichloro isonicotinic acid (INA) (He *et al.*, 1998), methyl jasmonate, ethylene (Schenk *et al.*, 2000) and by aluminum treatment (Sivaguru *et al.*, 2003). Whereas its expression was not altered by either high temperatures or high salt concentrations and was downregulated by wounding (He *et al.*, 1998).

Functions of WAKs in Arabidopsis

To assess the physiological functions of WAKs, several attempts were made to reduce expression by antisense methods (Anderson *et al.*, 2001). Initial attempts with the constitutive 35S CaMV35S promoter failed to yield any viable transgenic plants. Subsequent attempts were made with inducible promoters like PR1 promoter and/or glucocorticoid-inducible promoter (He *et al.*, 1998; Lally *et al.*, 2001).

The role of WAK1 in pathogen response was studied in detail since it was shown to be induced by pathogen, SA and INA. WAK1 was shown to be a pathogenesis related (PR) protein since its induction requires NPR1 (Nonexpressor of PR) genes, a positive regulator required for the SA-dependent activation of previously identified PR genes (Cao *et al.*, 1997; He *et al.*, 1998). Elimination of WAK1 mRNA induction *via* antisense WAK1 expression (under PR1 promoter) resulted in plants that could not survive on otherwise non-toxic levels of INA or SA. Similar results were obtained by the expression of dominant negative allele of WAK1 in *Arabidopsis*. Ectopic expression of the entire WAK1 or the kinase domain alone was shown to provide resistance to otherwise lethal SA levels (He *et al.*, 1998), but not to infection by *P. syringae*, suggesting that WAK1 may not be interacting with pathogen itself, but rather may be required by the plant during the response to pathogen. Overexpression of WAK1 in transgenic

Arabidopsis plants was shown to confer increased aluminium tolerance (Sivaguru *et al.*, 2003).

The later attempts to address this issue (functions of WAK) yielded the results that connect WAK function with cell enlargement (Wagner and Kohorn, 2001; Lally *et al.*, 2001). In both the studies, glucocorticoid (dexamethasone) inducible promoter was used to drive the expression of antisense WAK2 and WAK4 genes.

Lally *et al.* (2001) used a full-length antisense construct for WAK4. After the induction with dexamethasone, WAK protein gradually decreased, becoming undetectable 96 hours after induction of the antisense gene. In these experiments expression of all the WAK genes reduced since the full length antisense sequence included the highly conserved kinase domain. The induction of WAK4 antisense expression by dexamethasone resulted in a strong developmental arrest of *Arabidopsis* plants: seeds germinated adequately, but elongation of the hypocotyls and the root was blocked, as was the expansion of the leaves. Under the range of dexamethasone concentrations, from 0.001 mM to 1 mM, increasingly severe inhibition was obtained, and the severity of the growth arrest roughly correlated with the degree of suppression of WAK protein accumulation. Induction of antisense WAK4 in older plants similarly arrested growth and led to early senescence. In brief, the suppression of WAK protein expression led to the plants with small rosette leaves, condensed inflorescence stems, short siliques, unopened miniature flowers, and short primary roots. Microscope examinations revealed that cell enlargement was inhibited in the dexamethasone-treated plants. Lally *et al.*, (2001) also found that dexamethasone-treated seedlings had reduced levels of α -expansin. As expansins are key catalysts of cell-wall enlargement, this finding offers a potential explanation for the reduction in cell enlargement, that is, *via* a reduction in expansin gene expression.

Similarly, expression of an inducible antisense WAK2 in *Arabidopsis* leaf cells led to a 50 % reduction in WAK protein levels, with a subsequent loss of cell elongation, and hence dwarf plants (Wagner and Kohorn, 2001). These results indicate that WAKs are necessary for normal cell enlargement. It was unclear as to how WAKs interact with the biochemical processes that regulate plant cell enlargement. It was hypothesized that the WAKs may be affecting the synthesis and secretion of cell wall structural components and loosening enzymes, H⁺ pumping to reduce wall pH for optimal activity of wall-loosening enzymes and turgor regulation. Recent works indicate that the WAKs may be effecting the turgor regulation and/or secretion of cell wall materials. The

insertional mutation in WAK2 that reduced the expression of only WAK2 gene affected the rate of cell elongation in roots and hence reduced the overall root length. This insertional mutation in WAK2 also resulted in dependence on a combination of metabolized sugars and salts for seedling growth. These mutant plants also showed reduced vacuolar invertase activity, and a corresponding decrease in steady state levels of mRNAs specific for the most prominent of the vacuolar invertases (Kohorn *et al.*, 2006 a). Thus WAK2 was thought to regulate either directly or indirectly the transcription and activity of vacuolar invertase as one of the constituent of a mechanism modulating solute concentrations. As changes in the concentration of solutes are known to be involved in turgor regulation (Koch, 2004), this provides a possible mechanism for WAK to regulate cell expansion. Cells with insufficient vacuolar invertase activity would be compromised in one avenue of turgor maintenance, thus leaving them vulnerable to insufficient turgor during expansion, and hence slow or arrested growth. As a cell expands, there is increased need for a compensatory adjustment in turgor, and invertase activity can provide a critical source of organic solutes. So it is hypothesized that WAKs may sense cell wall expansion by their attachment to pectin, and provides a mechanism for transducing these signals to systems regulating solute changes. This in turn can influence the maintenance of turgor as the cell enlarges.

Wall-Associated Kinase like genes-Arabidopsis

Apart from WAKs, Verica and He (2002) reported the identification of 22 genes similar to WAKs in *Arabidopsis* by reiterative database searches (BLAST) using the WAK1 cDNA or WAK1 protein sequence as query. These genes were designated as WAK-like (WAKL) genes. Among these 22 genes, five genes (WAKL7, WAKL8, WAKL12, WAKL16 and WAKL19) were predicted to encode abbreviated WAKL proteins. The remaining 17 genes were predicted to encode a transmembrane protein containing a cytoplasmic STK (ser/thr kinase) and an extracellular region containing calcium-binding EGF-like domain (EGF-Ca²⁺ and/or an EGF-2 domain). It was reported that the EGF-like domains were slightly degenerate in some WAKL genes. The cysteine residues predicted to be involved in the formation of disulphide bridges were conserved, however, the spacing between them was off by one or two residues or other hallmark residues characteristic of EGF-like domains were changed. The effect of this degeneracy in EGF domains on the potential function of the WAKL proteins is still unclear. Several proteins were shown to have an auto phosphorylation activity and shown to localize in the cell

wall, suggesting that like WAKs, WAKLs were also tightly associated with the cell wall. All the 17 non-abbreviated WAKL genes were shown to have three exons and two introns, similar to the intron-exon organization of WAKs. Both the WAKs and WAKLs were grouped together to form a large WAK/WAKL gene family in *Arabidopsis*, consisting of 26 members.

WAK/WAKL gene family members in *Arabidopsis* were divided into four groups (Figure 2) based on the pair-wise comparisons of their predicted protein sequences. WAK1 to WAK5 containing EGF-Ca²⁺ domain with an overlapping Asp/Asn hydroxylation site and an EGF-2 domain were placed in Group I. Both the EGF domains were predicted to be completely encoded by the second exon.

Seven WAKL members that include WAKL1 to WAKL6 and WAKL22 were placed in Group II. In all these group II genes, the EGF-Ca²⁺ and EGF-2 domains are separated by a short gap of 15-18 aa and were reversed in order relative to group I. The EGF-Ca²⁺ domain is encoded by first exon and EGF-Ca²⁺ domain is encoded by second exon. No Asn/asp hydroxylation site was predicted.

Group III contains six members: WAKL9, WAKL10, WAKL11, WAKL13, WAKL17, and WAKL18. Their corresponding proteins all contain EGF-Ca²⁺ and EGF2 domains, and they are structurally similar to the Group II WAKLs. In WAKL13, the EGF-Ca²⁺ domain is degenerate. With the exception of WAKL17, all have degenerate EGF2 domains.

Fig. 2. Intron-exon structure is conserved between the WAK and WAKL genes in *Arabidopsis*. The diagram shows a standardized depiction of a WAK or WAKL gene from each of the four groups (I-IV). Exons are represented by boxes. Introns are represented as 'V's. Regions of each gene encoding functional domains are indicated with shaded boxes: N-terminal signal sequence (black), EGF2-like domain (red), calcium-binding EGF domain (blue), transmembrane domain (green), and Ser/Thr protein kinase active site (orange).

Group IV contains four members: WAKL14, WAKL15, WAKL20, and WAKL21. Each has an EGF2 domain encoded by the first exon. This domain is degenerate in both WAKL20 and WAKL21. All four members lack the EGF-Ca²⁺ domain. In addition, each has a cytoplasmic protein kinase ATP-binding domain (PS00107). The remaining sequences (WAKL7, WAKL8, WAKL12, WAKL16, and WAKL19) are predicted to encode abbreviated WAKL proteins. WAKL7, WAKL8 and WAKL19 are similar to various other WAKLs in their extracellular regions, and lack a transmembrane domain. WAKL8 and WAKL9 both contain an EGF-Ca²⁺ domain and WAKL19 contains a degenerate EGF2 domain. Neither of these domains is present in WAKL7. WAKL12 also contains an EGF-Ca²⁺ domain, but unlike WAKL8, it contains a trans-membrane domain. WAKL16 contains a transmembrane domain, an STK domain that is most similar to WAK3, and a short extracellular domain of eight amino acids that lacks both of the EGF-like domains.

There is a possibility that all these genes may play a role in the formation of an active WAKL receptor complex. A similar role has been proposed for SCR/SP11 (S cysteine-rich/ pollen S determinant) in the s-locus receptor kinase (SRK) receptor complex in self-incompatible *Brassica* spp. (Schopfer *et al.*, 1999; Takayama *et al.*, 2000). Alternatively, these sequences may encode nonfunctional WAKL isoforms. Bacterial expression of several WAKL members shows that they have auto-phosphorylation activity, and analysis of several of the WAKL proteins shows that they localize to the cell wall (Verica and He, 2002). This suggests that the WAKLs, like the WAKs, are protein kinases that are tightly associated with the cell wall.

The WAK and WAKL genes are distributed among all five chromosomes, with the majority being present on chromosome I. The remaining six genes are distributed more or less evenly among chromosomes II through V (Table 1). Closer examination of the *Arabidopsis* genome reveals several potential explanations for the expansion of the WAK gene family. For example, the arrangement of and the high sequence similarity between the genes in the WAKL1 through WAKL6 cluster suggest that they might have arisen *via* tandem duplication or by retrotransposons since the WAK/WAKL genes are flanked by copia-like elements. A second possibility may be because of extensive duplication of large chromosomal segments in *Arabidopsis* (*Arabidopsis* Genome Initiative, 2000; Blanc *et al.*, 2000) since several of the WAKL genes are contained within these regions. The high degree of sequence similarity between these genes is consistent with this possibility.

Table 1. The Ara WAK and WAKL genes are distributed among all five chromosomes of *Arabidopsis*.

Chromosome number	Genes localized
I	WAKL1-13, WAKL22, WAK1-5
II	WAKL14
III	WAKL15, WAKL16
IV	WAKL17, WAKL18, WAKL19
V	WAKL20, WAKL21

Verica *et al.*, 2003 have characterized group II members that are composed of a cluster of seven tandemly arrayed WAKL genes. The predicted WAKL proteins are highly similar in their cytoplasmic region but are more divergent in their predicted extracellular ligand-binding region. WAKL7 encodes a truncated WAKL isoform that is predicted to be secreted from the cytoplasm. Ratios of nonsynonymous to synonymous substitutions suggest that the extracellular region is subject to diversifying selection. Comparison of the WAKL and WAK gene clusters suggests that they arose independently. Protein gel-blot and immunolocalization analyses suggest that WAKL6 is associated with the cell wall. Histochemical analyses of WAKL promoters fused with the b-glucuronidase reporter gene have shown that the expressions of WAKL members are developmentally regulated and tissue specific. Unlike WAK members whose expressions were found predominately in green tissues. WAKL genes are highly expressed in roots and flowers. The expression of WAKL5 and WAKL7 can be induced by wounding stress and by the salicylic acid (SA) and its analog 2, 6-dichloroisonicotinic acid (INA) suggesting that they, like some WAK members, are wound inducible and can be defined as pathogenesis-related genes. WAKL4 was shown to have versatile role in *Arabidopsis* mineral nutrition responses. WAKL4 expression was induced by Na⁺, K⁺, Cu²⁺, Ni²⁺, and Zn²⁺. WAKL4 promoter impairment abolished WAKL4-induced expression by all the metal ions except the Ni²⁺. And this resulted in hypersensitivity of the plants to Na⁺, K⁺, Cu²⁺ and Zn²⁺, and better tolerance to Ni²⁺. WAKL4 also upregulates zinc transporter genes during zinc deficiency and the WAKL4 T-DNA insertion resulted in a reduction of Zn²⁺ accumulation in shoots (Hou *et al.*, 2005; Verica *et al.*, 2003).

Wall-Associated Kinase Gene Family-Rice

Zhang *et al.*, in 2005 had done much work on the evolutionary expansion, gene structure and expression of the rice Wall-Associated Kinase gene family. Detailed *in silico* analysis of rice genome (*japonica* cv

Nipponbare) revealed the presence of 125 genes belonging to WAK/WAKL (*OsWAK*) gene family. Out of these, 67 are *OsWAK*-RLKs (Receptor-like kinases) containing both extracellular EGF-like domains and an intracellular kinase domain; 28 are *OsWAK*-RLCKs (Receptor-like cytoplasmic kinases) containing only the kinase domain; 13 are *OsWAK*-RLPs (receptor-like proteins) with only the extracellular EGF-like domains; 12 are *OsWAK* short genes lacking both the domains but has > 40 % identity at the amino acid level with other *OsWAK* members; and five are pseudogenes (with either stop codons or frame shifts in coding region). Functions of these various *OsWAK*s are yet to be determined.

In addition to the availability of around 38 full length WAK cDNA sequences and 11 expressed sequence tags, expression analysis of 20 *OsWAK*s on chromosome 10 using tiling microarray analysis and the RT-PCR analysis of five *OsWAK*s indicate that the majority of these identified genes are likely expressed in rice (Zhang *et al.*, 2005). In our lab we isolated a new member of *OsWAK* from *indica* rice, designated as *OsiWAK1* (*Oryza sativa indica* wall associated kinase 1; Vydehi, 2007). It was found to show tissue specific expression of its transcript in the rice roots and was downregulated by various stress conditions like, salinity, drought and salicylic acid. However, application of exogenous proline increased the levels of *OsiWAK1* accumulation in rice seedlings. An attempt was made to functionally characterize this gene by transcriptional silencing of the endogenous *OsiWAK1* gene in rice. The results indicated that the root development was severely affected in the silenced plants, and were dwarf as compared to control wildtype plants. The silenced plants also exhibited sterility phenotype due to anther indehiscence and reduced pollen viability. So as in *Arabidopsis*, the *OsWAK*s from rice is also involved in the regulation of plant developmental processes.

The two-intron gene structure pattern of *Arabidopsis* WAK/WAKLs (Verica and He, 2002) was generally conserved in *OsWAK*s. However, *OsWAK*s with extra/missing EGF-like domain, an extra/missing intron was observed in their extracellular regions. Extra introns were also observed in the kinase domains of a few *OsWAK*-RLCKs. Intron variation in *OsWAK* demonstrates that *OsWAK* gene structure is not as conserved as it is in the *Arabidopsis* WAK/WAKLs and is the reflection of its expansion in rice.

Compared to the 26 *Arabidopsis* WAKs/WAKLs (Verica and He, 2002), the *japonica* rice genome has nearly a 5 times greater number of *OsWAK*s. Previous

comparative analyses between *Arabidopsis* and rice species of different gene families like CONSTANS-like genes (involved in *Arabidopsis* photoperiodic flowering, Griffiths *et al.*, 2003) and the Dof (DNA-binding with one finger) family (plant specific transcription factors, Lijavetzky *et al.*, 2003) did not reveal the magnitude of difference in family sizes that is seen with the *OsWAK*s. Therefore the evolutionary expansion of the *OsWAK* gene family does not appear to be simply due to the larger genome size in rice.

Phylogenetic analyses of *OsWAK*s, *Arabidopsis* WAK/WAKLs and Barley (*Hordeum vulgare*) *HvWAK*s showed that the *OsWAK*s clustered in distinct species-specific clades and also suggested that the *OsWAK* gene family expanded in the rice genome due to lineage-specific expansion of the family in monocots. The 125 *OsWAK*s are present on all 12 chromosomes and are mostly clustered. Localized gene duplications appear to be the primary genetic event in *OsWAK* gene family expansion unlike local tandem duplications and large-scale genome duplications in *Arabidopsis* (Verica and He, 2002), since many small *OsWAK* groups, located in the close proximity on the same chromosome have high sequence similarity.

FUTURE PERSPECTIVES

Versatile roles played by the *Arabidopsis* WAKs suggest their importance in various processes like signal transduction, pathogen resistance, heavy-metal tolerance, mineral nutrition response, cell elongation and in the overall plant development. Previous work has shown that a reduction in WAK protein levels leads to a loss of cell expansion, indicating that these receptor-like proteins have a role in cell shape formation. WAKs may thus provide a molecular mechanism linking cell wall sensing (via pectin attachment) to regulation of solute metabolism, which in turn is known to be involved in turgor maintenance in growing cells. How WAKs are involved in signaling from the pectin extracellular matrix in coordination with GRPs will be key to our understanding of the cell wall's role in cell growth.

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