WAKsing plant immunity, waning diseases

Christopher Stephens, Kim E. Hammond-Kosack, and Kostya Kanyuka*

Department of Biointeractions and Crop Protection, Rothamsted Research, Harpenden AL5 2JQ, United Kingdom

*Author for correspondence: kostya.kanyuka@rothamsted.ac.uk

Running title: WAKs in plant immunity

Highlight

Members of the wall-associated receptor-like kinase protein (WAK) family are emerging as important players in plant immunity, contributing to defence against a variety of pathogens with diverse lifestyles.

Abstract

With the requirement to breed more productive crop plants to feed a growing global population, compounded by increasingly wide-spread resistance to pesticides exhibited by pathogens, plant immunity is becoming an increasingly important area of research. Of the genes that contribute to disease resistance, the wall-associated receptor-like kinases (WAKs) are increasingly shown to play a major role, in addition to their contribution to plant growth and development or tolerance to abiotic stresses. Being transmembrane proteins, WAKs form a central pillar of a plant cells ability to monitor and interact with their extracellular environments. Found in both dicots and monocots, WAKs have been implicated in defence against pathogens with diverse lifestyles and contribute to plant immunity in a variety of ways. Whilst some act as cell surface-localised immune receptors recognising either pathogen- or plant-derived invasion molecules (e.g. effectors or damage-associated molecular patterns, respectively), others promote innate immunity through cell wall modification and strengthening, thus limiting pathogen intrusion. The ability of some WAKs to provide both durable resistance against pathogens and other agronomic benefits, makes this gene family important targets in the development of future crop ideotypes and important to a greater understanding of the complexity and robustness of plant immunity.

Keywords

Cell wall; disease resistance; immune receptor; pattern recognition receptor (PRR); plant immunity; plant-pathogen interactions; receptor-like kinase; wall-associated kinase.

Introduction

Phytopathogens are an endemic issue in modern agriculture, accounting for a significant proportion of lost yield (Schwessinger *et al.*, 2015). Recent restrictions on pesticide use and the emergence of resistance to those pesticides, combined with the requirement to feed a growing global population, make the study of plant immunity an area of increasing importance. The genetic basis for immunity in plants was first demonstrated by Flor *et al.* (1971) with the characterisation of the gene-for-gene interaction between a plant disease resistance gene and a corresponding pathogen avirulence gene. The cloning of the first genetic factors underpinning immunity led to the formulation of the zig-zag model (Jones and Dangl, 2006), which proposed two seemingly distinct branches of immunity: pathogen-associated molecular pattern (PAMP) triggered immunity (PTI) and effector-triggered immunity (ETI). PTI is moderated by extracellular pattern recognition receptors (PRRs) which detect conserved molecules, such as fungal chitin and bacterial flagellin, and provides broad spectrum resistance to non-adapted potential pathogens. By contrast, ETI is conferred by cytoplasmic resistance proteins which detect pathogen-specific secreted effector proteins either directly or through modifications of host proteins targeted by effectors (van der Biezen and Jones, 1998), and provides resistance to adapted pathogens.

In recent years this binary model of plant immunity has been challenged (Thomma *et al.*, 2011), with studies demonstrating the inter-interdependent nature of PTI and ETI responses (Yuan et al., 2021) and this has inspired the proposal of revised models. Cook *et al.* (2015) proposed to recognise all microbial and host damage-derived molecules as the invasion patterns, which are detected by the invasion pattern receptors, with all interactions existing on a spectrum of invasion pattern conservation and the strength of the immune response. This model has since been further refined to include a spatial dimension for the recognition of invasion molecules by immune receptors in the apoplast vs cytosol and simplified to cover interactions of plants only with their adapted pathogens (Kanyuka and Rudd, 2019). This ‘spatial invasion model’ categorised all immune receptors as either cell surface immune receptors (CSIRs) or intracellular immune receptors (IIRs). CSIRs were defined as membrane-bound proteins with extracellular domains involved in the recognition of all apoplastic invasion molecules, such as PAMPs, host-derived damage-associated molecular patterns (DAMPs), as well as pathogen effectors, and thus include a broader range of receptors compared to PRRs. The spatial invasion model emphasised the importance of CSIRs as an integral component of plant immunity. The diversity and complexity of this class of proteins is now better understood.

The first CSIR identified was Xa21 of rice, a receptor-like kinase (RLK) protein which confers resistance to the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*; Song *et al.*, 1995). The model plant *Arabidopsis thaliana* (hereafter referred to as Arabidopsis) RLKs, such as FLAGELLIN-SENSING 2 (FLS2; Gomez-Gomez and Boller, 2000) and ELONGATION FACTOR TU RECEPTOR (EFR; Zipfel *et al.*, 2006) were shown to interact with flg22 and elf18 peptides derived from the conserved bacterial proteins FLAGELLIN and ELONGATION FACTOR THERMO UNSTABLE (EF-Tu), respectively. Numerous RLKs and receptor-like proteins (RLPs), which are similar to RLKs but possess no intracellular kinase domain, have now been identified as CSIRs that recognise a wide range of invasion molecules. CSIRs could be grouped into several classes primarily based on the presence of specific motifs in their extracellular domains, including leucine-rich repeat (LRR) containing receptors (e.g. FLS2) which detect proteinaceous invasion molecules, lysin motif (LysM) containing receptors (e.g. CHITIN ELICITOR RECEPTOR KINASE 1, CERK1) which bind carbohydrates, and G-type lectin-containing receptors (e.g. I-3) which are capable of binding both glycans and proteins (Bouwmeester and Govers, 2009).

Detection of invasion molecules by CSIRs and the corresponding immune response mechanisms activated are complex and highly regulated. In Arabidopsis, FLS2 and EFR require SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK) coreceptors such as BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) for immune signalling. Signalling pathways for some CSIRs have been very well characterised and involve signal transfer to RECEPTOR-LIKE CYTOPLASMIC KINASES (RLCKs) and propagation by MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) cascades, influx of calcium (Ca^{2+}) and associated activation of CALCIUM-DEPENDENT PROTEIN KINASES (CDPKs) leading to the induction of reactive oxygen species (ROS) and WRKY transcription factors and upregulation of immune-related genes (Albert *et al.*, 2020). Stable genetic transformation (Schwessinger *et al.*, 2015) or engineering chimeric CSIR proteins (Wu *et al.*, 2019) has been utilised to express CSIRs from model plants in crop species. This has demonstrated the versatility and breadth of CSIR-mediated immunity and its potential utility in providing resistance to plant pathogens in agriculture.

Wall-associated receptor-like kinases or WAKs are only now coming into focus as major players in plant immunity (Kanyuka and Rudd, 2019). Already shown to be expanded in monocots (International Wheat Genome Sequencing Consortium, 2018), large variations in WAK gene sequences resulting from dramatic differences in the lengths of the first intron (see below) suggests an additional level of diversification of WAK proteins in monocots, through alternative splicing variants. A greater understanding of this gene family can help further elucidate the role of CSIRs in plant immunity, as well as provide many new candidate genes that may contributed to disease resistance.

Wall-associated receptor-like kinases (WAKs)

WAKs represent a significant separate sub-group within the RLK superfamily. Like other RLKs, WAKs possess an extracellular region characterised by the presence of sub-group specific domains including those potentially mediating interactions with the cell wall, a transmembrane helix, and an intracellular kinase domain (**Figure 1**). Some WAKs play roles in growth and development (Wagner and Kohorn, 2001) or in tolerance to abiotic stresses such as heat, salt and heavy metals (Hou *et al.*, 2005; Yin and Hou, 2017; Zhang *et al.*, 2019), while increasing number of reports demonstrate other WAKs have functions in plant immunity. And here, we will focus primarily on the role played by WAKs in plant defence against pathogens.

Arabidopsis *WALL-ASSOCIATED KINASE 1* (*AtWAK1*) was the first WAK-encoding gene identified and it is a member of a small gene cluster located on chromosome 1 (He *et al.*, 1999). A total of 22 genes were found to encode WAKs in the Arabidopsis genome (Verica and He, 2002). The AtWAK1 protein was isolated from fractions of proteolytically digested cell wall, suggesting its strong interaction with the cell wall *in vivo*. The direct connection between the cell wall and the cytosol, through the kinase domain, along with a short rosette leaves phenotype observed in the transgenic antisense *AtWAK1* and *AtWAK2* Arabidopsis plants, revealed a role for these genes in leaf cell expansion (He *et al.*, 1998; Wagner and Kohorn, 2001). AtWAK1 was also shown to bind with high affinity to oligogalacturonides (OGs), oligomers of α-1,4–linked galacturonic acid, derived from cell wall pectin and released during cell wall loosening and expansion or due to the action of pathogen-derived cell wall degrading enzymes (CWDEs) during infection (Decreux and Messiaen, 2005). OGs have long been understood as elicitors of plant defence, acting as DAMPs (Nothnagel *et al.*, 1983), and downstream defence signalling in response to OGs has been a subject of intense research (Ferrari *et al.*, 2013). Whilst expression of *AtWAK1* was shown to be upregulated by salicylic acid (SA) and wounding (He *et al.*, 1998), the direct interaction between AtWAK1 and OGs and the important role

for AtWAK1 in plant immunity was first demonstrated in elegant experiments involving transgenic Arabidopsis plants expressing a chimeric receptor-like protein comprising an ectodomain of AtWAK1 and a cytoplasmic kinase domain of EFR (Brutus *et al.*, 2010). Treatment of these plants with OGs induced an EFR kinase-mediated defence response. Furthermore, transgenic Arabidopsis plants ectopically expressing *AtWAK1* had enhanced resistance to the necrotrophic fungus *Botrytis cinerea* compared to the wild type, confirming a role for *AtWAK1* in plant immunity (Brutus *et al.*, 2010). Two other WAKs identified in Arabidopsis are strongly implicated in plant immunity. One is RESISTANCE TO FUSARIUM OXYSPORUM 1/WALL-ASSOCIATED KINASE LIKE 22 (RFO1/WAKL22), which was shown to confer quantitative resistance to necrotrophic root pathogens *Fusarium oxysporum* and *Verticillium longisporum* (Diener and Ausubel, 2005). The other is AtWAKL10, which contributes to *Pseudomonas syringae* resistance, with transgenic Arabidopsis lacking this WAK demonstrating increased susceptibility to this bacterial pathogen compared to the wild type (Bot *et al.*, 2019).

Immunity-related WAKs have been identified in other model and crop plant species, including dicots tomato (*Solanum lycopersicum*), cotton (*Gossypium hirsutum*) and oilseed rape (*Brassica napus*), and the monocot crops wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*), and barley (*Hordeum vulgare*) (**Table 1**). Several of these confer resistance to Ascomycete fungi of the Dothideomycetes class, which have either hemibiotrophic or latent necrotrophic lifestyles. However, WAKs conferring resistance to bacteria or fungal pathogens belonging to other classes have also been identified. The pathogens controlled by WAKs may have broad host ranges (e.g. *P. syringae*; Rosli *et al.*, 2013) or be restricted to a single plant species (e.g. *Sporisorium reilianum*; Zuo *et al.*, 2015). Moreover, WAKs were shown to be involved in defence against both extracellular pathogens (Saintenac *et al.*, 2018) and those which penetrate host cells (Li *et al.*, 2009). A WAK-mediated resistance against the floral diseases such as Fusarium head blight (Gadaleta *et al.*, 2019), root diseases such as blackleg (Larkan *et al.*, 2020), and foliar diseases such as Northern corn leaf blight (Hurni *et al.*, 2015) have all been documented (**Table 1**). These immunity-related WAKs confer resistance through a range of mechanisms, including pathogen or host-derived elicitor detection (Brutus *et al.*, 2010; Saintenac *et al.*, 2018) and cell wall restructuring (Yang *et al.*, 2019), whilst other WAKs have been identified as susceptibility factors, subverted by necrotrophic effectors (Liu *et al.*, 2012).

Domain architecture of WAK proteins

The extracellular regions of the WAK family show the highest diversity (Verica *et al.*, 2003) and are characterised by the presence of the galacturonan-binding (GUB_WAK_bind) domain frequently in combination with one or more additional domains. The intracellular regions of WAK proteins, which contain a kinase domain, appear to be more conserved.

The GUB_WAK_bind domain is implicated in the interaction with pectin in the extracellular cell wall matrix. Indeed, AtWAK1 forms both covalent and ionic bonds with pectin and remains firmly bound to the cell wall following plasmolysis. Some WAKs characterised from other plant species, such as rice OsWAK1 (Li *et al.*, 2009), OsWAK25 (Jo *et al.*, 2011) and XANTHOMONAS ORYZAE PV. ORYZAE RESISTANCE 4 (Xa4; Hu *et al.*, 2017), have also been shown to localise to the cell wall. However, other WAKs, such as TaWAK5 of wheat (Yang *et al.*, 2014) and WALL-ASSOCIATED KINASE-RECEPTOR LIKE KINASE 1/Htn1 (ZmWAK-RLK1/Htn1; Yang *et al.*, 2019) and WALL-ASSOCIATED KINASE/qHSR1 (ZmWAK/qHSR1; Zuo *et al.*, 2015) of maize, have been shown to separate from the cell wall and retract with the plasma membrane following plasmolysis, suggesting a

lack of covalent binding to the cell wall pectin. Interestingly, other WAKs, such as rice WALL-ASSOCIATED KINASE 91/ DEFECT IN EARLY EMBRYO SAC 1 (OsWAK91/OsDEES1; Wang *et al*., 2012) and wheat SEPTORIA TRITICI BLOTCH RESISTANCE 6 (Stb6; Stephens *et al.*, unpublished), could be found in both the plasma membrane and the cell wall, as well as within the Hechtian strands connecting the two following plasmolysis. The exact mechanism by which WAKs and pectin interact and what determines strong binding with the cell wall is yet to be fully understood (Kohorn, 2016), although amino acids, such as lysine and arginine, are suggested to be important in homogalacturonan-binding (Decreux *et al*., 2006). GUB_WAK_bind may play other important roles in WAKs. For example, susceptibility isoforms of the oilseed rape RESISTANCE TO LEPTOSPHAERIA MACULANS 9 (Rlm9), a WAK involved in the race-specific resistance against *Leptosphaeria maculans*, contain amino-acid changes in this domain relative to the resistance isoform (Larkan *et al*., 2020), suggesting a possible role for GUB_WAK_bind in effector recognition.

WAKs often possess other domains between the N-terminal GUB_WAK_bind and the transmembrane helix (**Figure 1**). For instance, many WAKs also contain one or more copies of epidermal growth factor (EGF)-like domains, some of which have been characterised as Ca^{2+}-binding. Others have been shown to contain a WAK-associated C-terminal domain (WAK_assoc_C), which lies downstream of GUB_WAK_bind. All these domains are rich in cysteine. EGF-like domains have been shown to form disulphide bridges stabilising the secondary protein structure and, in animals, they have been implicated in protein-protein interactions (Yarden and Sliwkowski, 2001). Some versions of EGF domains have been shown to bind a single Ca^{2+} ion (Selander-Sunnerhagen *et al.*, 1992). The latter appears to be essential for many interactions with the receptor ligands, with the ectodomains of AtWAK1 and *Brachypodium distachyon* BdWAK1, BdWAK10, and BdWAK42 shown to bind OGs in a Ca^{2+}-dependent manner (Decreux and Messiaen, 2005; Wu *et al*., 2020). EGF-mediated protein-protein interactions may be a common mode of action for WAKs that function in pathogen surveillance. However, some characterised immunity-related WAKs do not have EGF-like domains, including the wheat Stb6 (Saintenac *et al*., 2018) and maize ZmWAK-RLK1/Htn1 (Hurni *et al*., 2015) proteins which confer resistance to the fungal pathogens *Zymoseptoria tritici* and *Exserohilum turcicum*, respectively. Analyses of WAK-encoding genes in oilseed rape, tomato, poplar (*Populus trichocarpa*), and *B. distachyon,* also identified those that encode proteins with no EGF-like domains (Tocquard *et al*., 2014; Larkan *et al*., 2020; Sun *et al*., 2020; Wu *et al*., 2020).

Immunity-related WAKs could be classified as RLK/Pelle serine-threonine kinases (Lehti-Shiu and Shiu, 2012). Some WAKs involved in pathogen defence contain a conserved arginine (R) immediately preceding the catalytic aspartate (D) in the catalytic loop of the kinase domain, and thus are classified as 'RD' kinases. Both residues play vital roles in RD kinase activity, with aspartate acting as the catalytic residue, and the arginine being essential for orientation of the catalytic site and phosphotransfer to the kinase substrate (Krupa *et al*., 2004). Defence-related RD WAKs include Arabidopsis AtWAK1, RFO1/AtWAKL22 and AtWAKL10, as well as oilseed rape Rlm9, cotton GhWAK5A and GhWAK7A, rice OsWAK1, 14, 21.2, 25, 91, 92 and 112d, and tomato SlWAK1. However, RD kinases are more commonly linked to roles in growth and development, whereas all non-RD kinase characterised to date, including PRRs such as FLS2 and EFR, have been implicated in host immunity (Dardick *et al*., 2012). Also, so far, only non-RD WAKs related to plant immunity have been characterized from monocots. These include ZmWAK/qHSR1 and ZmWAK-RLK1/Htn1 of maize, Xa4 of rice, and Stb6, TaWAK5 and TaWAK6 of wheat. RD and non-RD kinases have different activation mechanisms, which likely affects their signalling pathways. For example, while non-RD receptor kinases FLS2 and EFR require BAK1 (an RD-kinase) for immune signalling, the RD receptor kinase CERK1 does not require BAK1 (Liebrand *et al*., 2014).

Recent studies have also identified a guanylyl cyclase (GC) motif embedded within the kinase domain of some WAKs (Bot *et al*., 2019). A web tool GCPred (http://gcpred.com/; Xu *et al*., 2018) predicts several immunity-related WAKs, including Rlm9, AtWAKL10, OsWAK25 and OsWAKL21.2, RFO1/AtWAKL22 and SlWAK1, as containing the GC functional centre (**Figure 1**). Interestingly, whilst kinase activity of OsWAKL21.2 is required for activation of pathogen resistance in rice, the GC activity is required for activation of immune responses when ectopically expressed in Arabidopsis (Malukani *et al*., 2020). This suggests that these WAKs modulate different signalling pathways, possibly allowing to carry out pleiotropic functions as well as immune responses. For example, AtWAKL10, which contains a GC motif within its kinase domain, has been shown to be involved in responses to both biotic and abiotic stresses, with the *atwakl10* mutant plants showing reduced tolerance to salt stress (Bot *et al*., 2019).

Exon-intron structure of WAK genes

The exon-intron structure of WAKs appears to be well conserved across monocot and dicots. The characterised immunity-related WAK genes comprise either three exons and two introns or four exons and three introns, with most genes typified by the presence of a long first exon and a very short second exon (**Figure 2**). The length of the first intron in the WAK genes is highly variable, ranging from 77-bp (*OsWAK92*) to 8334-bp (*ZmWAK-RLK1/Htn1*), with the increase in length thought to be at least in some cases attributed to insertion of transposons (Hurni *et al*., 2015). Interestingly, genes with the first introns longer than 150-bp have so far only been identified in monocots. Very long first introns may confer genetic diversity by spanning one or more alternative first exons, leading to splicing variants of the gene. Such is the case for *OsWAKL21* of rice, from which three alternatively spliced transcripts are produced, with only one (*OsWAKL21.2*) known to confer resistance to *Xoo* (Malukani *et al*., 2020).

The WAK gene family has been shown to be greatly expanded in monocots (International Wheat Genome Sequencing Consortium, 2018; Tripathi *et al*., 2020), and the presence of alternative splicing variants could represent an additional level of diversification of WAKs in monocot species. Despite the large variation in gene length, the size of the resulting WAK proteins is relatively consistent, with the majority comprising between 700-800 amino acids (aa). The smallest characterised is Stb6 (647-aa), whilst the largest is OsWAK112d (1015-aa), with most of the length diversity accounted for by variations in the extracellular regions (**Figure 1**).

The regulation of WAK genes

WAKs often confer or contribute to immunity at different growth stages and in different organs of plants. For example, the head smut resistance gene *ZmWAK/qHSR1* is most highly expressed in the mesocotyl of maize seedlings, which corresponds to the organ and growth stage in which *S. reilianum* infection most commonly occurs (Zuo *et al*., 2015). Similarly, *TaWAK5* was found to be substantially upregulated in roots but not in stems of wheat in response to the soil-borne pathogen *Rhizoctonia cerealis* (Yang *et al*., 2014). At a cellular level, the rice protein OsWAK25, whose overexpression enhances resistance to *Magnaporthe oryzae* and *Xoo* (Harkenrider *et al*., 2016), was found to be localised to plasmodesmata (Jo *et al*., 2011), which the fungus *M. oryzae* uses to spread cell-to-cell (Kankanala *et al*., 2007). Interestingly, the *Parastagonospora nodorum* susceptibility gene *STAGONOSPORA NODORUM NECROSIS 1* (*Snn1*) of wheat was found to have a circadian rhythm pattern of expression, with the *Snn1* transcript abundance increasing during darkness and decreasing

during daylight and sensitivity to the necrotrophic effector STAGONOSPORA NODORUM TOXIN 1 (SnTox1) demonstrating light dependence (Liu *et al*., 2012; Shi *et al*., 2016). Overexpression of *TaWAK6* was shown to enhance resistance to the leaf rust fungus *Puccinia triticina* only at the adult plant stage but not at the seedling stage (Dmochowska-Boguta *et al*., 2020). These spatial and temporal differences in expression can give clues to the function of WAKs and their potential molecular interactions and are correlated with the lifestyles of pathogens against which they confer resistance.

Pathogen challenge induces the upregulation of some WAKs, along with a wide range of other plant immunity-related genes (Dmochowska-Boguta *et al*., 2015). However, the speed, scale, and duration of gene upregulation in response to pathogens vary greatly between different WAKs. For some, upregulation occurs only transiently. Rice *OsWAK91* and *OsWAK92* peak in expression at only 4 hours post inoculation (hpi) with *M. oryzae* and 1 h after chitin infiltration. In both cases upregulation of these WAK genes is largely reduced after 24 h (Delteil *et al*., 2016). By contrast, wheat *TaWAK5* remains upregulated up to 21 days post inoculation with *R. cerealis* (Yang *et al*., 2014). The fastest increase in expression in response to pathogen inoculation is shown by rice *Xa4*, with a reported 30-fold increase after just 0.5-hpi with the incompatible isolate of *Xoo* (Hu *et al*., 2017). Some WAK genes show only a very modest upregulation in response to pathogens. For example, a two-fold increase in the expression was observed for *OsWAK25* and *ZmWAK/qHSR1* at 24-hpi (Wei *et al*., 2013; Zuo *et al*., 2015) with *M. oryzae* and *S. reilianum*, respectively.

During genetically compatible interactions, by contrast to WAK genes contributing to disease resistance, some susceptibility genes such as *Snn1* (Shi *et al*., 2016) and the negative regulator of immunity *OsWAK112d* (Delteil *et al*., 2016) have both been shown to be downregulated at 24-hpi with *P. nodorum* and *M. oryzae*, respectively. However, gene downregulation in response to pathogens appears not to be the universal feature of the WAKs involved in disease susceptibility. For instance, *SUSCEPTIBILITY TO BIPOLARIS SOROKINIANA-1* and *-2* (*Sbs1* and *Sbs2*) genes in barley were found to remain upregulated six-fold at 12-hpi with virulent isolates of *Bipolaris sorokiniana* (Ameen *et al*., 2020).

Recognition of pathogen effectors and other invasion molecules by WAKs

Three WAKs have so far been identified as having gene-for-gene interactions with specific pathogen effector proteins (**Figure 3**). Stb6 of wheat and Rlm9 of oilseed rape confer resistance to *Z. tritici* expressing AvrStb6 and *L. maculans* expressing AvrLm5-9, respectively (Brading *et al*., 2002; Larkan *et al*., 2016). However, a direct interaction between these disease resistance proteins and the corresponding fungal effectors has not been observed in either case (Saintenac *et al*., 2018; Larkan *et al*., 2020). The WAK protein Snn1 is a susceptibility factor in the interaction between wheat and *P. nodorum*. The effector protein SnTox1 induces cell death/necrosis specifically in wheat plants containing *Snn1* leading to disease, in what has been described as effector-induced susceptibility (Liu *et al*., 2012). Moreover, Shi *et al.* (2016) observed a direct interaction between Snn1 and SnTox1. The rice WAK protein Xa4 has been shown to control a race-specific resistance against *Xoo* (Hu *et al*., 2017), although the invasion molecule recognised by this resistance protein has not yet been identified.

Other WAKs contribute to plant immunity by monitoring for invasion molecules in a non-gene-for-gene manner (**Figure 3**). As discussed above, AtWAK1 is known to bind cell wall pectin and pectin-derived OGs that are recognised as DAMPs (Verica and He, 2002; Brutus *et al*., 2010). This ability to

detect DAMPs has led to AtWAK1 and other CSIRs being described as cell wall integrity sensors (Kohorn, 2016). The length of OGs appears to affect the character of the immunity response (Davidsson *et al*., 2017), whilst a decrease in pectin methylation correspondingly increases WAK sensitivity to OGs (Kohorn *et al*., 2014), illustrating a complex interaction between AtWAK1 and its ligands.

Detection of pathogen effectors and other invasion molecules may be utilised by other, as yet uncharacterised WAKs. Indeed, many WAKs have been shown to be upregulated in response to treatment with PAMPs (**Figure 3**). *OsWAKL21.2*, an alternative splice variant of rice *OsWAKL21*, is upregulated specifically by enzymatically active forms of the *Xoo* cell-wall degrading enzyme LIPASE A (LipA). Furthermore, suppression of the *OsWAKL21.2* expression reduces the LipA-induced immune responses (Malukani *et al*., 2020). Similarly, expression of *SlWAK1* in tomato is induced by flg22 and flg28 in a FLS2- and FLAGELLIN-SENSING 3 (FLS3)-dependent manner (Zhang *et al*., 2020), with *slwak1* knockout mutant plants showing compromised responsiveness to these PAMPs. The cotton *GhWAK7A* and rice *OsWAK14*, *OsWAK91* and *OsWAK92* genes were shown to be specifically induced by chitin (Delteil *et al*., 2016; Wang *et al*., 2020). Whilst *GhWAK7A* appears to contribute to chitin detection and signal transduction, the induction of *OsWAK14, OsWAK91 and OsWAK92* is likely to be part of downstream defence responses. Other WAKs induced by PAMPs include *AtWAKL10*, which is induced by chitin and flg22 (Diener and Ausubel, 2005) and *RFO1*/*AtWAKL22* which is upregulated in response to flg22 and NECROSIS AND ETHYLENE-INDUCING PEPTIDE 1–LIKE PROTEINS (NLPs) treatment (Qutob *et al*., 2006; Meier *et al*., 2010), both likely as part of downstream defence responses. Combined, the studies discussed above demonstrate the involvement of WAKs in defence responses induced by a wide range of plant- and pathogen-derived invasion molecules.

WAK-mediated defence responses

WAKs have been shown to induce a broad range of host defence responses. Stb6 and Rlm9 confer complete gene-for-gene resistance to isolates of *Z. tritici* and *L. maculans* (Brading *et al*., 2002; Larkan *et al*., 2016). While Rlm9-mediated resistance is associated with hypersensitive response (HR), *Stb6* controls disease without inducing host cell death. Other WAKs confer quantitative resistance to their respective pathogens. In maize, ZmWAK-RLK1/Htn1 reduces the number of successful penetrations of *E. turcicum* into the seeding epidermis (Yang *et al*., 2019) while ZmWAK/qHSR1 induces HR to restrict the growth of *S. reilianum* in the mesocotyl (Zhang *et al*., 2017). The disruption of many WAK genes, either through stable gene knockout, virus-induced gene silencing (VIGS), or targeting induced local lesions in genomes (TILLING) approaches, has been shown to result in increased susceptibility, determined by the number and size of lesions or the extent of pathogen proliferation (Delteil *et al*., 2016; Dmochowska-Boguta *et al*., 2020). Correspondingly, overexpression of WAKs in many cases (e.g. *OsWAK1*, *OsWAK25* and *GhWAKL*) results in enhanced disease resistance (Li *et al*., 2009; Harkenrider *et al*., 2016; Feng *et al*., 2020).

MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) cascades regulate multiple biological processes such as growth and development and also are implicated in immune responses (**Figure 3**). MPK3 and MPK6 are commonly associated with plant defence and both *AtWAK1*- and *GhWAK7A*-mediated defence involve these pathways, although in Arabidopsis, the MPK6 pathway has been proposed to be specifically required for defence (Kohorn and Kohorn, 2012; Wang *et al*., 2020). Upregulation of MPK3 has also been linked to the susceptibility responses mediated by Snn1 in wheat and Sbs1 and Sbs2 in barley (Liu *et al*., 2012; Ameen *et al*., 2020). Other MAPK pathways could also

be utilised by WAKs. For example, the *MEKK1* gene encoding a homologue of MITOGEN-ACTIVATED PROTEIN KINASE KINASE (MAPKK) located upstream of the MPK4 pathway, has been shown to be upregulated as part of the ZmWAK/qHSR1 mediated defence response (Zhang *et al*., 2017).

Plant defence responses are often orchestrated by hormone fluctuations, with several immunity-related WAKs upregulated by hormone treatments (**Figure 3**). For example, salicylic acid (SA) application induces the upregulation of *AtWAK1*, *OsWAK1*, *OsWAK25*, *GhWAKL* and sweet orange (*Citrus sinensis*) *CsWAKL08* (He *et al*., 1998; Li *et al*., 2009; Harkenrider *et al*., 2016; Feng *et al*., 2020; Li *et al*., 2020). Methyl jasmonate (MeJA), a derivative of jasmonic acid (JA), stimulates expression of *RFO1/AtWAKL22*, *OsWAK1* and *CsWAKL08* (Johansson *et al*., 2006; Li *et al*., 2009; Li *et al*., 2020), with *RFO1/AtWAKL22* also known to be induced by treatments with ethylene precursors. In turn, WAKs could induce or suppress hormone biosynthesis as part of their contribution to disease resistance. Some, such as *AtWAK1*, *CsWAKL08*, *Xa4*, *OsWAKL21.2* and *ZmWAK-RLK1*/*Htn1*, induce expression of the JA-biosynthesis related genes or JA accumulation (Moscatiello *et al*., 2006; Zuo *et al*., 2015; Yang *et al*., 2019; Li *et al*., 2020; Malukani *et al*., 2020), whilst others, such as *OsWAK25*, induce production of SA (Harkenrider *et al*., 2016). The silencing of cotton WAK genes, *GhWAK26* and *GhWAK77*, was shown to suppress the SA- and JA-related signalling in response to *Verticillium dahliae* infection (Yang *et al*., 2021). Pathways involved in JA, auxin, abscisic acid (ABA) and ethylene biosynthesis were all shown to be suppressed following pathogen challenge in maize overexpressing *ZmWAK/qHSR1*, whilst SA pathways were promoted (Zuo *et al*., 2015; Zhang *et al*., 2017). Interestingly, this trend is reversed in *ZmWAK/qHSR1* maize in the absence of a pathogen challenge. When ectopically overexpressed in Arabidopsis, *OsWAKL21.2* induced SA and suppressed JA biosynthesis (Malukani *et al*., 2020). Hormone pathways induced in response to infection vary based on the lifestyle of the infecting pathogen, with SA pathways commonly promoted in response to biotrophic pathogens and JA pathways induced following necrotrophs infection (Bari and Jones, 2009). It is likely that WAK-induced hormonal responses correspond to the lifestyles of the pathogens to which they confer resistance.

Other components of the plant immune system often associated with the WAK-induced signalling responses include cytoplasmic RLKs (CRLKs), which relay WAK kinase-induced defence signalling into the cytoplasm and nucleus (**Figure 3**). For example, two CRLKs, RESPONSE TO OGs 1 and 2 (ROG1 and ROG2), are phosphorylated following OG treatment, with OG responses reduced in transgenic Arabidopsis *rog2* knockout lines (Kohorn *et al*., 2016). Production of ROS, which has antimicrobial properties as well as acting in cell-to-cell signalling, is frequently observed in WAK-mediated defence responses, including those induced by Stb6 of wheat, GhWAK7A of cotton, or OsWAK91 of rice (Shetty *et al*., 2003; Delteil *et al*., 2016; Wang *et al*., 2020). Accumulation of another biotic stress signalling molecule, cyclic guanosine monophosphate (cGMP), is reported to be induced by AtWAKL10 in Arabidopsis following inoculation with *P. syringae* (Meier *et al*., 2010). The Ca^{2+} ion influx, another common feature of plant defence, is observed in the OG-treated Arabidopsis plants (Moscatiello *et al*., 2006). This is likely to be an AtWAK1-mediated process, which also involves the CALCIUM-DEPENDENT PROTEIN KINASES, CDPK5, 6 and 11 (Gravino *et al*., 2015).

Direct interactions between WAKs and other proteins

CSIRs are frequently identified as forming membrane complexes upon ligand recognition (Hohmann *et al*., 2017). BAK1 and other SERKs are common co-receptors frequently associated with various

CSIRs during immune responses (Liebrand *et al*., 2014), although these co-receptors are yet to be implicated in WAK-associated defence responses. The AtWAK1-mediated defence in response to OGs has been shown to be unaffected by the *P. syringae* effector AvrPto, which targets and inhibits the action of BAK1 and therefore negatively impacts the FLS2- and EFR-mediated immunity (Gravino *et al*., 2017). Similarly, BAK1 and SOBIR1 appear not to be involved in the Rlm9-mediated defence response (Larkan *et al*., 2020). Whether any of the WAKs require interactions with BAK1, SOBIR1, or other co-receptors for induction of immune responses is yet to be elucidated. Non-RD WAKs such as Stb6, Xa4 or ZmWAK-RLK1/Htn1 are more likely to form a complex with an RD kinase co-receptor to initiate defence signalling (Liebrand *et al*., 2014).

Interactions with proteins at the plasma membrane have been identified for some WAKs (**Figure 3**). For instance, SlWAK1 have been shown to interact with FLS2 and FLS3 upon recognition of flg22 and flg28, respectively (Zhang *et al*., 2020), with flagellin-induced defence responses shown to be compromised in tomato *slwak1* knockout lines. In rice, OsWAK91 and OsWAK92 were found to form homodimers and heterodimers, as well as heterodimers with OsWAK14, all mediated by their EGF-like domains, thus forming large receptor complexes at the plasma membrane (Cayrol *et al*., 2016). Cotton GhWAK7A was shown to interact *in vivo* with both GhCERK1 and LYSM RECEPTOR KINASE 5 (GhLYK5), and promote their dimerization (Wang *et al*., 2020), as well as phosphorylating GhLYK5. Compromised chitin-induced defence responses in *GhWAK7A*-silenced plants suggest an important role for GhWAK7A in chitin detection in cotton. The RFO1-mediated defence response has been demonstrated to be co-dependent on the presence of the receptor-like protein RFO2 (Diener and Ausubel, 2005; Shen and Diener, 2013), suggesting that RFO2 may act as a coreceptor.

Interactions between some WAKs and other proteins in cytosol or in the extracellular space have also been reported (**Figure 3**). In particular, AtWAK1 has been shown to interact in a resting state with the extracellular cell wall- and plasma membrane-localised GLYCINE-RICH PROTEIN 3 (AtGRP3) and the cytoplasmic plasma membrane-localised KINASE-ASSOCIATED PROTEIN PHOSPHATASE (KAPP), forming an approximately 500 kDa complex (Park *et al*., 2001; Gramegna *et al*., 2016). AtGRP3 has been suggested to act as a negative regulator of AtWAK1-mediated cell expansion, whilst a complex allosteric model is proposed for AtGRP3 regulation of AtWAK1–OG interaction (Mangeon *et al*., 2017). OsWAK1 phosphorylation of the transcriptional regulator, RING FINGER PROTEIN 1 (OsRFP1; Li *et al*., 2009), possibly represents a first stage in the OsWAK1-mediated defence response pathway in rice. A direct interaction between GhWAK7A and the heat-shock chaperone protein GhDNAJ1 has also been reported (Feng *et al*., 2020), with GhDNAJ1 shown to be required for GhWAK7A-mediated immunity. OsWAK25 of rice has been suggested to act as a positive regulator of Xa21-mediated immunity, indicating a possible interaction between these two receptor proteins (Seo *et al*., 2011). Cytoplasmic protein-protein interactions involving the kinase domains of WAKs, may also occur to relay a defence signal to the nucleus or, conversely, to regulate the WAK signalling capacity.

Cell wall modifications induced by WAKs

Many immunity-related WAKs confer the ability to modify the cell wall composition, providing additional strength to the barrier and preventing pathogen penetration, both constitutively and during a pathogen attack. The defence responses directed by *OsWAKL21.2* and *SlWAK1* to minimise the pathogen infection include the deposition of callose (Malukani *et al*., 2020; Zhang *et al*., 2020), while *ZmWAK-RLK1*/*Htn1* induces upregulation of lignin biosynthesis genes (Yang *et al*., 2019). Both

callose and lignin are polymers that reinforce the cell wall, strengthening it and limiting the penetration by pathogens. In wheat, *TaWAK2* has been proposed to negatively modulate the expression of *PECTIN METHYL-ESTERASE 1* (*PME1*) leading to a more rigid cell wall, which limited the penetration and spread of *Fusarium graminearum* (Gadaleta *et al*., 2019). It has also been observed that de-esterified pectin competes with OGs for binding to AtWAK1 in Arabidopsis, with increased concentrations of de-esterified pectin resulting in decreased OG sensitivity (Ferrari *et al*., 2013). It is therefore possible that the downregulation of *PME1* in wheat may lead to the increased OG-sensitivity and consequently to the cell wall reinforcement. Rice Xa4-mediated resistance to *Xoo* was associated with the upregulation of genes encoding the CELLULOSE SYNTHASE A (CesA) family enzymes, thus strengthening the cell wall barrier, and the downregulation of cell wall-loosening expansins (Hu *et al*., 2017). Interestingly, the expression of this cell wall strengthening phenotype has thus far only been linked to the action of WAKs involved in defence against non-vascular pathogens (Hu *et al*., 2017; Yang *et al*., 2019; Malukani *et al*., 2020; Zhang *et al*., 2020).

This ability to modulate cell wall characteristics means that some immunity-related WAKs also have additional roles in plant growth, development and/or responses to abiotic stresses. In rice, Xa4 expression is highest in the stem, where it strengthens cell walls and reduces cell length, conferring a small reduction in plant height associated with an increased resistance to lodging, thus preventing yield losses from mechanical stresses (Hu *et al*., 2017). Knockout rice lines of *oswak91/osdees1* show not only increased susceptibility to *M. oryzae* but also reduced fertility (Wang *et al*., 2012; Delteil *et al*., 2016), revealing an additional role for this WAK in plant sexual reproduction. ZmWAK/qHSR1 of maize, which contributes to head smut resistance, promotes cell growth in the absence of pathogen challenge (Zhang *et al*., 2017) and is proposed to be involved in cell turgor regulation and osmotic stress tolerance (Zuo *et al*., 2015). As well as enhancing resistance to *P. syringae* pv. *tomato*, *AtWAKL10* is upregulated in response to treatments with the abiotic stress signalling factor S-nitroso-L-cysteine (a donor of nitric oxide). The *atwakl10* knockout mutant plants demonstrate improved tolerance to drought-stress but reduced salinity stress tolerance (Bot *et al*., 2019) and also show a reduced branching phenotype, suggesting an additional role for *AtWAKL10* in growth and development. These pleiotropic effects often provide additional agronomic benefits for crops possessing these WAK genes.

Trade-offs between disease resistance to different types of plant pathogens

In many aspects of plant immunity, it has been observed that resistance to pathogens with one lifestyle may confer susceptibility to those with others. For example, the *MILDEW RESISTANCE LOCUS O* (*MLO*) genes, characterised in plants including Arabidopsis, tomato, wheat and barley, confer susceptibility to the obligate biotrophic fungi which cause powdery mildew disease, with knockout lines showing enhanced disease resistance (Wang *et al*., 2014). However, Arabidopsis *mlo2 mlo6 mlo12* triple mutants show enhanced susceptibility to the hemibiotrophic pathogens *F. oxysporum* and *P. syringae* pv. *maculicola* (Acevedo-Garcia *et al*., 2017). Interestingly, while transgenic rice lines overexpressing *OsWAK25* show enhanced resistance to the hemibiotrophic pathogens *Xoo* and *M. oryzae*, at the same time these lines display enhanced susceptibility to the necrotrophic pathogens *Cochliobolus miyabeanus* and *Rhizoctonia solani* (Harkenrider *et al*., 2016). Disease resistance conferred by other WAKs may also be associated with trade-off between responses to different diseases. For example, although *ZmWAK-RLK1*/*Htn1* of maize confers resistance to Northern corn leaf blight (Hurni *et al*., 2015), it has also been shown to associate with a decrease in the accumulation

of benzoxazinoids, indole-derived plant metabolites that function in defence against aphids and other pests (Yang *et al*., 2019).

Other WAKs have been identified as susceptibility factors or negative regulators of immunity. The wheat *Snn1* is the best characterised WAK gene that confers susceptibility to *P. nodorum* (Liu *et al*., 2012), triggered upon recognition of the corresponding fungal effector SnTox1. Under yeast two-hybrid conditions, a direct interaction was observed between SnTox1 and a region of the Snn1 ectodomain between the GUB_WAK_bind and the EGF-like domains (Shi *et al*., 2016). As Snn1-mediated susceptibility is associated with the elevated expression of pathogenesis-related genes *PR-1-A1*, *chitinase,* and *thaumatin* and involves activation of host cell death, it has been hypothesised that *P. nodorum* hijacks wheat pathways involved in resistance to biotrophic pathogens, utilising host cell death to gain nutrients and to sporulate (Lorang *et al*., 2012). Leaves of transgenic rice lines overexpressing *OsWAK25* often develop disease mimic lesions (Harkenrider *et al*., 2016) suggesting constitutive activation of the defensive host cell death pathways, which may explain the observed enhanced resistance to hemibiotrophic pathogens and enhanced susceptibility to necrotrophic pathogens.

Another recently identified susceptibility factor is the barley *Rcs5* locus, which encodes the two WAKs, Sbs1 and Sbs2 (Ameen *et al*., 2020). The Sbs1/Sbs2-mediated susceptibility is thought to be dependent on the recognition of an unknown non-proteinaceous effector secreted by *B. sorokiniana*. Mutants in the rice *OsWAK112d* gene and the transgenic lines overexpressing this WAK gene have been shown to have enhanced resistance and susceptibility, respectively, to *M. oryzae* (Delteil *et al*., 2016). However, unlike Snn1 and Sbs1/Sbs2, OsWAK112d does not appear to be activated by a pathogen effector but rather acts as a negative regulator of defence, interacting with other proteins including an RLK related to the disease resistance protein Xa21 (Rohila *et al*., 2006). It is conceivable that whilst OsWAK112d negatively regulates immune responses, this WAK may also be involved in other essential processes in a way similar to the Arabidopsis RLK FERONIA, which plays a negative role in defence against *P. syringae* whilst also being a key signalling factor in pollen delivery during fertilisation (Escobar-Restrepo *et al*., 2007).

Final remarks

WAKs are increasingly recognised as conferring manifold agronomic benefits to crop species, not only in growth and development, but also in abiotic and biotic stress resistance. Expansion of the WAK gene family in monocots suggests that WAKs play an important role in pathogen monitoring and immunity in cereal crops such as wheat, maize, and rice, the most widely grown crops worldwide. Immunity-related WAKs appear to utilise diverse strategies: acting as monitors for pathogen- or host-derived invasion molecules or restructuring and reinforcing the cell wall to limit pathogen penetration and spread. Some of these WAKs have already been characterised as broad-spectrum disease resistance genes (Brutus *et al*., 2010) and shown to be durable in the field (Hu *et al*., 2017). Moreover, some of these genes confer additional beneficial traits for resilient, productive crop plants (Hu *et al*., 2017; Zhang *et al*., 2017). WAKs, therefore, represent an important breeding and biotechnology target in the quest to meet future challenges of sustainable food production and feeding the growing world population.

The majority of immune-related WAKs have been cloned/characterised only recently, during the past 5-6 years. This demonstrates a rapid increase in the identification and characterisation of genes conferring disease resistance in plants, providing ever more breeding targets for crop improvement.

This increased rate of discovery has been facilitated by the development of new strategies and tools for the genome-wide identification of gene families and the rapid characterisation of their function. Traditional approaches, such as map-based cloning, are now supplemented by reverse genetics tools such as VIGS, TILLING, and genome editing to aid the rapid identification of disease resistance-conferring genes (Bouton *et al*., 2018; Saintenac *et al*., 2018; Hahn *et al*., 2020). Global plant transcriptome analyses during pathogen challenge are increasingly used to identify candidate genes involved in plant immunity in diverse species of agriculturally important plants from Chinese cabbage to apple trees (Zhang *et al*., 2019; Zuo *et al*., 2019). An increased genomic sequencing capacity, dramatically improved sequencing technologies, and the ability to selectively sequence genes from specific families has allowed for the development of extensive gene libraries for crop species (Tripathi *et al*., 2020). These libraries also provide many new candidates for disease resistance genes, especially where the genomic location of genes and disease resistance QTLs overlap. It is likely that these technological advancements will further our understanding of the multifaceted roles played by WAKs in the coming years.

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Table 1. Characteristics of wall-associated kinase (WAK) genes implicated in plant immunity.

WAK gene	Plant species	Disease / pathogen species	Differentially regulated genes / pathways	Induced by invasion patterns	Induced by defence signalling molecules	Induced by other signals	Other roles	Protein interactions / associations	Citations
Isolate specific (gene-for-gene) resistance									
Rlm9	Oilseed rape (*Brassica napus*)	Stem canker / *Leptosphaeria maculans*	Hypersensitive response	AvrLm5-9				AvrLm5-9	Larkan *et al.*, 2019
Stb6	Wheat (*Triticum aestivum*)	Septoria tritici blotch / *Zymoseptoria tritici*	ROS production in substomatal cavity	AvrStb6				AvrStb6	Brading *et al.*, 2002 Shetty *et al.*, 2003 Saintenac *et al.*, 2018
Isolate non-specific resistance									
AtWAK1	Thale cress (*Arabidopsis thaliana*)	Grey mould / *Botrytis cinerea* Bacterial speck / *Pseudomonas syringae* Bacterial soft rot / *Pectobacterium carotovorum*	JA and ET expression *MAPK3* expression Ca^{2+} influx	OGs	SA		Requires for cell expansion	AtGRP3 (apoplastic) KAPP (cytoplasmic) NPR1-dependent defence response	He *et al.*, 1998 Wagner and Kohorn, 2001 Kohorn *et al.*, 2006 Moscatiello *et al.*, 2006 Brutus *et al.*, 2010
AtWAKL10	Thale cress (*A. thaliana*)	Bacterial speck / *Pseudomonas syringae*	Increase in cGMP	Chitin flg22 OGs		Nitric acid	Increases branching and germination under salt stress Negatively regulates drought tolerance		Meier *et al.*, 2010 Bot *et al.*, 2019
GhWAK7A	Cotton (*Gossypium hirsutum*)	Fusarium wilt / *Fusarium oxysporum* Verticillium wilt / *Verticilium longisporum* *V. dahliae*	Upregulation of *GhMPK3, GhMPK6* and *WRKY30* Production of ROS	Chitin				GhCERK1 (independent of chitin treatment) and GhLYK5	Wang *et al.*, 2020
GhWAKL	Cotton (*G. hirsutum*)	Verticillium wilt / *Verticilium dahliae*			SA			GhDNAJ1 (GhWAKL	Feng *et al.*, 2020

								kinase-dependent)	
OsWAK1	Rice (*Oryza sativa*)	Rice blast / *Magnaporthe oryzae*			SA JA	Wounding		OsRFP	Li *et al.*, 2009
OsWAK14	Rice (*O. sativa*)	Rice blast / *M. oryzae*		Chitin				OsWAK14 and OsWAK92	Cayrol *et al.*, 2016 Delteil *et al.*, 2016
OsWAKL21.2	Rice (*O. sativa*)	Rice blight / *Xanthamonas oryzae*	Upregulation of JA genes Callose deposition	LipA			Upregulation of SA genes Suppression of JA genes		Malukani *et al.*, 2019
*OsWAK25**	Rice (*O. sativa*)	Rice blight / *X. oryzae* Rice blast / *M. oryzae*	Upregulation of SA-related genes			Wounding	Mimic lesions (OE-lines)	Positive regulator of *Xa21*, XB15	Jo *et al.*, 2011 Seo *et al.*, 2011 Harkenrider *et al.*, 2016
OsWAK91 / OsDEES1	Rice (*O. sativa*)	Rice blast / *M. oryzae* Root rot / *Rhizoctonia solani*	ROS production Upregulation of defence genes Decrease in *M. oryzae* cell-to-cell movement	Chitin			Reduced fertility (KO-lines)	OsWAK92	Cayrol *et al.*, 2016 Delteil *et al.*, 2016
OsWAK92	Rice (*O. sativa*)	Rice blast / *M. oryzae*		Chitin				OsWAK91	Cayrol *et al.*, 2016 Delteil *et al.*, 2016
RFO1 / AtWAKL22	Thale cress (*A. thaliana*)	Fusarium wilt / *Fusarium oxysporum* Verticillium wilt / *Verticilium longisporum* *V. dahliae*		NLPs flg-22	JA ethylene			RFO2, RFO4, RFO6	Verica *et al.*, 2002 Diener and Ausubel, 2005 Johansson *et al.*, 2006
SlWAK1	Tomato (*Solanum lycopersicum*)	Bacterial speck / *Pseudomonas syringae*		flg22 flg28			Response to salt stress	FLS2 and FLS3 (required for flg22 PTI response)	Rosli *et al.*, 2013 Meco *et al.*, 2020 Zhang *et al.*, 2020

TaWAK2	Durum wheat (*Triticum durum*)	Fusarium head blight / *Fusarium graminearum*	Suppression of PME Reinforcement of cell wall					Gadaleta *et al.*, 2019
TaWAK6	Wheat (*T. aestivum*)	Wheat leaf rust / *Puccinia triticina*				Adult resistance only		Dmochowska-Boguta *et al.*, 2015 Dmochowska-Boguta *et al.*, 2020
Xa4	Rice (*O. sativa*)	Rice blight / *X. oryzae*	Accumulation of JA-Ile Upregulation of CesA genes and phytoalexins Downregulation of expansins		Temperature-dependent resistance	Reduction in height Increase in mechanical strength Higher cellulose content Decrease in lodging		Hu *et al.*, 2017
ZmWAK / qHSR1	Maize (*Zea mays*)	Head smut / *Sporisorium reilianum*	Suppression of auxin, JA, ABA and ethylene Promotion of SA Upregulation of *MEK1*			Promotes cell growth Controls turgor pressure		Zuo *et al.*, 2015 Zhang *et al.*, 2017
Htn1 / ZmWAK-RLK1	Maize (*Z. mays*)	Northern corn leaf blight / *Exserohilum turcicum*	Upregulation of JA and ethylene pathways Lignin biosynthesis			Downregulation of BXs		Hurni *et al.*, 2015 Yang *et al.*, 2019
Susceptibility factors								
*OsWAK25**	Rice (*O. sativa*)	Root rot / *R. solani* Brown spot / *Cochliobolus miyabeanus*	Upregulation of SA-related genes		Wounding	Mimic lesions (OE-lines)	Positive regulator of *Xa21*, XB15	Jo *et al.*, 2011 Seo *et al.*, 2011 Harkenrider *et al.*, 2016
OsWAK112d	Rice (*O. sativa*)	Rice blast / *M. oryzae*		Chitin (down-regulated)			Os02g12450 (related to *Xa21*)	Rohila *et al.*, 2006 Delteil *et al.*, 2016
Snn1	Wheat (*T. aestivum*)	Septoria nodorum blotch / *Parastagonospora nodorum*	ROS production Upregulation of *PR* genes DNA laddering Upregulation of *TaMAPK3*			Follows circadian expression cycle Light-dependence for susceptibility	SnTox1	Liu *et al.* 2012 Shi *et al.*, 2016

Other defence related WAKs							
CsWAKL08	Sweet orange (*Citrus sinensis*)	Bacterial canker / *Xanthomonas citri ssp. citri*	Upregulation of JA biosynthesis and JA-responsive genes ROS production		SA MeJA		Li *et al.*, 2020
GhWAK5A	Cotton (*G. hirsutum*)	Fusarium wilt / *Fusarium oxysporum* Verticillium wilt / *Verticilium longisporum V. dahliae*	Silencing induces increased resistance			Negative regulator	Wang *et al.*, 2020
GhWAK26	Cotton (*G. hirsutum*)	Verticillium wilt / *Verticilium longisporum V. dahliae*	Silencing leads to reduced resistance Reduced lignin content, nitric oxide and ROS		SA JA		Yang *et al.*, 2021
GhWAK77	Cotton (*G. hirsutum*)	Verticillium wilt / *Verticilium dahliae*	Silencing leads to reduced resistance Reduced lignin content, nitric oxide and ROS		SA		Yang *et al.*, 2021
Sbs1 and Sbs2	Barley (*Hordeum vulgare*)	Root rot / *Bipolaris sorokiniana*	ROS production Hypersensitive response	Non-proteinaceous effector		Susceptibility factor	Ameen *et al.*, 2020
TaWAK5	Wheat (*T. aestivum*)	Root rot / *R. solani*	Elevated expression in resistant lines Silencing has no impact on resistance		SA ABA JA		Yang *et al.*, 2014

*OsWAK25 has been described as a resistance or a susceptibility WAK in different studies

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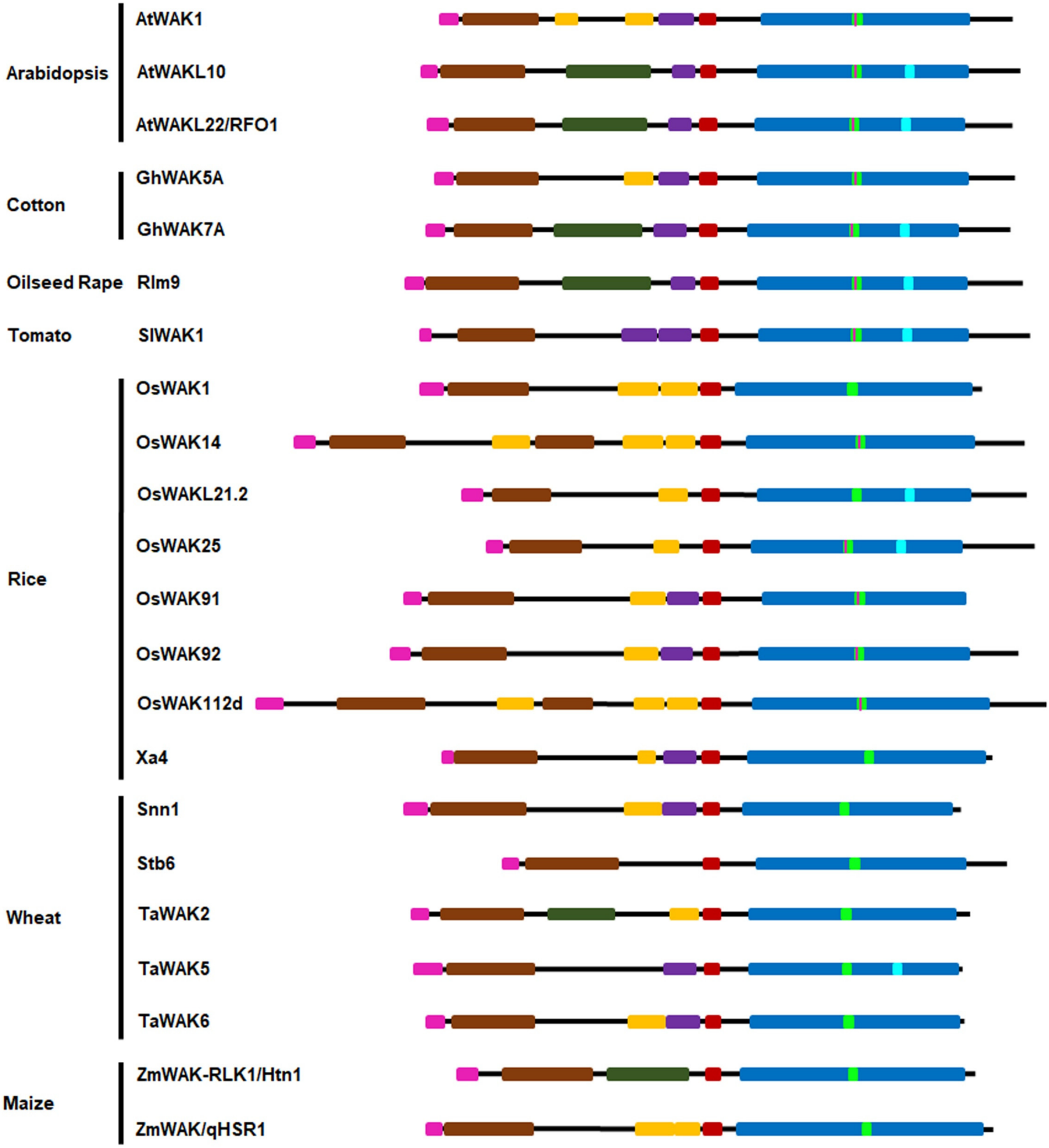

Figure 1. Domain architecture of immunity-related WAKs from both dicot and monocot species. The extracellular domains of WAKs show a high degree of diversity, with many possessing galacturonan-binding domains (GUB_WAK_bind; brown), implicated in binding cell wall pectin and epidermal growth factor (EGF)-like (orange) and $Ca2^{+}$-dependent EGF (purple) that may play a role in protein-protein interactions. The WAK intracellular domains, including a serine/threonine kinase (blue) are more well conserved, with immunity-related WAKs possessing a non-arginine aspartate (RD) motif so far identified exclusively in monocots. Other domains/motifs: signal peptide (pink), WAK_assoc_C (dark green), transmembrane helix (red), serine/threonine kinase (blue), RD motif (pink bar), kinase active site (green), and guanylyl cyclase (cyan).

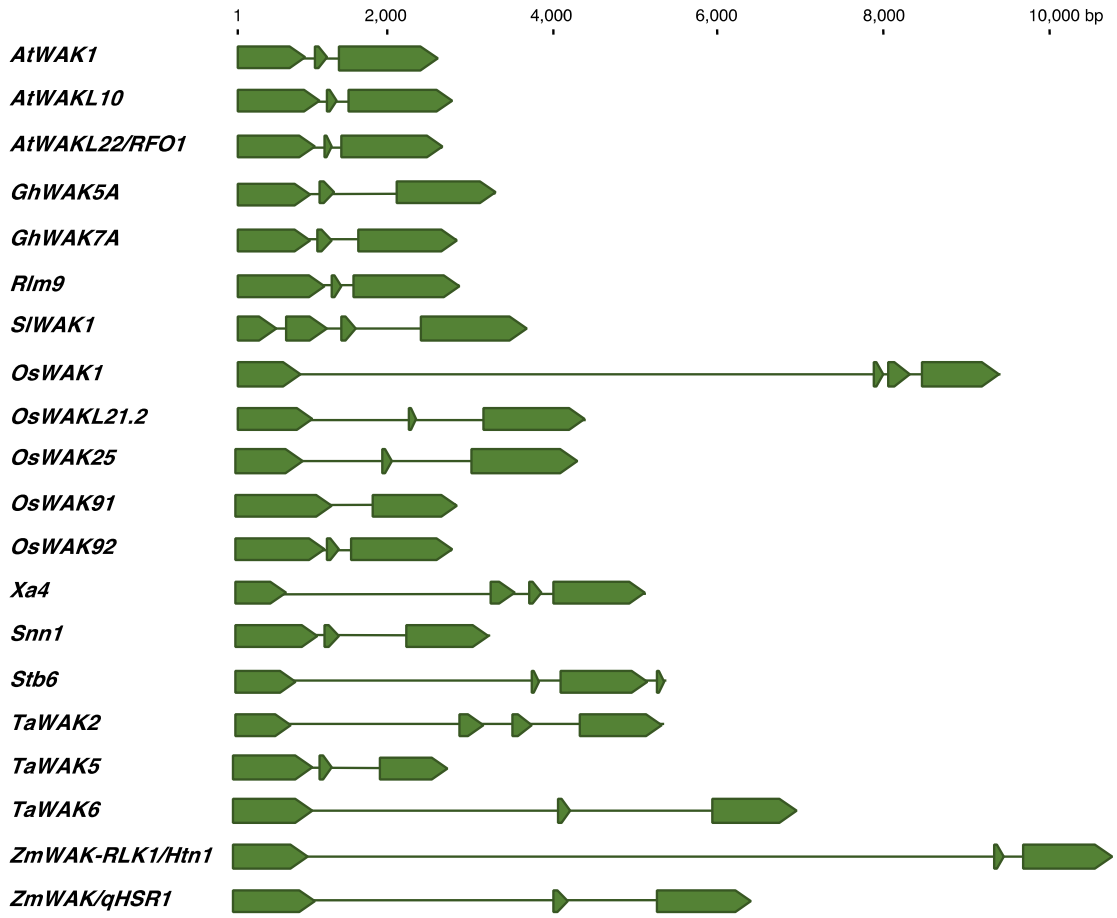

Figure 2. Exon-intron structure of immunity-related WAK genes. The majority of WAK genes comprise three or four exons (green boxes), with a short second exon. In monocots, the length of the first intron (green connecting lines) varies considerably and possibly contains alternative exons for splicing variants of WAK genes. Despite the considerable difference in length of different WAK genes, the length of the WAK proteins they encode is more consistent. Gene length in base pairs is shown above.

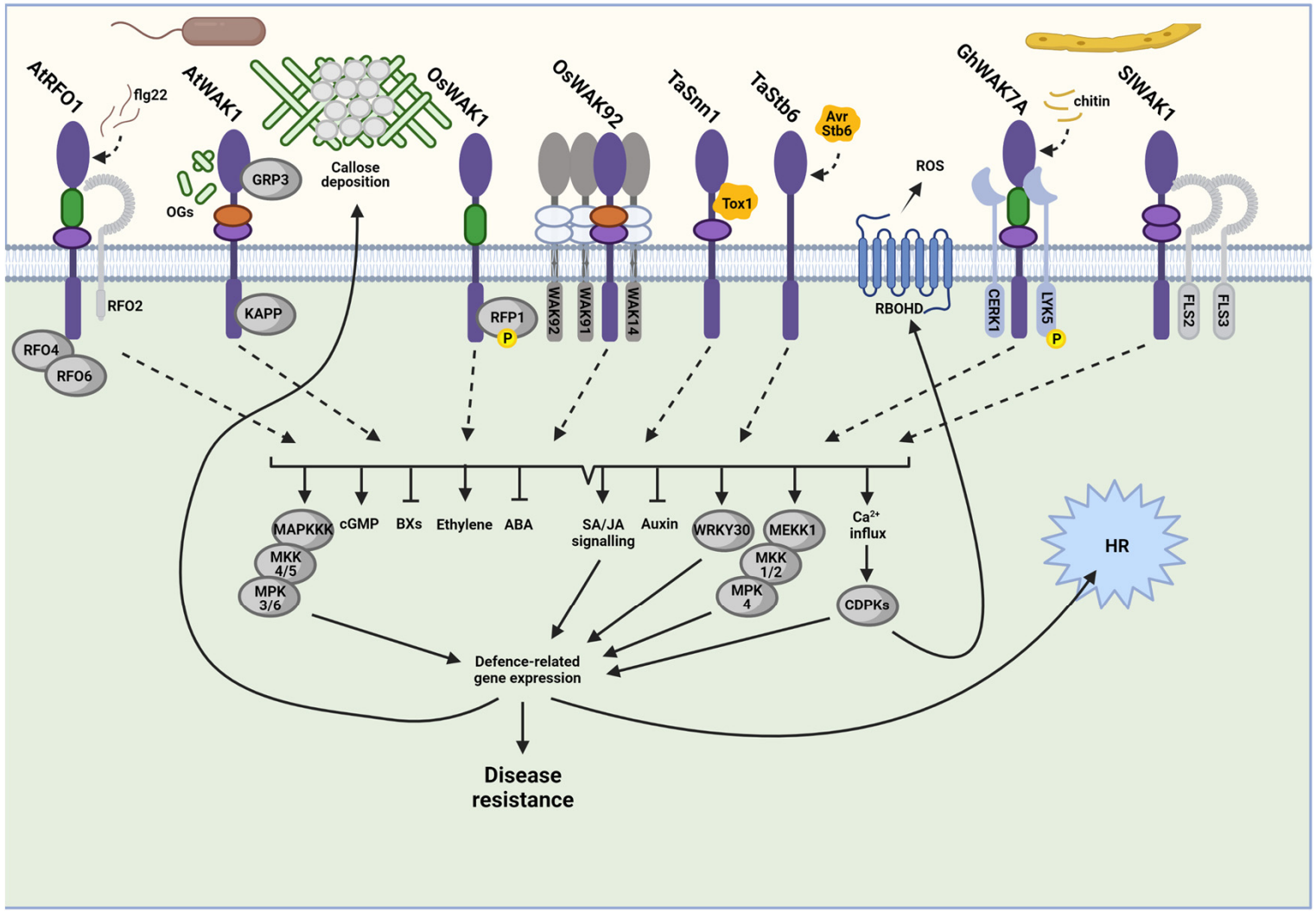

Figure 3. Schematic diagram illustrating the downstream pathways employed by some of the better characterised immune-related WAKs, as part of their defence responses. Immune responses directed by different WAKs vary considerably and often involve protein-protein interactions with other membrane-bound proteins or cytoplasmic proteins which leads to activation of mitogen-activated protein kinase (MAPK) cascades, hormone fluctuations, the production of reactive oxygen species (ROS), and in some cases a hypersensitive response (HR). Figure created with BioRender.com.