OXI1 protein kinase is required for plant immunity against Pseudomonas syringae in Arabidopsis

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Abstract

Expression of the Arabidopsis Oxidative Signal-Inducible1 (OXI1) serine/threonine protein kinase gene (At3g25250) is induced by oxidative stress. The kinase is required for root hair development and basal defence against the oomycete pathogen Hyaloperonospora parasitica, two separate H2O2-mediated processes. In this study, the role of OXI1 during pathogenesis was characterized further. Null oxi1 mutants are more susceptible to both virulent and avirulent strains of the biotrophic bacterial pathogen Pseudomonas syringae compared with the wild type, indicating that OXI1 positively regulates both basal resistance triggered by the recognition of pathogen-associated molecular patterns, as well as effector-triggered immunity. The level of OXI1 expression appears to be critical in mounting an appropriate defence response since OXI1 overexpressor lines also display increased susceptibility to biotrophic pathogens. The induction of OXI1 after P. syringae infection spatially and temporally correlates with the oxidative burst. Furthermore, induction is reduced in atrbohD mutants and after application of DPI (an inhibitor of NADPH oxidases) suggesting that reactive oxygen species produced through NADPH oxidases drives OXI1 expression during this plant–pathogen interaction.

Key words: Hyaloperonospora parasitica, plant defence, Pseudomonas syringae, reactive oxygen species, signal transduction.

Introduction

Plant immunity to the wide variety of potential pathogens involves a complicated web of components ranging from preformed defence barriers to signalling molecules such as reactive oxygen species (ROS), protein kinases, and hormones to elicit appropriate end responses (Thomma et al., 2001; Ingle et al., 2006; Torres et al., 2006). The current viewpoint is that there are two major branches of plant immunity as reviewed by Jones and Dangl (2006). The first encompasses a general immune response triggered by the recognition of evolutionary conserved pathogen-associated molecular patterns (PAMPs), for example, bacterial flagellin, lipopolysaccharides, and fungal chitin. This PAMP-triggered immunity (PTI) activates a series of inducible basal defence mechanisms such as callose deposition and defence gene expression and is successful against non-host pathogens. Virulent pathogens suppress PTI via pathogen effector molecules which can target components of the basal defence mechanism and induce effector triggered susceptibility (ETS). This enables virulent pathogens to cause disease on susceptible host plants (Jones and Dangl, 2006). The second layer of immunity occurs when the host plant harbours a resistance protein to detect either the presence and/or activity of one or more effectors resulting in the rapid activation of plant defence responses and disease resistance known as effector triggered immunity (ETI) (Mackey et al., 2002; Jones and Dangl, 2006). Although ETI responds faster to pathogen infection, PTI and ETI share many regulatory components (Ingle et al., 2006).

Central to plant immunity against biotrophic pathogens is the accumulation of ROS, which apart from direct functions in toxicity (Kepler et al., 1989) and oxidative cross-linking of plant cell walls (Bradley et al., 1992; Fry...
et al., 2000) serve a signalling role in mounting the defence response (Grant and Loake, 2000). A key feature of ROS signalling is regulation of the hypersensitive response (HR) characterized by rapid localized cell death at the infection site as well as the induction of defence-related genes (Levine et al., 1994; Lamb and Dixon, 1997; Grant and Loake, 2000). Chemical inhibition of ROS accumulation following pathogen challenge in Arabidopsis led to a reduction in the HR and inhibited expression of the defence gene glutathione-S-transferase1 (Alvarez et al., 1998). Conversely, elevation of H$_2$O$_2$ levels either through suppression of antioxidant enzyme activity, such as in transgenic tobacco plants deficient in peroxisomal catalase activity (Channonpol et al., 1998), or expression of enzymes required for ROS production, such as in transgenic potato plants expressing glucose oxidase (Wu et al., 1997), resulted in a primed immune response with accumulation of salicylic acid (SA), expression of defence-related genes, and enhanced resistance to a broad range of pathogens. More recently, Arabidopsis ascorbate-deficient mutants were found to exhibit microlesions, constitutive Pathogenesis Related (PR) gene expression, and increased resistance to Pseudomonas syringae infection (Pavet et al., 2005) providing further evidence for the role of ROS accumulation in disease-resistance responses.

Genetic evidence points to a role for the respiratory burst NADPH oxidase as the principal source of ROS production during pathogen challenge (Torres et al., 2002). Arabidopsis mutants lacking either or both of the respiratory burst oxidase genes, AtRbohD and AtRbohF, which encode catalytic subunits of the NADPH oxidase, displayed a reduction in H$_2$O$_2$ accumulation and the HR in response to avirulent P. syringae pv. tomato DC3000avrRpm1 infection compared to wild-type Arabidopsis (Torres et al., 2002). However, following challenge with a virulent Hyaloperonospora parasitica strain (formerly known as Peronospora parasitica; Constantinescu and Fatehi, 2002), the atrbohF mutant displayed an enhanced HR and increased resistance to this pathogen (Torres et al., 2002) indicating that the HR is differentially regulated by ROS accumulation depending on the invading pathogen. Alternative mechanisms for ROS production during pathogen attack have been demonstrated, for example, pharmacological inhibition of peroxidase activity but unaffected by NADPH oxidase.

Despite the strong correlation between ROS accumulation and disease resistance, current understanding of the discriminators of ROS signalling is sorely limiting. The OXIDATIVE SIGNAL-INDUCIBLE1 (OXII) protein kinase has emerged as a potential player linking ROS accumulation to disease resistance in response to virulent H. parasitica attack (Rentel et al., 2004). OXII is not only induced by the exogenous application of H$_2$O$_2$ and challenge with virulent H. parasitica Emc5 but the oxil null mutant also displayed increased susceptibility compared to wild-type Arabidopsis following infection with Emc5 (Rentel et al., 2004). Furthermore, OXII is required for the partial activation of MPK3 and MPK6 in response to treatment with H$_2$O$_2$ and cellulase, mimicking pathogen attack (Rentel et al., 2004). Both MPK3 and MPK6 are involved in the mitogen-activated protein kinase cascade activated following recognition of bacterial flagellin by the receptor-like kinase FLS2 (Asai et al., 2002) which initializes the induction of defence genes such as WRKY22/29 and GST and is effective in defence responses against both bacterial and fungal pathogens (Gomez-Gomez et al., 2001; Asai et al., 2002; Chinchilla et al., 2006). In this report, a role for OXII in Arabidopsis is further extended to plant immunity against the bacterial pathogen P. syringae and NADPH-produced ROS is shown to drive expression of OXII during this plant–pathogen interaction. Interestingly, regulation of OXII expression levels appears important in mediating an appropriate defence response, since both down-regulation and overexpression of OXII results in enhanced susceptibility to biotrophic pathogens.

### Materials and methods

#### Plant growth conditions

Arabidopsis thaliana plants were grown on a 1:1 (v/v) soil mix composed of peat (Jiffy Products, International AS, Norway) and vermiculite in a controlled environment under a 16/8 h light/dark cycle at 21°C, 55% relative humidity, and fluorescent light of 80–100 μmol photon m$^{-2}$ s$^{-1}$.

#### Plant lines

Wild-type Arabidopsis seeds were acquired from Lehle Seeds (Lehle, Texas, USA). The oxil null mutant, OXII complemented, and OXII::GUS transgenic lines were the same as those used in Rentel et al. (2004). The atrbohD T-DNA mutant line used was that described in Torres et al. (2002).

#### Generation of 35S::OXI1 and 35S::OXI1-YFP constructs

A 1.4 kb DNA fragment of OXII including the entire coding region and its intron was PCR amplified from genomic DNA from the Ws-2 ecotype with the primers 5’-GGCCCTGAGGTCGACATTATGCTAGAGGG-3’ and 5’-GGCCGGATCCCATACACCATAGCCATTCCATAGAC-3’. The 2.5 kb OXII–YFP protein fusion comprising a 1.4 kb OXII DNA fragment, a 1.1 kb YFP coding region, and a c-myc epitope tag, was PCR amplified from the pBluescript SK+ plasmid harbouring the OXII-YFP-cmyc construct (Rentel, 2002) with the primers 5’-GGCCGGATCCGGTCGACATTATGCTAGAGGG-3’ and 5’-GGCCCGGGGCAAGACCGGCAACAGGATTC-3’.

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Both PCR products were cloned into the pUC2X3SS plasmid containing two 35S CaMV promoters with the restriction enzymes PstI and BamHI for OXI1 and BamHI and XmaI for OXII-YFP-cmyc, respectively, followed by subcloning into the pBINPLUS binary vector through the unique restriction sites AscI and PacI. Both vectors were a gift from Malcolm Campbell (Department of Botany, University of Toronto, Canada). The resulting plasmids were transformed into the C58C1 strain of Arabidopsis plants of the Ws-2 ecotype by the floral dip method (Clough and Bent, 1998). 25 µg ml⁻¹ kanamycin was used for selection of homozygous lines.

Pathogen infections

Inoculations with virulent Pseudomonas syringae pv. tomato DC3000 and avirulent P. syringae harbouring the avrB gene were performed as described in Murray et al. (2002). The avirulent strain was maintained and grown on King’s broth media (King et al., 1954) supplemented with 50 µg ml⁻¹ rifampicin and 50 µg ml⁻¹ kanamycin. Inoculation and assessment of Hyaloperonospora parasitica sporulation was determined as described in Rentel et al. (2004). All pathogen infection experiments were repeated at least three times.

In vivo histochemical GUS and DAB staining

GUS staining of Arabidopsis leaves was performed as previously described by Rentel et al. (2004). The presence of H₂O₂ was detected by gently shaking leaves submerged in a 1 mg ml⁻¹ 3,3'-diaminobenzidine (DAB) solution for 2–3 h at room temperature until a reddish-brown precipitate was observed. Images for both GUS and DAB staining were obtained by scanning the leaves with a Canon 8400F Scanner.

Northern blot analysis

Total RNA was extracted using either the RNasey Plant Total RNA kit (Qiagen, UK) as per the manufacturer’s instructions or a guanidinium thiocyanate-phenol-chloroform extraction protocol (Chomczynski and Sacchi, 1987). Electrophoresis and transfer of RNA onto nylon membrane was performed as previously described by Murray et al. (2007). DNA probes were labelled with 32P using a Megaprime DNA labelling kit (Amersham, UK) and hybridized to total RNA in hybridization buffer composed of 5× SSC, 50% (v/v) formamide, 0.5% (v/v) SDS, 5× Denhardt’s solution, and 100 µg ml⁻¹ denatured salmon sperm DNA. A full-length 1.4 kb DNA probe of OXI1 (At3g25250) was obtained through restriction digestion of OXI1 cloned into the pUC2X3SS plasmid with the enzymes PstI and BamHI. The VSP1 (At5g24780) template of approximately 300 bp was amplified by PCR of genomic DNA with the primers 5’-GGGATCCATCGTTCCAGCGTC-3’ and 5’-CTAGAGAAGGAGCTGTC-3’. The PR-1 (At2g14610) probe was amplified from genomic DNA using primers previously described by Denby et al. (2005).

Results

OXI1 is necessary for full resistance to P. syringae

Given the requirement for OXI1 in the basal defence response to virulent H. parasitica (Rentel et al., 2004), it was investigated whether OXI1 is required for defence against other virulent plant pathogens. The oxil null mutant, wild type (Ws-2), and the oxil mutant complemented with the wild-type OXI1 gene (oxil+OXII) transgenic line were challenged with virulent P. syringae pv. tomato DC3000 (Pst DC3000). The oxil mutant exhibited increased susceptibility at 2 and 3 d post-inoculation (dpi) compared with the wild type (Fig. 1A; see Supplementary Fig. S1A at JXB online). Importantly, the complemented line exhibited wild-type bacterial titres, demonstrating that the increased susceptibility phenotype of the oxil mutation was due to the lack of OXI1 expression (Fig. 1A; see Supplementary Fig. S1B at JXB online). OXI1 is therefore required for basal resistance against both an oomycete (H. parasitica) and a bacterial (Pst DC3000) biotrophic pathogen. Despite strong induction after infection with Botrytis cinerea (see Supplementary Fig. S2 at JXB online), oxil mutants did not show altered susceptibility to this necrotrophic pathogen (data not shown).

It was also found that OXI1 is necessary for full resistance against an avirulent isolate of P. syringae which carries the avrB gene (Pst DC3000 avrB) (Fig. 1B; see Supplementary Fig. S1C at JXB online). Again, the complemented line contained bacterial titres similar to the wild type. The requirement for OXI1 for full resistance was confirmed using an additional avirulent isolate of P. syringae (Pst DC3000 carrying avrRpt2) (see Supplementary Fig. S3 at JXB online). Hence, although defence against avirulent H. parasitica isolates is OXI1-independent (Rentel, 2002), OXI1 is required for full resistance against both virulent and avirulent P. syringae.

Overexpression of OXI1 results in increased susceptibility to biotrophic pathogens

Having demonstrated that oxil mutants are more susceptible to P. syringae, it was tested whether increased expression of OXII could lead to enhanced resistance. Two independent overexpressor lines were generated; both drive OXII expression from the 35S CaMV promoter but one contains OXII fused to the reporter gene YFP. Both lines show increased OXII expression at the mRNA level (Fig. 2). Surprisingly, these overexpressor lines displayed enhanced susceptibility to both virulent and avirulent isolates of P. syringae (Fig. 3A, B). Since both overexpressor lines showed the same phenotype, the increased susceptibility was not due to the position of the transgene or as a consequence of the YFP fusion. Due to this unexpected result, and as oxil mutants show increased susceptibility to virulent H. parasitica (Rentel et al., 2004), the susceptibility of these overexpressing lines to the virulent H. parasitica isolate Emco5 was tested (Fig. 3C). Again, the 35S::OXII over-expressor showed enhanced susceptibility (as seen by
increased sporulation) compared to the wild type. Sporulation in the 35S::OXI1-YFP line was highly variable, hence, although the average susceptibility was increased, the result was not statistically significant. From these results it was concluded that modulation of OXI1 expression levels (either increased or knocked out) causes increased susceptibility to virulent and avirulent P. syringae as well as to virulent H. parasitica isolates.

Expression of two defence marker genes is uncompromised in the oxi1 null mutant

As OXI1 is required for resistance against at least two biotrophic pathogens, an attempt was made to establish a functional basis for this requirement. However, expression of the classic defence gene PR-1 was not compromised in the oxi1 mutant following infection with avirulent P. syringae (Fig. 4A; see Supplementary Fig. S4A at JXB online). Given that OXI1 is required for full activation of MPK3 and MPK6 in response to ROS and cellulase treatment (Rentel et al., 2004), it was investigated whether OXI1 regulates the expression of MPK6-dependent Vegetative Storage Protein1 (VSP1) in response to pathogen challenge. VSP1 was induced only 48 h after infection with virulent P. syringae, again this induction was not affected in the oxi1 mutant (Fig. 4B; see Supplementary Fig. S4B at JXB online). As expected, VSP1 was not induced in response to challenge with avirulent P. syringae in either wild-type or oxi1 mutant plants (data not shown).

The oxidative burst mediates induction of OXI1 expression

Expression of OXI1 is known to be induced in response to ROS (H2O2) and in cells adjacent to H. parasitica hyphae (Rentel et al., 2004). As ROS production is one of the earliest plant responses to pathogen infection (Lamb and Dixon, 1997), it was investigated whether ROS accumulation was responsible for the induction of OXI1 gene expression after P. syringae infection. After infection with either virulent or avirulent P. syringae, GUS expression driven by the OXI1 promoter increased and was confined to the regions of ROS accumulation in the leaf (Fig. 5A). Two methods were used to reduce the rapid oxidative burst which occurs after avirulent P. syringae infection and to determine the effect on...
OXI1 expression. As expected, an atrbohD mutant failed to accumulate H2O2 during infection with avirulent Pst DC3000 avrB (Fig. 5B). OXI1 expression in this mutant background was reduced compared to the wild-type control background was reduced compared to the wild-type control (Fig. 5C), suggesting that ROS generated through NADPH oxidase is at least partly responsible for the induction of OXI1 during ETI. This conclusion was strengthened by reduced GUS activity in leaves of OXI1::GUS plants co-infiltrated with Pst DC3000avrB and 10 μM diphenylene iodonium (DPI), a chemical inhibitor of NADPH oxidase, compared to leaves infiltrated with Pst DC3000avrB alone (Fig. 5D; see Supplementary Fig. S5 at JXB online).

Discussion

Pathogen-induced OXI1 expression is the result of ROS accumulation, produced at least in part, via the AtrbohD NADPH-oxidase mechanism (Fig. 5) and OXI1 clearly contributes to both basal and effector-triggered resistance to the bacterial pathogen P. syringae (Fig. 1). It is thought that the regulation of PTI and ETI resistance responses overlaps considerably, with ETI being an accelerated and amplified PTI response (Jones and Dangl, 2006). Large-scale expression profiling provided evidence that ETI is qualitatively similar to PTI as the expression profiles, as well as the level of induction of genes, during the early stages of infection with P. syringae pv. maculicola (Psm) harbouring the avirulence avrRpt2 gene were similar to those produced during the late stages of infection with virulent Psm (Tao et al., 2003). Furthermore, Arabidopsis mutant analysis has identified many molecular components in the defence signalling network involved in both PTI and ETI. For example, a mutation in the ENHANCED...
DISEASE SUSCEPTIBILITY1 (EDS1) gene results in eds1 mutants being more susceptible to both virulent and avirulent isolates of *H. parasitica* (Parker et al., 1996). Similarly, the *Arabidopsis* mutants eds5, npr1, sid2, and pad4 are compromised in their resistance responses to both virulent and avirulent isolates of *H. parasitica* and/or *P. syringae* (Cao et al., 1994; Zhou et al., 1998; Nawrath and Métraux, 1999). Silencing of MPK6, a component of the *Arabidopsis* MAPK cascade induced by flagellin during PTI, also leads to enhanced susceptibility to virulent and avirulent isolates of *P. syringae* (Menke et al., 2004). Our data indicate that OXI1 represents another shared component between PTI and ETI since the oxi1 mutant exhibits enhanced bacterial titres compared to the wild type following infection with both virulent and avirulent *P. syringae*. OXI1 is likely to trigger phosphorylation events that result in the activation of defence responses that serve to restrict or slow the process of pathogen growth.

In contrast to infection by *P. syringae*, a role for OXI1 could only be discerned in the defence against a virulent
H. parasitica isolate and not the avirulent isolate Emoy2 (Rentel et al., 2004). Only one avirulent isolate of H. parasitica has been tested and it is possible that OXI1 may play a role in ETI against isolates with different effector complements. Given that effectors themselves target different components of the defence system (which are then unavailable for signalling), it is unlikely that ETI signalling will be identical in response to all effectors.

While infection with the necrotrophic pathogen B. cinerea resulted in increased expression of OXI1 in Arabidopsis (see Supplementary Fig. S1 at JXB online), lack of OXI1 did not increase susceptibility compared with the wild type (data not shown). Induction of OXI1 in response to B. cinerea appears to be a consequence of ROS accumulation during this interaction, without an active role in defence. Hence our results currently limit the role of OXI1 to disease resistance against biotrophic pathogens.

A surprising feature of OXI1 is that reduced expression and overexpression of OXI1 both led to enhanced susceptibility to biotrophic pathogens (Fig. 3). It is unlikely that a single kinase plays both a positive and a negative role during the same defence response. Hence it is proposed that the level of OXI1 protein is crucial for the appropriate signalling, and modulation of these levels (either higher or lower) disrupts OXI1 function. As transcript levels do not necessarily correlate with protein levels, it is possible that the OXI1 overexpression lines either have lower or higher OXI1 protein levels compared to wild type since these lines mirror the loss of function mutant. In the first instance, if the protein levels are actually reduced in the overexpression lines relative to the wild type, it could be that prolonged expression of OXI1 protein from a constitutive promoter (35S) might lead to enhanced activation of pathways naturally present to regulate the OXI1 protein negatively, i.e. by protein degradation. Alternatively, it would be more orthodox and parsimonious to assume that protein levels are higher in the overexpression lines than in the wild type. It is not uncommon for plants overexpressing proteins to show the same phenotypes as loss of function mutants, for example, FIP1 and EBS, also in Arabidopsis (Pineiro et al., 2003; Chen et al., 2007). In these cases the perceived wisdom is that these proteins operate in complexes in which the stoichiometry is crucial and regulated by the levels of protein expression. Therefore, both under- or over-expression would lead to suboptimal complex formation, leading to reduced function. Therefore the absolute level of abundance of such components is crucial and OXI1 may be one such component. Furthermore, OXI1 levels appear to be tightly controlled in planta; 35S::OXI1-YFP lines show low levels of YFP protein compared with lines expressing YFP-aequorin protein in single root cell types, and treatment of seedlings with the proteasome inhibitor MG132 for 1 h results in dramatically increased protein levels indicating a short half-life (data not shown). This rapid turnover of OXI1 protein together with the importance of timing in pathogen responses presents another scenario whereby the key factor could also be one of appropriate timing. For example, having either OXI1 protein or transcript already present when plants are challenged with pathogen may be detrimental to establishing an optimal defence response.

Despite the demonstration of MPK6 as a downstream component of OXI1 in response to H2O2 and cellulase treatment (Rentel et al., 2004) and the fact that the defence phenotype of mpk6 silenced Arabidopsis mutants resembles that of oxi1 (Menke et al., 2004), MPK6-dependent expression of VSP1 during PTI appears to be independent of OXI1 (Fig. 4B). Similarly, despite the importance of SA signalling to disease resistance against biotrophic pathogens, OXI1 was not required for expression of the SA marker gene PRI (Fig. 4A) or development of systemic acquired resistance (see Supplementary Fig. S6 at JXB online). OXI1 interacts directly with, and can phosphorylate, the Ser/Thr protein kinase PTI1-2 (Anthony et al., 2006). PTI1-2 is activated in response to various stress treatments and is dependent on OXI1 for its activation in response to flagellin and H2O2 (Anthony et al., 2006). Given the homology between PTI1-2 and the tomato Pto kinase, which confers resistance to avirulent P. syringae carrying the avrPto gene (Zhou et al., 1995), it is tempting to speculate that OXI1 promotes defence against P. syringae through the activation of PTI1-2. However, no targets of PTI1-2 have been identified and, unlike Pto, OXI1 is likely to function downstream of ROS. Hence, the identification of additional direct targets of OXI1 will be vital in elucidating the function of this ROS-responsive kinase during Arabidopsis-Pseudomonas/Hyaloperonospora interactions and in addressing how specificity of ROS signalling is achieved.

Supplementary data

Supplementary data are available at JXB online.

Supplementary Fig. S1. The oxi1 null mutant is more susceptible to both virulent and avirulent P. syringae (A, C) and this genotype of the null mutant is rescued in the complemented line which exhibits wild-type bacterial titres when challenged with P. syringae (B, C).

Supplementary Fig. S2. OXI1 expression is induced following infection of Arabidopsis leaves with the necrotrophic pathogen Botrytis cinerea.

Supplementary Fig. S3. The oxi1 null mutant is more susceptible than the wild type to the avirulent strain Pst DC3000 avrRpt2.

Supplementary Fig. S4. Expression of PR-1 and VSP1 is unaffected in the oxi1 null mutant in response to pathogen challenge.

Supplementary Fig. S5. DPI treatment alone has no effect on OXI1::GUS expression.

Supplementary Fig. S6. The oxi1 mutation does not affect the development of systemic acquired resistance.

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References


Chammongpol S, Willekens H, Moeder W, Langebartels C, Anthony RG, Khan S, Costa J, Pais MS, Bo¨ gre L.

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References


Parker JE, Holub EB, Frost LN, Falk A, Gunn ND, Daniels MJ. 1996. Characterization of eds1, a mutation in Arabidopsis suppressing...
resistance to *Peronospora parasitica* specified by several different *RPP* genes. *The Plant Cell* 8, 2033–2046.


