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Arabidopsis MLO2 is a negative regulator of sensitivity to extracellular ROS

Running title: Role of MLO2 in ozone- and abiotic stresses

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ABSTRACT

The atmospheric pollutant ozone (O₃) is a strong oxidant that causes extracellular reactive oxygen species (ROS) formation, has significant ecological relevance, and is used here as a non-invasive ROS inducer to study plant signalling. Previous genetic screens identified several mutants exhibiting enhanced O₃-sensitivity, but few with enhanced tolerance. We found that loss-of-function mutants in Arabidopsis *MLO2*, a gene implicated in susceptibility to powdery mildew disease, exhibit enhanced dose-dependent tolerance to O₃ and extracellular ROS, but a normal response to intracellular ROS. This phenotype is increased in a *mlo2 mlo6 mlo12* triple mutant, reminiscent of the genetic redundancy of *MLO* genes in powdery mildew resistance. Stomatal assays revealed that enhanced O₃ tolerance in *mlo2* mutants is not caused by altered stomatal conductance. We explored modulation of the *mlo2*-associated O₃ tolerance, powdery mildew resistance, and early senescence phenotypes by genetic epistasis analysis, involving mutants with known effects on ROS sensitivity or antifungal defence. Mining of publicly accessible microarray data suggests that these *MLO* proteins regulate accumulation of abiotic stress response transcripts, while transcript accumulation of *MLO2* itself is O₃-responsive. In summary, our data reveal *MLO2* as a novel negative regulator in plant ROS responses, which links biotic and abiotic stress response pathways.

KEYWORD INDEX

ROS signalling, *MLO2*, ozone, immunity, abiotic stress, unequal genetic redundancy

INTRODUCTION

Mildew Locus O (*MLO*) genes code for polytopic integral membrane proteins of unknown biological function (Acevedo-Garcia et al. 2014). *Mlo* was originally identified in barley (*Hordeum vulgare*), but is conserved in plants in small to medium-sized gene families (Büschges et al. 1997; Kusch et al. 2016). Loss-of-function alleles of specific *MLO*s confer resistance against powdery mildew disease in various plant species, thus they are considered negative regulators of plant immunity or alternatively as “susceptibility” genes co-opted by powdery mildew pathogens to promote pathogenesis (van Schie, C. C. N. & Takken 2014; Panstruga 2005). The *Arabidopsis thaliana* (*Arabidopsis*) genome contains a 15-member *MLO* family. *MLO2* (At1g11310), *MLO6* (At1g61560) and *MLO12* (At2g39200) form a phylogenetic group (clade V *MLO*s) most similar to barley *Mlo* (Devoto et al. 2003). These genes co-function with unequal redundancy in the regulation of immunity against *Arabidopsis* powdery mildew pathogens such as *Golovinomyces orontii*. Accordingly, *mlo2* single mutants are partially and *mlo2 mlo6 mlo12* triple mutants fully resistant (Consonni et al. 2006; Acevedo-Garcia et al. 2017). Reminiscent of penetration resistance typical for non-host interactions (Humphry et al. 2006), immunity conferred by loss of *MLO* function is broad spectrum, durable, and halts fungal ingress prior to the sporeling breaching the host cell wall (Acevedo-Garcia et al. 2014). Penetration resistance is associated with deposition of cell wall appositions (papillae) at the attempted entry sites. Among other constituents, papillae contain callose, which locally reinforces the cell wall (Ellinger et al. 2013; Voigt 2014). Papilla formation often spatially and temporally coincides with an apoplastic burst of reactive oxygen species (ROS), which further strengthens cell walls by oxidative crosslinking (Hückelhoven et al. 2001; Mellersh et al. 2002). In addition to its immunity phenotypes, the *Arabidopsis mlo2*

single and *mlo2 mlo6 mlo12* triple mutants also exhibit premature leaf senescence with associated spontaneous, ectopic callose deposition and ROS accumulation (Consonni et al. 2006; Acevedo-Garcia et al. 2017; Consonni et al. 2010). Beyond *MLO2*, *MLO6* and *MLO12*, few functions of other Arabidopsis *MLO* genes are known. *MLO4* and *MLO11* are involved in modulating root thigmomorphogenesis (Chen et al. 2009; Bidzinski et al. 2014) and *MLO7* in the regulation of pollen tube growth (Kessler et al. 2010).

ROS act as versatile signalling intermediates in a variety of cellular pathways. Signalling specificity is determined largely by the site of ROS production and the chemical properties of each species, such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), or singlet oxygen (1O_2) (Gadjev et al. 2006). In plant development, ROS are involved in growth, specifically in cell wall loosening, cell expansion, and cell cycle progression (Gapper & Dolan 2006; Swanson & Gilroy 2010; Polyn et al. 2015). Other developmental events, such as fertilization, mechanosensing in thigmomorphogenesis, senescence, and developmentally programmed cell death are also regulated by ROS (Huysmans et al. 2017; Rogers & Munné-Bosch 2016; Duan et al. 2014). Importantly, ROS signalling is a prominent feature in plant responses to both abiotic and biotic stresses. ROS act as signalling intermediates in the abscisic acid (ABA) pathway, a key regulator of stomatal closure and adaptation to heat, cold, desiccation, and osmotic stresses (Tuteja 2007). Additionally, ROS function in ABA-independent pathways in the response to these stresses. In plant immunity, defence responses are coordinated by a rapid and transient peak in ROS production termed the oxidative burst (Torres 2010). The oxidative burst is characteristic of multiple forms of disease resistance, playing an active role in basal defences, or so-called PAMP-triggered immunity (PTI), as well as coordinating the activation of defence signalling

and cell death in gene-for-gene resistance, or effector-triggered immunity (ETI; Jones & Dangl 2006). ROS production in plant immune responses occurs typically on the extracellular side, where plasma membrane NADPH oxidases, dubbed in plants as respiratory burst oxidase homologs (RBOHs), or apoplastic peroxidases, produce superoxide into the apoplastic space (Qi et al. 2017).

Ozone (O₃) is an atmospheric pollutant and itself a form of ROS (Overmyer et al. 2009). Entering leaves through open stomata, O₃ breaks down in the apoplast to form other ROS, which in turn trigger an active oxidative burst within the plant (Wohlgemuth et al. 2002). Thus, O₃ exposure has been utilized as a tool to induce the oxidative burst, in the absence of other pathogen signals, for the study of downstream ROS signalling (Vainonen & Kangasjärvi 2015). The level of sensitivity to O₃ within Arabidopsis is subject to natural genetic variation (Brosché et al. 2010). In addition, a number of induced mutants with enhanced O₃ sensitivity have been identified (Vainonen et al. 2015; Overmyer et al. 2008; Rao et al. 2002; Overmyer et al. 2000; Saji et al. 2017; Saji et al. 2008). These are defective in various physiological pathways that likely are interconnected functionally with each other (Overmyer et al. 2008). However, only few mutants with enhanced O₃ tolerance are known (Booker et al. 2004; Overmyer et al. 2000; Joo et al. 2005).

Prompted by apparent links of MLO proteins to physiological processes involving ROS, we took a genetic approach using O₃ exposure to address the role of MLOs in ROS signalling.

MATERIALS AND METHODS

Plant growth

Arabidopsis thaliana (*Arabidopsis*) was grown on 1:1 mixture of peat (B2, Kekkilä, Finland) and vermiculite with 5 plants per 8 x 8 cm pot. Plants were cultivated in controlled environment chambers (Weiss 1300; Weiss Technik UK, Loughborough, U.K.) under short day conditions (12 h light/12 h dark) with 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance at 22/18°C (day/night) and 70/90% relative humidity. Mutant seeds were obtained from the Nottingham Arabidopsis Stock Centre (NASC; www.arabidopsis.info) unless otherwise indicated. Double mutant genotypes were confirmed by PCR in the F₁, F₂, and F₃ generations prior to use. Mutants and primer sequences for genotyping are listed in Table S1A. Barley (*Hordeum vulgare*) was cultivated in a controlled growth room under 18/14°C day/night temperatures, with 60% constant humidity, 18 h days, and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance. The near-isogenic genotypes included in this study comprised wild type (*Mlo*) and *mlo-1* mutant plants in the genetic background of cultivar Ingrid. The X-ray-induced *mlo-1* loss-of-function mutation causes an amino acid exchange in *Mlo* (tryptophan 162 to arginine) that leads to an unstable protein variant (lack of detectable protein accumulation; Freisleben 1942; Büschges et al. 1997; Müller et al. 2005).

ROS treatments

For all ROS treatments, 15- to 25-day-old plants were used. ROS-treated and control plants were grown under identical conditions and randomly selected into experimental groups. Standard O₃ exposures comprised 250 nl l⁻¹ to 400 nl l⁻¹ O₃ for 6 h unless otherwise noted, with parallel “clean air” controls as described (Overmyer et al. 2000). “Clean air” controls were exposures to unfiltered ambient air with normal background

O₃ concentrations (typically 10-20 nl l⁻¹), which are known not to have any effect on plants. *In vitro* superoxide exposures were generated enzymatically with xanthine (0.5 mM) and xanthine oxidase 0.1 unit ml⁻¹; Sigma-Aldrich, Munich, Germany) in sodium phosphate buffer (10 mM, pH 7.0) delivered by vacuum infiltration into detached, fully expanded middle-aged Arabidopsis leaves, or 3 cm segments of ten-day-old barley primary leaves, and incubated 8-20 h at 22°C in closed 50 ml tubes, as described (Overmyer et al. 2000; Jabs et al. 1996).

ROS-induced cell death was measured by electrolyte leakage of 2-5 detached leaves into 5 ml or whole rosettes into 12 ml milli-Q water for 1 h followed by quantification with a conductivity meter (Model FE30; Mettler Toledo, Gießen, Germany). Electrolyte leakage data is expressed as % total ions (determined after disrupting leaves by boiling) and are the means of 5-10 replicates. Methyl viologen response was measured from eight- to ten-day-old *in vitro* seedlings aseptically grown on 0.5x MS media agar (0.8% agar, 0.05% MES, at pH 5.7, and 1% sucrose) in the presence or absence of methyl viologen (0.01 µM; Sigma; www.sigmaaldrich.com). Seedlings were grown in the vertical position and photographed with a size reference, root length was then quantified from photos using ImageJ software (<https://imagej.nih.gov/ij/>)

Stomatal measurements

Steady state stomatal conductance and photosynthesis rate was measured under controlled conditions using a GFS-3000 gas exchange system (Walz, Effeltrich, Germany) using a whole Arabidopsis rosette cuvette. Stomatal conductance was also measured using a Delta-T Device porometer with a clip on cuvette (Model AP4; www.delta-t.co.uk). For O₃-induced stomatal closure and the diurnal stomatal aperture experiments, gas-exchange was monitored with a custom-built gas-exchange device,

as previously described (Kollist et al. 2007). Briefly, plants were inserted to experimental chambers, when stomatal conductance had stabilized at ~65-75% relative humidity and $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ light, O_3 (400 nl l^{-1} for 3 min) was applied. Water loss was measured by monitoring the weight of detached leaves.

qRT-PCR analysis

Control (“clean air”) and O_3 -treated plants (10-15 plants each) were harvested at 1 h of O_3 exposure (350 nl l^{-1}). RNA was extracted according to the manufacturer’s instructions (GeneJET kit; Thermo Fisher Scientific, Bartlesville, OK, USA). DNaseI-treated RNA (3 μg) was used for reverse transcription with RevertAid Premium Reverse Transcriptase (Thermo Fisher Scientific). A 25 μl cDNA synthesis reaction was diluted to 100 μl , and 1 μl thereof used as a template in the HOT FIREPol EvaGreen qPCR Mix (Solis BioDyne, Tartu, Estonia) with three technical replicates. Primer sequences are given in Table S1B. The raw cycle threshold values were analysed in qBase (Hellemans et al. 2007) using three reference genes (Table S1B).

GO enrichment and cluster analysis

Lists of genes misregulated in the *mlo2* mutant previously identified by Consonni *et al.* (Consonni et al. 2010) were analysed using Gene Ontology Consortium Online enrichment analysis tool (www.geneontology.org), using Bonferroni correction for multiple testing. (Yi et al. 2013). Bootstrapped Bayesian Hierarchical Clustering was conducted as described (Wrzaczek et al. 2010). MLO2-regulated genes (Table S2A; sourced from Consonni et al. 2010), were subjected to clustering analysis with publicly available transcriptomics datasets as described previously (Blomster et al. 2011; Brosché et al. 2014). Affymetrix gene expression data accession numbers and pre-processing was described previously (Georgii et al. 2012). Briefly, data from

ArrayExpress (www.ebi.ac.uk/microarrayas/ae/), Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo/), The Integrated Microarray Database System (<http://ausubellab.mgh.harvard.edu/imds/>) and NASC Arrays (<http://arabidopsis.info/affy>) were used. Differential expression profiles were computed from log₂ ratios of mean treatment and control expression across biological replicates, following Robust Multi-array Average (RMA) normalization. Profiles were combined with custom oligo microarray data (Array Express accession E-MTAB-662), using pre-processing as described previously (Blomster et al. 2011; Brosché et al. 2014).

Powdery mildew infection assays

Powdery mildew infection assays were performed as described (Acevedo-Garcia et al. 2017). Briefly, ca. six-week-old plants grown in short day conditions (8 h light, 22°C; 16 h dark, 20°C; 80 µmol m⁻² s⁻¹) were heavily inoculated with *G. orontii* by leaf-to-leaf contact. At 48 h post inoculation, host cell entry rates per germinated conidiospore were microscopically assessed by the presence of secondary hyphae.

Callose staining

Plants were grown in short day conditions (8 h light, 22°C; 16 h dark, 20°C; 80 µmol m⁻² s⁻¹) in a controlled environment until the age of five, six, seven or eight weeks. Rosette leaves were harvested and callose staining performed as previously described (Adam & Somerville 1996). Epifluorescence microscopy was performed with a Keyence BZ-9000 digital microscope (Keyence, Düsseldorf, Germany) and a UV filter set. Each leaf sample was semi-quantitatively assigned to one of the following four categories: 0, no signs of callose deposits; 1, sporadic occurrence of callose deposits; 2, prevalent occurrence of callose deposits; 3, massive occurrence of callose deposits.

212 The experiment was performed four times, with three-four plants per genotype and
213 age.

214 **Statistical analysis**

215 Single representative experiments were tested by two-way ANOVA with *post-hoc*
216 analysis using Tukey's honest significant difference test, or a T-test for pairwise
217 comparisons. Pooled data from several independent biological repeats were fitted in a
218 linear model by genotype, treatment and their interaction terms. Model contrasts and
219 their significances were estimated with multcomp package in R (Version 3.03; Bretz et
220 al. 2011). Gene expression and percentage data were log-transformed prior to
221 statistical analysis.

222 **RESULTS**

223 **The *mlo2* mutant exhibits enhanced tolerance to extracellular ROS**

224 To explore a potential role of *MLO2* in the ROS signalling, we exposed Col-0 wild-type
225 and *mlo2-5* mutant plants (in the genetic background of Col-0) to O₃, which induces
226 extracellular ROS production. Plants were subjected 350 nl l⁻¹ O₃, which caused visible
227 lesions in Col-0 as well as in *mlo6-2* and *mlo12-1*, but not *mlo2-5* mutants (Figure 1a).
228 To quantify this response, we measured electrolyte leakage as a proxy for cell death
229 in O₃-exposed plants, including *mlo6-2* and *mlo12-1* mutants, as additional candidates,
230 and the highly sensitive *radical induced cell death1-4* (*rcd1-4*) mutant (Overmyer et al.
231 2000), as a control (Figure 1b). In this assay, the *mlo6-2* mutant had a sensitivity level
232 similar to Col-0 wild-type (~10% ion leakage), while *mlo2-5* was significantly more
233 tolerant, with lower levels of O₃-induced electrolyte leakage (~6.5% ion leakage). As
234 expected, the *rcd1-4* mutant showed strongly enhanced O₃ sensitivity (~26% ion
235 leakage). The *mlo12-1* mutant was moderately O₃-sensitive in this experiment,

exhibiting electrolyte leakage levels higher than Col-0 but lower than *rcd1-4* (~18% ion leakage; Figure 1b).

The enhanced ROS tolerance phenotype is specific for *mlo2*

T-DNA/transposon knockout mutants are available for all fifteen Arabidopsis *MLO* paralogs, except *MLO8* (Consonni et al. 2006). To address whether the observed enhanced O₃ tolerance is specific for *mlo2-5*, their ROS sensitivity was tested and quantified by electrolyte leakage in O₃ exposures at 350 nl l⁻¹, revealing that only *mlo2-5* conferred significantly enhanced ROS tolerance (~5% ion leakage as compared to ~13% ion leakage for Col-0; Figure S1a). Additionally, some *mlo* mutants (*mlo4-4*, *mlo7-1*, *mlo9-2* and *mlo12-1*) showed a tendency towards slightly increased O₃-sensitivity in this experiment (>16% ion leakage). The enhanced tolerance phenotype of *mlo2* was further confirmed by testing the ROS sensitivity of eight independent *mlo2* alleles, including three different T-DNA insertions and five ethyl methane sulfonate (EMS)-derived lines leading to various missense mutations in *MLO2*. All examined *mlo2* mutants showed a similar level of ion leakage (~4-8%), which was clearly lower than that of the Col-0 wild-type control (~17%; Figure S1a).

The O₃ tolerance phenotype is dose-dependent and further enhanced in the *mlo2 mlo6 mlo12* triple mutant

To characterize further the role of *MLO2* in the O₃ response, electrolyte leakage was monitored in a dose-response experiment (0, 250, 300, 350 and 400 nl l⁻¹ O₃), confirming the high sensitivity of the control *rcd1-4*, moderate sensitivity of *mlo12-1*, and enhanced tolerance of *mlo2-5* (Figure 1c). In this experiment, the *mlo6-2* mutant exhibited O₃ tolerance similar to *mlo2-5* at lower concentrations and tolerance intermediate to *mlo2-5* and Col-0 at 400 nl l⁻¹ (Figure 1c). The observed trend towards

enhanced O₃-tolerance in *mlo6-2* suggests possible genetic redundancy between *MLO* paralogs in the regulation of extracellular ROS responses. *MLO2*, *MLO6* and *MLO12* form a phylogenetic clade of *Arabidopsis* co-orthologs with the highest sequence similarity to barley *Mlo* and were previously shown to exhibit unequal genetic redundancy in the regulation of immunity against powdery mildew disease (Consonni et al. 2006). To test for potential functional overlap in the ROS response, *mlo2-5*, *mlo6-2*, *mlo12-1* and the respective triple mutant (*mlo2-5 mlo6-2 mlo12-1*) were exposed to very high O₃ levels (400 and 500 nl l⁻¹). At 400 nl l⁻¹, *mlo2-5* and the triple mutant were more tolerant, exhibiting lower electrolyte leakage levels (~7-8% as compared to ~19% for Col-0 wild-type; Figure 1d). The 500 nl l⁻¹ exposure exceeded the damage threshold for *mlo2-5*, which resulted in high electrolyte leakage (~25%). However, the *mlo2-5 mlo6-2 mlo12-1* triple mutant remained undamaged at this O₃ concentration, as seen by its low level (~8%) of electrolyte leakage, indicating it has a higher ROS tolerance than the *mlo2-5* single mutant (Figure 1d).

Enhanced tolerance to extracellular ROS is not restricted to O₃ and also occurs in a barley *mlo* mutant

To test whether the observed phenotype is specific for exposure to O₃ or more general for elevated ROS levels, we monitored cell death as electrolyte leakage in leaves treated *in vitro* with an enzymatic superoxide generation system comprised of xanthine and xanthine oxidase (XXO; Overmyer et al. 2000). In this assay, Col-0 exhibited ~60% electrolyte leakage, while the *mlo2-5* single mutant and the *mlo2-5 mlo6-2 mlo12-1* triple mutant showed a reduction in ion leakage (~50% and ~45%, respectively; Figure 2a). To address the question whether the elevated ROS tolerance is a specific feature of *Arabidopsis mlo2* plants or a more general phenomenon of *mlo* mutants, we included a barley (*Hordeum vulgare*) *mlo* mutant in our experiments. O₃ exposure and

285 XXO treatment of wild-type (*Mlo*) cultivar Ingrid and a near-isogenic *mlo* null mutant
286 (*mlo-1*) revealed that similar to the results in Arabidopsis, the *mlo* mutant genotype
287 exhibited lower levels of ozone-induced visible damage and XXO-induced cell death,
288 ~23% ion leakage for *mlo-1* versus ~36% for wild-type *Mlo* (Figure S2).

289 ***mlo2* mutants show an unaltered response to intracellular ROS**

290 To analyse the response of the Arabidopsis *mlo* mutants to intracellular ROS
291 production, ten-day-old seedlings were grown in the presence and absence of the
292 herbicide methyl viologen (MV), which induces light-dependent ROS production in the
293 chloroplast. As a positive control, we included the *rcd1-4* mutant, which shows
294 enhanced tolerance to intracellular ROS (Ahlfors et al. 2004a). Results of these root
295 growth assays revealed no difference between Col-0, the *mlo2-5* single mutant and
296 the *mlo2-5 mlo6-2 mlo12-1* triple mutant (root length each ~10% of the controls as
297 compared to ~48% for *rcd1-4*; Figure 2b), suggesting that these MLOs specifically
298 modulate responses to extracellular ROS, but not intracellular (chloroplast-derived)
299 ROS.

300 ***mlo2* mutants show unaltered stomatal responses upon O₃ exposure**

301 O₃ enters leaves through open stomata; thus stomatal mutant phenotypes may result
302 in an altered O₃ response (Brosché et al. 2010). To assess the potential contribution
303 of stomatal aperture to the O₃ tolerance phenotype of the *mlo2* mutant, we measured
304 a number of stomata-related phenotypes. The stomatal conductance of Col-0, the
305 *mlo2-5* single mutant and the *mlo2-5 mlo6-2 mlo12-1* triple mutant was first tested
306 under control growth conditions using a clip-on cuvette. In this experiment, we
307 deployed the *ost1-3* mutant (Vahisalu et al. 2010), which has constitutively open
308 stomata, as a positive control. This line showed the expected elevated stomatal

conductance, and a respective *mlo2-6 ost1-3* double mutant was not significantly different from the *ost1-3* single mutant ($\sim 600 \mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ each), while the *mlo2-6* single mutant and the *mlo2-5 mlo6-2 mlo12-1* triple mutant revealed a wild-type-like stomatal conductance of $\sim 200 \mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (Figure 3a). We extended this analysis using cuvettes able to fit an entire *Arabidopsis* rosette. The photosynthesis rate and stomatal conductance were tested for Col-0, the *mlo2-6* single mutant and the *mlo2-5 mlo6-2 mlo12-1* triple mutant, which revealed no differences between genotypes (Figure S4a and b, respectively). Additionally, we assessed the diurnal cycle of stomatal conductance over a 24 h period with Col-0 and three *mlo2* null mutant alleles (*mlo2-5*, *mlo2-6* and *mlo2-7*), yielding comparable curves (Figure S4c). To test O_3 -induced stomatal closure, we monitored stomatal conductance in intact whole plants following a single 3 min pulse of O_3 (400 nl l^{-1} ; Kollist et al. 2007), which resulted in similar stomatal closure kinetics in *mlo2-5* and Col-0 wild-type plants (Figure 3b). Water loss from detached leaves, which is another common assay for induced stomatal closure, also revealed no differences between the wild-type, the *mlo2-5* single mutant and the *mlo2-5 mlo6-2 mlo12-1* triple mutant (water loss $\sim 25\%$, as compared to $\sim 70\%$ in *ost1-3*; Figure 3c). Taken together, these data demonstrate that *mlo2* mutants have normal stomatal responses, excluding the possibility that the enhanced tolerance of *mlo2* mutants to extracellular ROS is conditioned at the level of stomatal aperture.

Modulation of O_3 phenotypes by *MLO2* in selected double mutants

The enhanced O_3 tolerance phenotypes of *mlo2* mutants is only seen at very high O_3 concentrations ($\geq 300 \text{ nl l}^{-1}$; Figure 1c and d) that are arguably not physiologically relevant. To evaluate the effect of *MLO2* at lower O_3 concentrations, double mutants of *mlo2-6* were created with the ROS (O_3)-hypersensitive mutants, *rcd1-4* (Jaspers et

al. 2009), *reticulata-8* (*re-8*; Overmyer et al. 2008), *open stomata1-3* (*ost1-3*, also
 known as *snrk2.6* and *snrk2e*; Vahisalu et al. 2010) and *vitiminc1-1* (*vtc1-1*; Conklin et
 al. 1997). Following exposure to 250 nl l⁻¹ O₃, which induced cell death in *re-8*, *ost1-3*
 and *vtc1-1*, there was no statistically significant difference between Col-0 wild-type
 plants and the *mlo2-6* mutant. However, presence of the *mlo2-6* mutation conferred a
 reduction in ROS sensitivity in the respective double mutants with *re-8*, *ost1-3* and
vtc1-1 (Figures 4a and S5). Unexpectedly, the *mlo2-6* mutation enhanced the ROS
 sensitivity of the *rcd1-4* mutant under these conditions, with the *mlo2-6 rcd1-4* double
 mutant showing significantly more O₃-induced electrolyte leakage (~25%) than *rcd1-4*
 (~18%; Figure 4a and S5). Jasmonic acid (JA)-insensitive mutants exhibit strongly
 enhanced sensitivity to O₃ exposure (Overmyer et al. 2000). Thus, double mutants of
mlo2-11 with mutants *jasmonate resistant1* (*jar1-1*) and *coronatine insensitive1-16*
 (*coi1-16*), which are defective in JA signalling (Ellis & Turner 2002; Staswick et al.
 1992), were also tested. The *mlo2-11* mutation conferred enhanced ROS tolerance in
 the context of these JA signalling mutants, indicated by a dramatic reduction in
 electrolyte leakage induced at 250 nl l⁻¹ O₃, in both the *mlo2-11 jar1-1* and *mlo2-11*
coi1-16 double mutants (Figure 4b and S5). No role for ethylene signalling was found
 using the *ethylene insensitive2* (*ein2-1*; Alonso et al. 1999) double mutant; *mlo2-11*
ein2-1 retained enhanced ROS tolerance (Figure 4b and S5). Taken together, these
 results indicate that (1) the threshold for MLO2-dependent effects can be lowered
 when the *mlo2* mutation is in O₃-sensitive mutant backgrounds; (2) that *mlo2* is an
 enhancer of *rcd1-4*-associated ROS sensitivity; and (3) that JA signalling is not
 required for enhanced ROS tolerance in *mlo2* mutants.

The *mlo2* mutant requires components of non-host resistance and salicylic acid (SA)
 signalling for complete immunity against powdery mildew disease (Consonni et al.

2006). Thus, the role of these components and the stress hormone SA in the *mlo2* ROS phenotype was also tested using genetic epistasis analysis. Mutations in the components of non-host resistance, *PENETRATION1* (*PEN1*; Collins et al. 2003), *PEN2* (Lipka et al. 2005) and *PEN3* (Bednarek et al. 2009) as single mutants had no effect on O₃ sensitivity and each slightly enhanced ion leakage in the *mlo2* mutant context; however, this increase was not statistically significant (Figure 4c). The role of SA was assessed with *salicylic acid induction deficient2* (*sid2-1*) and *phytoalexin deficient4* (*pad4-4*), which are SA biosynthesis (Wildermuth et al. 2001) and signalling mutants (Zhou et al. 1998), respectively. The enhanced O₃ tolerance of *mlo2* was lost in both *mlo2-6 sid2-1* and *mlo2-6 pad4-1* (Figure 4d and S5), indicating SA is required in the ROS signalling pathway regulated by MLO2.

To investigate further the role of ROS signalling, additional *mlo2* double mutants involved in ROS and defence signalling were created and tested. These included double mutants with the NAD(P)H oxidase, *respiratory burst oxidase homolog C* (*rbohC*; *rhd2-4* transposable element insertion allele; Foreman et al. 2003), the ROS signalling MAP kinases, *mitogen activated kinase3* (*mpk3-1*) and *mpk6-2* (Ahlfors et al. 2004b), the ABA biosynthesis mutant *abscisic acid deficient3* (*aba3-1*; Xiong et al. 2001), the kinase mutant *oxidative signal-inducible1* (*oxi1-2*; Rentel et al. 2004), and the co-receptor mutant, *bri1-associated receptor kinase1-4* (*bak1-4*; Li et al. 2002). The *rbohC*, *oxi1-2*, *mpk6-2*, *aba3-1* and *bak1-4* mutations suppressed the enhanced O₃ tolerance of *mlo2-6*; *mlo2-6 rbohC*, *mlo2-6 oxi1-2*, *mlo2-6 mpk6-2*, *mlo2-6 aba3-1* and *mlo2-6 bak1-4* exhibited equal or greater O₃-induced electrolyte leakage than Col-0 wild-type (Figure 4e and f). The *aba3-1* mutant is O₃-sensitive with higher electrolyte leakage than Col-0 wild-type (~10% vs. ~5%). The *mlo2-6 aba3-1* mutant exhibited full O₃ sensitivity with electrolyte leakage levels similar to the *aba3-1* mutant (~12%).

Unaltered *mlo2*-conditioned O₃ tolerance was seen in *mlo2-6 mpk3-1* (Figure 4e), indicating that mutations in *MPK3* do not affect the *mlo2-6* phenotype.

Powdery mildew infection and spontaneous callose deposition phenotypes of double mutants harbouring *mlo2*

The newly generated *mlo2* double mutants described in the previous sections and their respective single mutants were then assayed for powdery mildew (*Golovinomyces orontii*) infection phenotypes. However, none of the tested single or double mutants had any statistically significant difference in *G. orontii* host cell entry to their respective controls, which were Col-0 (~90% host cell entry) for the single mutants and *mlo2-6* (~50% host cell entry) for the double mutants (Figure S6). These results indicate that these loci are not involved in pre-invasive basal defence or *mlo2*-conditioned powdery mildew resistance.

In addition to enhanced resistance against powdery mildew attack, the *mlo2* mutant exhibits developmentally controlled premature leaf senescence, which is preceded by spontaneous ectopic callose deposition in rosette leaves (Consonni et al. 2006; Consonni et al. 2010). Since in our conditions callose deposition, visualized microscopically in aniline blue-stained samples, was the more robust marker, we assayed the same set of single and double mutants for this phenotype in a time-course experiment. Rosette leaf samples were collected during weeks five to eight, *i.e.* time-points before (five weeks), during (six-seven weeks) and after (eight weeks) full development of the callose deposition phenotype in *mlo2* genotypes. Following aniline blue staining, these specimens were assessed semi-quantitatively for the occurrence of callose deposits. Similar to the *mlo2-6* mutant, the *aba3-1* and in particular the *vtc1-1* single mutant showed early spontaneous callose deposition (Figure 5), while all other

single mutants (*bak1-4*, *mpk3-1*, *mpk6-2*, *oxi1-2*, *rbohC*, *rcd1-4*, *re-8* and *ost1-3*) had phenotypes similar to Col-0 wild-type during the time-course (Figure 5a). The callose deposition phenotypes of *aba3-1* and *vtc1-1* were additive with that of *mlo2-6*, resulting in earlier and more pronounced callose deposits in *mlo2-6 aba3-1* and *mlo2-6 vtc1-1* double mutants (Figure 5). Remarkably, the *rcd1-4* mutant completely suppressed the *mlo2-6* callose phenotype, with the *mlo2-6 rcd1-4* double mutant behaving indistinguishable from Col-0 wild-type, even in eight-week-old plants (Figure 5b and c). All other *mlo2-6* double mutants showed strong and consistent callose deposition (Figure 5b). In summary, while mutants involved in ROS and defence signalling did not impact the powdery mildew resistance phenotype of the *mlo2-6* mutant, some of the respective mutants either phenocopied/enhanced (*aba3-1*, *vtc1-1*) or fully suppressed (*rcd1-4*) the spontaneous callose deposition phenotype of the *mlo2-6* mutant.

Deregulated genes in the *mlo2 mlo6 mlo12* triple mutant comprise abiotic stress response genes

To identify and further analyse genes that are deregulated in a *mlo* mutant context we mined publicly available gene expression data of the *mlo2-6 mlo6-2 mlo12-1* mutant utilising lists of genes (Table S2a) with altered expression in the triple mutant at the age of five, six, and seven weeks previously identified by Consonni and co-workers (Consonni et al. 2010). Enriched gene ontology (GO) terms were identified among the selected genes (Table S2). Consistent with known phenotypes of this mutant, most enriched GO terms are related to immunity, senescence, biotic stress signalling, cell death, and antimicrobial compound synthesis (Table S2b, c, d). This confirms in part the results of an earlier study (Consonni et al. 2010), however, we also found novel gene expression patterns in this dataset that are related to abiotic stress and not previously discussed. The GO classification “response to ROS” (GO: 0000302) was

433 enriched in the six week gene set, while “response to hydrogen peroxide” (GO:
434 0042542), “response to ROS” (GO:0000302), and “response to oxidative stress” (GO:
435 0006979) were enriched in the seven week gene set. This pattern underscores the
436 involvement of ROS in processes regulated by MLO2, MLO6, and MLO12 and
437 suggests that ROS-signalling may be pre-activated during development in the *mlo2-5*,
438 *mlo6-2 mlo12-1* triple mutant.

439 Unexpectedly, some GO classes related to abiotic stress response were also enriched;
440 these included “cold acclimation” (GO: 0009631), “response to abiotic stimulus” (GO:
441 0009628 - see downregulated genes at seven weeks; Table S2f), and “response to
442 ABA” (GO: 0009737, at seven weeks). Four out of five genes comprising the genes
443 enriched for GO: 0009631 “cold acclimation” are known targets of the ABA-
444 independent abiotic stress signalling pathway regulated by the transcription factor
445 DREB2a (Table S2; Sakuma et al. 2006), prompting further examination of a potential
446 overlap between MLO-dependent and DREB2a-regulated gene sets. Respectively, 19,
447 60, and 33 genes were found in common between datasets for a transgenic line
448 overexpressing constitutively active DREB2A and the five, six and seven week
449 datasets of the *mlo2-5 mlo6-2 mlo12-1* triple mutant (Table S2g). Together, these
450 represent a significant enrichment for DREB2a-regulated genes (Table S2h) among
451 the clade V MLO-regulated genes. This finding suggests that there is overlap between
452 DREB2a-associated signalling and the pathways downstream of these MLOs.

453 To further explore MLO-regulated genes, we analysed the hierarchical clustering of
454 expression profiles for MLO2-, MLO6- and MLO12-regulated genes across a collection
455 of publicly available transcript profiling experiments related to ROS, abiotic stress,
456 biotic stress, and hormone signalling. In total, these MLOs regulate 1646 genes during
457 leaf rosette development (Consonni et al. 2010). To facilitate analysis, we reduced the

gene number by selecting, from the week six gene set, 106 genes with a fold induction of >6 in at least one experiment and show below that these are a representative set of MLO-regulated genes. The highest expression levels of these genes were seen in experiments related to abiotic stress, such as salt and osmotic stresses, and processes involving ROS production, such as biotic stress treatments (PAMPs, *Botrytis*, virulent and avirulent bacterial pathogens; Figure 6). Notably, O₃, paraquat (methyl viologen) and H₂O₂ treatment also induced the expression of MLO-regulated genes. Generally, genes upregulated in the *mlo* triple mutant were also upregulated by stress treatments and those downregulated in the *mlo* triple mutant were likewise downregulated by stress. However, there is a block of experiments that exhibit predominately reduced expression of all MLO2 regulated genes; these are high light treatments, mitochondrial stress (rotenone treatment), the *constitutive triple response1 (ctr1)* mutant, and the *rcd1-4* mutant (Figure 6).

The week six data used here has previously been used as a representative dataset for MLO-regulated genes (Consonni et al. 2010). To test how representative our results are, we utilized all genes from each of the three time points separately (Figures S7-S9). Comparison of results from week five, six and seven demonstrates that, although these gene sets are largely distinct, all MLO-regulated genes exhibited similar expression behaviour across the selected experiments. Together these findings suggest that these MLOs act in the regulation of abiotic stress-dependent gene expression.

***MLO2* transcript accumulation is ROS-responsive**

To find out if *MLO2* gene expression is ROS-responsive, publicly available microarray data from O₃-exposed plants (Blomster et al. 2011) was mined. *MLO2* transcript

482 accumulation was found to be significantly (~3-fold; $P=0.03$) elevated in O_3 -treated
483 samples (Figure S3a). To experimentally verify this result, we assessed *MLO2*
484 transcript accumulation by quantitative reverse transcription polymerase chain reaction
485 (qRT-PCR) based on leaf samples that were exposed for 1 h to either O_3 (300 nl l^{-1}) or
486 “clean air” (ambient O_3 concentrations) as control. We confirmed that the *MLO2*
487 transcript accumulates to significantly (~3-fold) higher levels in the O_3 -treated Col-0
488 samples (Figure S3b). Furthermore, *MLO2* transcript accumulation was slightly, but
489 significantly, higher in the *rcd1-4* mutant, which is hypersensitive to O_3 and exhibits
490 enhanced ROS accumulation. Thus, we conclude that *MLO2* is responsive to
491 extracellular ROS.

492

DISCUSSION

MLO2 negatively regulates ROS-induced cell death

O₃-tolerance in the *mlo2* mutant suggests that MLO2 modulates ROS responses. Relative to known O₃-tolerant genotypes, the *mlo2 mlo6 mlo12* triple mutant counts among the most tolerant. Although many O₃-sensitive mutants have been studied (reviewed in Vainonen et al. 2015), few O₃-tolerant genotypes are known (Joo et al. 2005; Brosché et al. 2010; Booker et al. 2004; Overmyer et al. 2000). Interestingly, these include *gpa1*, which is defective in the heterotrimeric G protein α subunit, while the G β subunit mutant (*agb1*) shows enhanced O₃ sensitivity (Joo et al. 2005; Booker et al. 2004). Antagonistic activity of *MLO2* and *AGB1* has been previously noted in relation to powdery mildew immunity (Lorek et al. 2013).

Altered ROS responses in leaves is common to MLO2, MLO6, and MLO12, but not to other MLOs (Figure 1a-c; Figure S1a). These clade V MLOs were previously shown to regulate powdery mildew immunity with unequal genetic redundancy (Consonni et al. 2010). Here, similar redundancy in the response to ROS was shown (Figure 1d). Specifically, the pattern of ROS tolerance in these mutants, i.e. partial ROS tolerance in *mlo2*, slight sensitivity or no phenotype in *mlo6* and *mlo12*, and strong tolerance in the triple mutant, indicates unequal genetic redundancy of *MLO* functions for this trait. The shared genetic architecture underlying the regulation of ROS and immune responses suggests mechanistic overlap between the physiological programs governing ROS tolerance and antifungal immunity.

In double mutant contexts, loss of *MLO2* function conferred enhanced tolerance to a variety of ROS-sensitive mutants representing various mechanisms of sensitivity (Figure 4). These include *ost1-3*, which has more open stomata; *vtc1-1*, which is

517 deficient in ascorbate and thus ROS scavenging; and *re-8*, which has an altered
518 chloroplast redox response. MLO2's involvement in many ROS-related processes
519 suggests it acts to dampen ROS signals in general, likely high up in the ROS signalling
520 cascade.

521 O₃ enters leaves through open stomata and forms ROS in the apoplast; thus O₃
522 tolerance may be explained by altered stomatal responses. We comprehensively
523 addressed this by measuring the following, steady-state stomatal conductance by two
524 methods, the diurnal pattern of stomatal movements, water loss from detached leaves,
525 and O₃-induced stomatal closure. All assays indicate wild-type-like phenotypes in *mlo*
526 mutants (Figures 3a-c and S4c). Also the photosynthesis rate was assessed; as
527 previously shown by chlorophyll fluorescence (Consonni et al. 2010), *mlo2* mutants
528 exhibited wild-type photosynthesis rates (Figure S4A). Stomata in *ost1* are
529 constitutively open; thus unchanged water loss and stomatal conductance but
530 enhanced O₃-tolerance in the *ost1 mlo2* double mutant provides further genetic
531 evidence that *mlo2*-mediated ROS tolerance is independent of stomata (Figure 3b).
532 Finally, infiltration with an *in vitro* XXO superoxide generating system, which delivers
533 ROS in a non-stomatal-dependent manner, further demonstrates this point (Figure 2a).
534 Together, these results indicate that the MLO2 regulates tolerance to extracellular
535 ROS independent of stomatal regulation. Meta-analysis of MLO2-regulated genes
536 (Table S2c, d) demonstrates an enrichment among upregulated genes for GO classes
537 related to ROS signalling. This indicates that the *mlo2* mutant is primed for O₃
538 responsiveness and thus MLO2 operates as a negative regulator of ROS-induced
539 gene expression. This inference implies that MLO2 modulates the threshold for ROS-
540 induced cell death. We therefore conclude that MLO2 is a regulator of ROS responses,
541 including cell death. This finding is consistent with the previous notion that barley Mlo

acts as a negative regulator of localized ROS production at fungal attack sites and mesophyll cell death (Piffanelli et al. 2002).

MLO2 functions in ROS signalling and immunity can be genetically uncoupled

Epistasis analysis indicated that ROS tolerance conditioned by loss of MLO2 function requires several known mediators of ROS, stress, and immune signalling. These results further support the role of MLO2 as a regulator of stress-induced cell death and allow dissection of the divergent processes regulated by MLO2. Consistent with the role of ROS in immunity, some processes are needed for both *mlo2*-mediated enhanced immunity against powdery mildew (Consonni et al. 2006) and enhanced ROS tolerance (Figure 4). These include SA accumulation, and SA-dependent signal transduction. By contrast, three *PEN* proteins are necessary for enhanced immunity (Consonni et al. 2006), but not ROS tolerance (Figure 4c). These proteins function in the synthesis and delivery of antimicrobial compounds; as such, they are not expected to modulate ROS/cell death pathways.

Other processes required for enhanced ROS tolerance (Figure 4), but not enhanced immunity (Figure S6), included stress responsive ABA production; ROS production from RBOHC; the MAP kinase MPK6; and the BAK1 co-receptor, required for the function of hormone and PAMP receptor complexes. These results suggest that MLO2 regulation of ROS signalling occurs upstream of at least two distinct signalling pathways, which can be genetically uncoupled; one regulating immunity and the other controlling cell death and other ROS processes. This suggests MLO2 is an integrator of multiple signals, modulating various physiological responses (Figure 7).

The *mlo2* mutant is immune against powdery mildew and appears to have pre-activated ROS responses (Figure S6 and Table S2). This is in agreement with the

566 previous observation that O₃ can induce SA accumulation and immunity, for instance
567 against the hemibiotrophic pathogen *Pseudomonas syringae* pv. *maculicola* in
568 susceptible *Arabidopsis* and the biotrophic pathogen *Blumeria graminis* f. sp. *tritici* in
569 susceptible wheat (*Triticum aestivum*) (Sharma et al. 1995; Pazarlar et al. 2017). Loss
570 of MLO2 function also confers enhanced susceptibility to the hemibiotrophic pathogen
571 *Magnaporthe oryzae* (Lyngkjær et al., 2000) and the necrotrophic pathogen *Bipolaris*
572 *sorokiniana* (Kumar et al. 2001). Accordingly, O₃ is a known enhancer of susceptibility
573 to necrotrophic fungi (Manning and von Tiedemann, 1995). However, the *mlo2* mutant
574 is a notable exception to the general positive correlation between plant O₃ sensitivity
575 and susceptibility to necrotrophic pathogens (Manning and von Tiedemann, 1995) and
576 the interaction between O₃ and hemibiotrophic pathogens appears to be variable.

577 The original barley *mlo* mutant shares features with so-called lesion-mimic mutants,
578 which ectopically trigger cell death, exhibit enhanced callose deposition, and confer
579 enhanced immunity (Piffanelli et al. 2002). However, *mlo*-associated developmental
580 cell death resembles premature senescence, as suggested by early pigment decay
581 (Piffanelli et al. 2002). It remains unclear if enhanced cell death and callose deposition
582 are required for immunity in barley *mlo* genotypes. *Arabidopsis mlo2* and *mlo2 mlo6*
583 *mlo12* mutants also exhibit the same phenotypes (Consonni et al. 2010).
584 Transcriptomic studies of these mutants suggest the activation of a true senescence
585 program (Consonni et al. 2010). *MLO2* regulates resistance bearing many hallmarks
586 of non-host penetration resistance (Humphry et al. 2006), which lacks cell death,
587 further supporting that *mlo*-mediated resistance does not require cell death. Finally,
588 the O₃-sensitive *rcd1-4* mutant completely suppresses *mlo2*-associated spontaneous
589 callose deposition, but had no effect on enhanced immunity (Figure 4 and S6). Thus,
590 MLO2 may modulate the threshold for cell death activation in a ROS signalling pathway

independent of processes related to immunity. These data further support previous results demonstrating uncoupling of *mlo2* phenotypes. Premature callose deposition was separated from immunity in the *mlo2 powdery mildew resistant 4 (pmr4)* double mutant (Consonni et al. 2010). *PMR4* is a *GLUCAN SYNTHASE-LIKE* gene required for wound- and pathogen-induced callose biosynthesis (Jacobs et al. 2003; Nishimura et al. 2003). As previously noted, these results suggest the possibility of engineering the favourable enhanced immunity of *mlo2* genotypes without the undesirable premature senescence (Consonni et al. 2010).

MLO2 function is linked to abiotic stress responses

Similar to *mlo2*, several O₃-sensitive mutants (*aba3-1*, *re-8*, and *vtc1-1*) exhibited enhanced callose deposition (Figure 5), underscoring the role of ROS in this process. The *aba3-1* mutant was of particular interest, as it suggests a role for abiotic stress signalling pathways in this context. Analysis of MLO2-, MLO6-, MLO12-regulated genes indicates enrichment of the GO category “cold acclimation”, and targets of the DREB2a transcription factor, indicating misregulation of abiotic stress marker genes (Table S2b, e, h). DREB2 is a known RCD1-interaction partner (Jaspers et al. 2009). DREB2A and RCD1 are involved in non-ABA-dependent abiotic stress signalling and senescence, further implicating MLOs in abiotic stress. This connection suggests targets of further research, which will be required to reveal the role of MLO2 in abiotic stress response. Finally, these results in Arabidopsis further support the previously suggested link of barley Mlo to abiotic stress responses (Piffanelli et al. 2002).

A common role for MLO proteins as regulators of ROS responses?

The biochemical function of MLO proteins remains unknown (Acevedo-Garcia et al. 2014; Kusch et al. 2016). Here we demonstrate that clade V MLOs act, in unequal

615 genetic redundancy, as negative regulators of ROS signalling. This raises the intriguing
616 possibility that involvement in ROS signalling could be a general feature of MLO
617 proteins. ROS signalling has roles in various aspects of plant life, including immunity,
618 senescence and abiotic stress responses, which are all associated with clade V MLO
619 functions. Additionally, ROS regulate developmental processes governed by other
620 *Arabidopsis* MLOs. In plant sexual reproduction, the pollen tube grows to, and bursts
621 at, synergid cells of the female gametophyte to release its sperm cells and mediate
622 dual fertilization. MLO7 and the receptor-like kinase, FERONIA (FER), act in pollen
623 tube guidance and reception (Kessler et al. 2010). In *mlo7* mutants, pollen tubes grow
624 past the synergid cells, or arrive but fail to release the sperm cells. Remarkably, the *fer*
625 mutant exhibits a powdery mildew resistance phenotype. ROS generated by an
626 NADPH oxidase act in concert with a calcium gradient, both of which are essential for
627 rapid polar pollen tube growth (Potocký et al. 2007; Li et al. 1999). Notably, FER was
628 recently shown to regulate high-level ROS accumulation, near the entrance to the
629 female gametophyte, which is required to induce calcium-dependent pollen tube
630 rupture and sperm release (Duan et al. 2014).

631 MLO4 and MLO11 are involved in modulating root thigmomorphogenesis. In *mlo4* and
632 *mlo11* mutants, roots grow in a tightly curled pattern; this anisotropic chiral root
633 expansion phenotype requires polar auxin transport and calcium signalling (Bidzinski
634 et al. 2014; Chen et al. 2009). Mechanical stimulation of *Arabidopsis* roots results in a
635 specific signature of calcium transients that further triggers apoplastic alkalinisation
636 and *RBOHC*-dependent ROS accumulation, transmitting touch stimuli into appropriate
637 growth responses (Monshausen et al. 2009).

638 Taken together, MLO function is linked to ROS responses in a variety of physiological
639 processes. However, the connection between MLO function and ROS signalling can

640 be in part genetically uncoupled. It remains to be discovered whether this interplay is
641 indeed tied to the core biochemical activity of MLO proteins and if so, how it is
642 established at the mechanistic level.

643

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657

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883

FIGURE LEGENDS

Figure 1: *mlo2* mutants show enhanced O₃ tolerance.

(a) Phenotype of Col-0, *mlo2-5*, *mlo6-2*, and *mlo12-1* plants that had been exposed to O₃ (350 nl l⁻¹) for 6 h and photographed at 48 h post begin of exposure. Arrows indicate examples of O₃-damaged leaves. Scale bar= 1 cm. (b) Cell death assayed as electrolyte leakage from plants shown in (a). (c) Electrolyte leakage from an O₃ (0, 250, 300, 350, and 400 nl l⁻¹) dose-response experiment for Col-0, *mlo2-5*, *mlo6-2*, *mlo12-1*, and the highly O₃-sensitive *radical-induced cell death1-4* (*rcd1-4*) mutant as a control. (d) Electrolyte leakage upon high O₃ concentration (400 and 500 nl l⁻¹) exposure, exceeding the tolerance threshold of *mlo2-5*, for Col-0, *mlo2-5*, *mlo6-2*, *mlo12-1* single mutants and the for the *mlo2-5 mlo6-2 mlo12-1* triple mutant. All experiments were performed 3-6 times in independent biological repeats; with one representative experiment shown. All data presented are means ± standard deviation (SD; n=5) for electrolyte leakage expressed as % of total ions determined after disrupting leaves by boiling. Statistical significance (P<0.05) tested by two-way ANOVA with *post-hoc* analysis. In (b), letters indicate significance groups were genotypes with different letters are significantly different and in c) and (d) asterisks show significant difference from Col-0 for each ozone concentration. See also Figure S1.

Figure 2: *mlo2* mutants are more tolerant to extracellular, but not to intracellular ROS.

(a) Electrolyte leakage from leaves of Col-0, *mlo2-6*, and *mlo2-5 mlo6-2 mlo12-1* plants, treated *in vitro* with an enzymatic superoxide generating system comprised of

1 mM xanthine and 0.1 unit ml⁻¹ XXO. Data are presented as means $\pm$ SD (n=5) for ion leakage expressed as % of total ions determined after disrupting leaves by boiling. (b) Root growth assay to determine MV sensitivity of Col-0 as well as the *mlo2-5* and *mlo2-5 mlo6-2 mlo12-1* mutants. The highly MV-tolerant *rcd1-4* mutant was included as a control. Root length was quantified using ImageJ (<https://imagej.nih.gov/ij/>) from photographs of nine-day-old seedlings grown on 0.5x MS plates (with 1% sucrose) in the presence or absence of 0.1 μ M MV. Results are presented as means $\pm$ SD of the ratio between MV-treated and control seedlings, expressed as a percentage (% of control). All experiments were performed three times in independent biological replicates and letters show statistical significance groups (P<0.05). In (a) significance tested by two-way ANOVA with *post-hoc* analysis and a single representative experiment is shown, in (b) results from all experiments were pooled and analysed (n=36 in total) and *post-hoc* analysis was used by computing contrasts from linear models and subjecting the P values to single-step error correction.

Figure 3: *mlo2* mutants have unaltered stomatal responses.

(a) Stomatal conductance of Col-0, *mlo2-6*, *mlo2-5 mlo6-2 mlo12-1*, *open stomata1-3 (ost1-3)*, and *mlo2-6 ost1-3*, measured with a Delta-T Device porometer (b) Fast stomatal closure response to O₃ in wild-type Col-0 and *mlo2-5* mutant plants. The time course of stomatal conductance (expressed in relative units) of three- to four-week-old plants was recorded. At the time point indicated with an arrow, 400 nl l⁻¹ O₃ was applied. Data points represent means $\pm$ standard error of the mean (SEM; n=12). (c) Water loss measured from excised leaves of Col-0, *mlo2-6*, *mlo2-5 mlo6-2 mlo12-1*, *open stomata1-1 (ost1-3)*, and *mlo2-6 ost1-3*. All experiments performed at least three times

in independent biological repeats. Representative experiments (a, c) or pooled data from all experiments (b) are presented. In (a) and (c), different letters indicate statistically different groups based on two-way ANOVA with *post-hoc* analysis. See also Figure S4.

Figure 4: O₃ phenotypes of *mlo2* double mutants.

In all experiments, plants were exposed to 6 h of O₃ at the indicated concentrations and electrolyte leakage was quantified as a proxy for cell death at 24 h post-exposure. All experiments were repeated in at least four independent biological replicates, with data from a representative experiment shown. All data presented are means $\pm$ SD (n=5) for electrolyte (ion) leakage expressed as % of total ions determined after disrupting leaves by boiling. Significance groups are indicated with lowercase letters, where genotypes with different letters show significant differences (P<0.05) tested by two-way ANOVA with *post-hoc* analysis. (a) O₃-sensitive mutants, *rcd1-4*, *re-8*, *ost1-3*, and *vtc1-1* and their respective *mlo2-6* double mutants exposed to 250 nl l⁻¹ O₃. (b) The O₃-sensitive jasmonate signalling mutants, *jar1-1* and *coi1-16*, the O₃-tolerant ethylene insensitive *ein2-1* mutant, and their respective *mlo2-5* or *mlo2-11* double mutants were exposed to 250 nl l⁻¹ O₃. (c) The mildew susceptible mutants, *pen1-1*, *pen2-1*, *pen3-1*, and their respective *mlo2-5* or *mlo2-6* double mutants were exposed to 375 nl l⁻¹ O₃. (d) The SA signalling defective mutants, *sid2-1*, *pad4-1*, and their respective *mlo2-5* or *mlo2-6* double mutants were exposed to 325 nl l⁻¹ O₃. (e) The ROS signalling mutants, *rbohC*, *mpk3-1*, *mpk6-2*, and their respective *mlo2-6* double mutants were exposed to 325 nl l⁻¹ O₃. (f) The ABA deficient *aba3-1* mutant, the immune and hormone signalling co-receptor mutant, *bak1-4*, the early ROS signalling mutant

oxi1-2, and their respective *mlo2-6* double mutants were exposed to 300 nl l⁻¹ O₃. Data from three separate exposures are plotted together and separated by vertical lines on the graph; comparisons should only be made within each exposure, each of which has its own wild-type Col-0 control. See Figure S5 for photographs of O₃-exposed plants of selected genotypes.

Figure 5: Some *mlo2* double mutants exhibit altered spontaneous callose deposition.

(a) Semi-quantitative assay of callose deposition. Plants of the indicated genotypes at five-, six-, seven-, and eight-weeks of age were stained for callose visualization with analine blue and visually scored for the presence of callose deposits under an epifluorescence microscope. Semi-quantitative assessment of callose deposition was performed as described in Materials and Methods. The experiment was performed four times, with three to four samples per genotype and age, and data pooled. Shown are the mean $\pm$ SD of the four experiments. Asterisks indicate a statistically significant difference ($P < 0.05$) compared to Col-0 (upper panel) or *mlo2-6* (lower panel) based on *post-hoc* analysis by computing contrasts from linear models and subjecting the P values to single-step error correction. (b) Representative photos of selected mutant phenotypes. Scale bar, 500 μ m, is valid for all micrographs.

Figure 6: MLO genes regulate abiotic stress response genes.

(a) Hierarchical clustering analysis of expression profiles for MLO-regulated genes. We selected 106 representative MLO2-, MLO6-, MLO12-regulated genes from the week six gene set (Consonni et al. 2010) with a fold induction of >six in at least one experiment (see Table S2i for gene list) and subjected their expression profiles to

hierarchical clustering analysis. A collection of publicly available transcript profiling experiments related to ROS, abiotic stress, biotic stress, hormone signalling, and transcriptional regulators was used (see Table S2i for list of experiment accession numbers). The same analysis was performed with all MLO-regulated genes and the week five, six, and seven data is presented in Supplemental Figures S7-S9, respectively.

Figure 7: Model of MLO2 as an integrator of abiotic and biotic stress responses.

O₃ enters leaves through open stomata, indicated by two guard cells (GC), a process that is not regulated by MLO2, and reacts in the apoplast to form other reactive oxygen species (ROS). We hypothesize that MLO2 integrates various inputs from abiotic and biotic stress sensors, such as extracellular ROS, e.g. derived from O₃ and microbe-associated molecular patterns (MAMPs) originating from pathogens. ROS signals are perceived and transmitted into the cell (denoted by the box) by yet unknown mechanisms. We suggest that MLO2 acts to attenuate a common ROS signalling pathway that splits downstream to regulate various processes, such as tolerance to extracellular ROS, spontaneous callose deposition, antifungal immunity and cell death. MLO2-regulated processes are inferred from *mlo2* mutant phenotypes and the divergent pathways from the different loci genetically modulating *mlo2* mutant phenotypes. Genes negatively contributing to a given pathway are indicated with *. Diagrams inside the box denote signalling events inside the plant cell without implying information on their subcellular localization.

NahG
sen.JA_SA_ET.NG
sen.JA_SA_ET.Co
sen.JA_SA_ET.Ei
B.cinerea_48h
coi1
Col.senescence
ein2
ABA_Exp2
Salt.shoot.3h
ABA_3h
Salt.shoot.24h
Salt.shoot.12h
Salt.shoot.6h
Cold.shoot.12h
Cold.shoot.24h
Cold.shoot.3h
Cold.shoot.6h
Drought.shoot.30min
Cold.shoot.1h
Drought.shoot.1h
Osmotic.shoot.1h
MeJA_30min
Drought.shoot.6h
Drought.shoot.3h
jin1.9.MeJA
MeJA
Drought.shoot.12h
OPDA
ABA_1h
BTH_npr1_Exp2
shoot_hypoxia_total
shoot_hypoxia_p35S
shoot_hypoxia_pRBCS
ET.3hr
MLO_7
Norflurazon
ZAT12
Brassinolid_1uM_1h
X1uM_IAA_for_1h
X1uM_IAA_for_3h
Brassinolid_1uM_3h
MLO_6
MLO_5
Brassinolid_1uM_30min
MeJA_2h
MeJA_6h
Rotenone_3h
rcd1_vs_Col_control_2h
rcd1_vs_Col_control_1h
rcd1_vs_Col_control_8h
rcd1_vs_Col_control_0h
rcd1_vs_Col_control_4h
rcd1_vs_Col_control_24h
high_light_W40
Avr_4h
Avr_8h
Vir_16h
Vir_8h
H2O2
PQ_12h
root_hypoxia_pWOL
root_hypoxia_pCO2
root_hypoxia_pSCR
root_hypoxia_pSHR
root_hypoxia_pSUC2
roottip_hypoxia_p35S
PQ_Exp2
HrpZ_1h
Flg22_1h
NPP1_1h
GST_4h
LPS_4h
ctr1
HL_OE40
HL_W40
Vir_24h
HL_OE63
high_light_OE63
BTH_Exp1
E.cichoracearum
Salt.root.1h
Salt.root.6h
Salt.root.3h
Salt.root.12h
Salt.root.24h
X17days.leaf2
X17days.leaf4
Osmotic.shoot.6h
Osmotic.shoot.3h
Osmotic.shoot.12h
Osmotic.shoot.24h
PQ_24h
B.cinerea_18h
Flg22_4h
HrpZ_4h
NPP1_4h
Syringolin_12h
Ozone
AgNO3
B.graminis

a13550060
a14431800
a15304640
a13501190
a11960090
a15554490
a14431280
a14329760
a15651600
a13644260
a15521020
a11519020
a11980840
a11676050
a15527420
a11668020
a15552760
a12251850
a11661800
a13626830
a11984360
a1552390
a15517860
a14431650
a11675090
a15591080
a11632360
a13601830
a11635210
a11919250
a12630770
a144910500
a13650770
a11630700
a15634610
a14412500
a13643840
a14423150
a11625420
a15505240
a12619190
a136118250
a15639900
a11951890
a13626220
a11666100
a13654400
a1566100
a13622120
a11662890
a15639810
a15639500
a14416260
a14423800
a11651090
a12937870
a15662310
a11616890
a15639820
a11609350
a13617420

Figure S1

Figure S1: Enhanced O₃ tolerance is specific for *mlo2* mutants. (a) O₃-induced electrolyte leakage from mutants for 14 of the 15 *Mlo* gene family members in Arabidopsis, including *mlo1-5*, *mlo2-5*, *mlo3-2*, *mlo4-4*, *mlo5-2*, *mlo6-2*, *mlo7-1*, *mlo9-2*, *mlo10-5*, *mlo11-4*, *mlo12-1*, *mlo13-2*, *mlo14-7*, and *mlo15-3*. Plants were exposed to O₃ (350-400 nl l⁻¹) for 6 h and cell death assayed as electrolyte leakage. Experiments were repeated in three independent biological replicates, with data of a representative experiment shown. All data presented are means ± SD (n=5) for electrolyte leakage expressed as % of total ions determined after disrupting leaves by boiling. Letters indicate statistically different groups based on two-way ANOVA with *post-hoc* analysis. (b) O₃ exposure phenotypes of eight *mlo2* mutant alleles in comparison to Col-0 wild-type. Lines *mlo2-5*, *mlo2-6* and *mlo2-7* comprise T-DNA insertion mutants, while *mlo2-8* (G66R), *mlo2-9* (S220F), *mlo2-10* (D253N), *mlo2-11* (D287N), and *mlo2-13* (P431S) are missense point mutations. O₃ exposure and data presentation are the same as in (a).

Figure S2

Figure S2: Enhanced tolerance of a barley *mlo* mutant to extracellular ROS. (a) Electrolyte leakage from primary leaves of barley (*Hordeum vulgare*) wild-type (*Mlo*) and a near-isogenic *mlo* mutant (*mlo-1*) in cultivar “Ingrid”, treated *in vitro* with an enzymatic superoxide generating system comprised of 1 mM xanthine and 0.1 unit ml⁻¹ xanthine oxidase (XXO). Data are presented as means $\pm$ SD (n=5) for ion leakage expressed as % of total ions determined after disrupting leaves by boiling. The asterisk indicates a statistically significant difference between XXO treated wild-type Ingrid *Mlo* and XXO treated mutant Ingrid *mlo-1* based on a student t-test. (b) Photos of the basal section from leaf number two of two-week-old wild-type *Mlo* or mutant *mlo-1* cultivar “Ingrid” barley exposed for two days to 350 nl l⁻¹ O₃ and photographed 72 h after the beginning of the first exposure. Photos of each genotype were cropped from a single larger photo and are separated by a black line. The black scale bar in the bottom right (= 2 cm) is valid for both photos. The experiments were repeated in three independent biological replicates, with data or photos of a single representative experiment shown.

Figure S3

Figure S3: *MLO2* gene expression. (a) Expression of *MLO2* mined from a publicly available O₃ transcriptomics dataset (Blomster et al. 2011). The figure depicts the time course of *MLO2* expression in Col-0 plants, presented as log₂-fold change in comparison to a common control, over the course of a 6 h exposure to 300 nl l⁻¹ O₃. (b) Quantification of *MLO2* transcript levels by qRT-PCR from four-week-old Col-0 wild-type and *rcd1-4* mutant plants, exposed to either 1 h of 250 nl l⁻¹ O₃ (ozone) or to “clean air” (control). Results are presented in relative units as means ± SD of two biological replicates and were pooled and analysed. Significance groups (P < 0.05) are indicated by letters based on two-way ANOVA with *post-hoc* analysis. See Table S1 for a list of reference genes and primers used.

Figure S4

Figure S4: Stomatal and photosynthesis measurements. (a) Photosynthetic rate, indicated as $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, of Col-0, *mlo2-6*, *mlo2-5 mlo6-2 mlo12-1*. Statistical analysis revealed no difference between genotypes. (b) Stomatal conductance [G_s], indicated as $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, of Col-0, *mlo2-5*, *mlo2-5 mlo6-2 mlo12-1*. Statistical analysis revealed no difference between genotypes. (c) Diurnal time course of stomatal conductance of Col-0 and *mlo2* mutant plants. Stomatal conductance, presented in relative units, was recorded for two consecutive days and circadian stomatal conductance patterns for the second day are shown. Two data points each for three alleles of *mlo2* (*mlo2-5*, *mlo2-6*, and *mlo2-7*) are displayed.

Figure S5

Figure S5: Photos depicting the O₃ phenotypes of *mlo2* double mutants. Three-week-old plants of the indicated genotypes were exposed to O₃ for 6 h at the same concentrations as in Figure 4. Specifically, the O₃-sensitive mutants, *rcd1-4*, *re-8*, *ost1-3*, and *vtc1-1* and their respective *mlo2-6* double mutants exposed to 250 nl l⁻¹ O₃. The O₃-sensitive jasmonate signalling mutants, *jar1-1* and *coi1-16*, the O₃-tolerant and ethylene insensitive *ein2-1* mutant, and their respective *mlo2-5* or *mlo2-11* double mutants were also exposed to 250 nl l⁻¹ O₃. The SA signalling defective mutants, *sid2-1*, *pad4-1*, and their respective *mlo2-5* or *mlo2-6* double mutants were exposed to 325 nl l⁻¹ O₃. Plants were photographed at 48 h after the beginning of the exposure. All experiments were repeated in at least two independent biological replicates, with data from a representative experiment shown. Separate photos that have been composited are separated by white lines. The white scale bar (=2 cm) in the top left is valid for all images.

Figure S6

Figure S6: The *mlo2* double mutants exhibit unaltered powdery mildew entry rates. Six-week-old plants of the indicated genotypes were challenged with *G. orontii*. The proportion of cells of the indicated genotypes successfully entered by the fungal pathogen at 48 h after inoculation are shown. Results are presented as the means $\pm$ SD of pooled data from five independent biological replicates. At least 100 interaction sites were manually scored by microscopic examination of three to five leaves per biological replicate. The absence of an asterisk indicates no significant difference of single mutants (apart from *mlo2-6*) to Col-0 (a) or *mlo2-6* double mutants compared to the *mlo2-6* single mutant (b). Note that the same Col-0 and *mlo2-6* controls are plotted in both graphs for reference. Significance of pooled data was calculated using *post-hoc* analysis by computing contrasts from linear models and subjecting the P values to single-step error correction.

Figure S7

Figure S7: Clustering analysis of MLO-regulated genes: week five time point genes. Hierarchical clustering analysis of expression profiles for MLO-regulated genes from the five-week-old time point. We used all 297 MLO2-, MLO6-, MLO12-regulated genes from the week five gene set (Consonni et al. 2010; see Table S2a for gene list) and subjected their expression profiles to hierarchical clustering analysis. A collection of publicly available transcript profiling experiments related to ROS, abiotic stress, biotic stress, hormone signalling, and transcriptional regulators was used (see Table S2i for list of experiment accession numbers).

Figure S8

Figure S8: Clustering analysis of MLO regulated genes: week six time point genes. Hierarchical clustering analysis of expression profiles for MLO-regulated genes from the six-week-old time point. We used all 1134 MLO2-, MLO6-, MLO12-regulated genes from the week six gene set (Consonni et al. 2010; see Table S2a for gene list) and subjected their expression profiles to hierarchical clustering analysis. A collection of publicly available transcript profiling experiments related to ROS, abiotic stress, biotic stress, hormone signalling, and transcriptional regulators was used (see Table S2i for list of experiment accession numbers).

Figure S9

Figure S9: Clustering analysis of MLO regulated genes: week seven time point genes. Hierarchical clustering analysis of expression profiles for MLO-regulated genes from the seven-week-old time point. We used all 413 MLO2-, MLO6-, MLO12-regulated genes from the week seven gene set (Consonni et al. 2010; see Table S2a for gene list) and subjected their expression profiles to hierarchical clustering analysis. A collection of publicly available transcript profiling experiments related to ROS, abiotic stress, biotic stress, hormone signalling, and transcriptional regulators was used (see Table S2i for list of experiment accession numbers).

Supplemental Table S1: Primers used in this study

Genotyping primers

Name	Full name	Allelic to	AGI code
<i>rbohC</i>	<i>respiratory burst oxidase homolog c</i>	<i>rhd2 -4</i>	At5g51060
<i>rcd1 -4</i>	<i>radical-induced cell death1</i>		At1g32230
<i>oxi1 -2</i>	<i>oxidative signal-inducible1</i>		At3g25250
<i>ost1 -3</i>	<i>open stomata 1-3</i>	<i>srk2e</i>	At4g33950
<i>mpk3 -1</i>	<i>mitogen-activated protein kinase 3-1</i>		At3g45640
<i>mpk6 -2</i>	<i>mitogen-activated protein kinase 6-2</i>		At2g43790
<i>vtc1 -1</i>	<i>vitamin c deficient 1</i>		At2g39770
<i>aba3 -1</i>	<i>aba deficient3-1</i>		At1g16540
<i>bak1 -4</i>	<i>bri1-associated receptor kinase</i>		At4g33430
<i>re -8</i>	<i>reticulata-8</i>	<i>lcd1</i>	At2g37860

References

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JL, Conklin PL, Norris SR, Wheeler GL, Williams EH, Smirnov N, Last RL. 1999. Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Torres MA, Jaspers P, Blomster T, Brosché M, Salojärvi J, Ahlfors R, Vainonen JP, Re Nakagami H, Soukupová H, Schikora A, Zárský V, Hirt H. 2006. A Mitogen-Overmyer K, Kollist H, Tuominen H, Betz C, Langebartels C, Wingsle G, K Rentel MC, Lecourieux D, Ouaked F, Usher SL, Petersen L, Okamoto H, Ki Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S. 2007. Stomatal Develop Yoshida R, Hobo T, Ichimura K, Mizoguchi T, Takahashi F, Aronso J, Ecke

Mutant type	Phenotypes	genotyping
Transposon insertion		PCR
GABI_229D11	O ₃ sensitive; smaller rosette, short petioles, distorted leaves with serrated margins	PCR
GABI_355H08		PCR
SALK_008068		PCR
SALK_151594		PCR
SALK_073907		PCR
EMS	O ₃ sensitive; short root and much more root hairs with seedlings. smaller rosette and narrower leaves with adult plants	CAPS/Styl
EMS	small rosette size and permeable cuticle	CAPS/MnII
SALK_116202		PCR
EMS	O ₃ sensitive; reticulate chlorosis	CAPS

JDG, Felix G, Boller T. 2007. A flagellin-induced complex of the receptor FLS2 and BAK1
19. Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis
, Linstead P, Costa S, Brownlee C, Jones JDG, et al. 2003. Reactive oxygen species production
Reddy RA, Immink R, Angenent G, Turck F. 2009. Unequally redundant RCD1 and SRO1
activated Protein Kinase Kinase Kinase Mediates Reactive Oxygen Species Homeostasis
Kangasjärvi S, Brader G, Mullineaux P, Kangasjärvi J. 2008. Complex phenotypic profile
Wright H, Peck SC, Grierson CS, Hirt H, et al. 2004. OXI1 kinase is necessary for oxidative
development and Patterning Are Regulated by Environmentally Responsive Mitogen-Activated Protein Kinase
Wright JR, Shinozaki K. 2002. ABA-Activated SnRK2 Protein Kinase is Required for Dehydration

Forward primer

TAGCTTCTCCATGTGACCGCG

AGGTGTGAAGATGGATTCCG

CCTTTCCAAACAAAGCAAGTG

TTTGCCGATTATGCACGATA

CGAGCAATCTTCTGTTGAACG

GCCTCAGATGCCTGGGATTGAGAATATTC

TGCATTTTCAGGAAAAGGAGTT

TCATTCTTTCTACTGCTCCTGATTT

CTATCTCTCCTACACCGCCATC

TGTGGCATTGTAGGTCAAGG

initiates plant defence. *Nature* **448**: 497–500.
ynthesis. *Proceedings of the National Academy of*
roduced by NADPH oxidase regulate plant cell g
1 mediate stress and developmental responses at
stasis in Arabidopsis. Journal of Biological Ch
es leading to ozone sensitivity in Arabidopsis thali
ive burst-mediated signalling in Arabidopsis. *Natu*
rotein Kinases in Arabidopsis. *The Plant Cell On*
tion Stress Signaling in Arabidopsis. *Plant and Ce*

Reverse primer	Source or reference
ATCTAAAGCTAGATGCCTTAGC	Foreman et al., 2003
GGGGTTTTTGGCTCCTGTTTC	Jaspers et al., 2009
AAGAAACGTCTCTTCCGCTTC	Rentel et al., 2004
AGGAGCGATGTAAGCAGGAG	Yoshida et al., 2002
GCTGCTGCACTTCTAACCGTATG	Nakagami et al., 2006
AGAGTGGCTTACGGTCCATT	Nakagami et al., 2006
TTAGCAAAATCAACAAGGGGCCTTG	Conklin et al., 1999
GGTGAAGCAAATGAACTTATGATG	Xiong et al., 2001
CTTGCAGATCCCAACGCAATACG	Chinchilla et al., 2007
CACCTGCAATTAATCAACCA	Overmyer et al., 2008

Sciences **96**: 4198–4203.
 rowth. *Nature* **422**: 442–446.
 nd interact with transcription factors. *The Plant Journal* **60**: 268–279.
chemistry 281: 38697–38704.
 ana mutants. *Plant, Cell & Environment* **31**: 1237–1249.
ire **427**: 858–861.
line **19**: 63–73.
all Physiology **43**: 1473–1483.

Supplemental Table S2i: Genes and Experiments used in Figure 6.

[Click here to jump to Experiments below.](#)

[‡]Gene descriptions sourced from from TAIR (<https://www.arabidopsis.org/tools/>)

[†] Data sources are from: The National Center for Bioinformatics Gene expression data; The Nottingham Arabidopsis Stock Centre Arrays (NASC Arrays); and the Harvard

Genes [‡]			
<i>AGI codes</i>	<i>Gene Model Name</i>	<i>Gene Model Description</i>	<i>Gene Model Ty</i>
AT4G24380	AT4G24380.1	dihydrofolate reductase;(source:Araport11)	
AT2G20670	AT2G20670.1	sugar phosphate exchanger, putative (DUF50	
AT1G66100	AT1G66100.1	Predicted to encode a PR (pathogenesis-rela	
AT4G12500	AT4G12500.1	Bifunctional inhibitor/lipid-transfer protein/see	
AT3G04640	AT3G04640.1	glycine-rich protein;(source:Araport11) proteir	
AT4G29780	AT4G29780.1	nuclease;(source:Araport11) protein_codin	
AT1G68620	AT1G68620.1	alpha/beta-Hydrolases superfamily protein;(s	
AT3G28220	AT3G28220.1	TRAF-like family protein;(source:Araport11)	
AT2G32190	AT2G32190.1	cysteine-rich/transmembrane domain A-like p	
AT3G54400	AT3G54400.1	Eukaryotic aspartyl protease family protein;(s	
AT3G48640	AT3G48640.1	transmembrane protein;(source:Araport11)	
AT1G66090	AT1G66090.1	Disease resistance protein (TIR-NBS class);(	
AT3G01830	AT3G01830.1	Calcium-binding EF-hand family protein;(sour	
AT5G39520	AT5G39520.1	hypothetical protein (DUF1997);(source:Arap	
AT1G56060	AT1G56060.1	cysteine-rich/transmembrane domain protein	
AT1G16850	AT1G16850.1	transmembrane protein;(source:Araport11)	
AT5G52760	AT5G52760.1	Copper transport protein family;(source:Arap	
AT5G38900	AT5G38900.1	Thioredoxin superfamily protein;(source:Arap	
AT1G19020	AT1G19020.1	CDP-diacylglycerol-glycerol-3-phosphate 3-ph	
AT2G37870	AT2G37870.1	Bifunctional inhibitor/lipid-transfer protein/see	
AT4G16260	AT4G16260.1	Encodes a putative beta-1,3-endoglucanase 1	
AT4G39670	AT4G39670.1	Glycolipid transfer protein (GLTP) family prote	
AT1G35210	AT1G35210.1	hypothetical protein;(source:Araport11) protei	
AT3G02840	AT3G02840.1	ARM repeat superfamily protein;(source:Arap	
AT3G13950	AT3G13950.1	ankyrin;(source:Araport11) protein_coding	
AT2G44240	AT2G44240.1	NEP-interacting protein (DUF239);(source:Ar	
AT1G51890	AT1G51890.1	Leucine-rich repeat protein kinase family prot	
AT5G52390	AT5G52390.1	PAR1 protein;(source:Araport11) protein_cod	
AT3G18250	AT3G18250.1	Putative membrane lipoprotein;(source:Arap	
AT1G26420	AT1G26420.1	FAD-binding Berberine family protein;(source	
AT1G30700	AT1G30700.1	FAD-binding Berberine family protein;(source	
AT1G51090	AT1G51090.1	Heavy metal associated domain containing pr	
AT4G12490	AT4G12490.1	Encodes a member of the AZI family of lipid t	
AT5G38710	AT5G38710.1	Methylenetetrahydrofolate reductase family p	
AT1G32960	AT1G32960.1	Subtilase family protein;(source:Araport11)	
AT4G23810	AT4G23810.1	member of WRKY Transcription Factor; Grou	
AT2G43570	AT2G43570.1	chitinase;(source:Araport11) protein_coding	
AT5G45340	AT5G45340.1	Encodes a protein with ABA 8'-hydroxylase a	

AT4G11280	AT4G11280.1	encodes a a member of the 1-aminocycloprop
AT5G24420	AT5G24420.1	Encodes a cytosolic 6-phosphogluconolacton
AT1G32350	AT1G32350.1	alternative oxidase 1D;(source:Araport11)
AT1G33960	AT1G33960.1	Identified as a gene that is induced by avirule
AT3G12500	AT3G12500.1	encodes a basic chitinase involved in ethylen
AT5G20230	AT5G20230.1	Encodes a Al-stress-induced gene. Along with
AT3G61190	AT3G61190.1	Encodes a protein with a C2 domain that bind
AT3G48360	AT3G48360.1	encodes a protein (BT2) that is an essential c
AT5G17860	AT5G17860.1	calcium exchanger 7;(source:Araport11) prot
AT5G42380	AT5G42380.1	calmodulin like 37;(source:Araport11) protei
AT1G76650	AT1G76650.1	calmodulin-like 38;(source:Araport11) protei
AT3G50770	AT3G50770.1	calmodulin-like 41;(source:Araport11) protei
AT5G27420	AT5G27420.1	Encodes CN11 (Carbon/Nitrogen Insensitive1
AT4G15610	AT4G15610.1	Uncharacterized protein family (UPF0497);(sc
AT3G44260	AT3G44260.1	Encodes one of the homologs of the yeast CC
AT3G22120	AT3G22120.1	cell wall-plasma membrane linker protein hom
AT1G29395	AT1G29395.1	Integral membrane protein in the inner envel
AT2G42540	AT2G42540.2	A cold-regulated gene whose product is target
AT4G23600	AT4G23600.1	Encodes cystine lyase which is expected to b
AT4G23150	AT4G23150.1	Encodes a cysteine-rich receptor-like protein
AT2G30770	AT2G30770.1	putative cytochrome P450 protein_coding
AT4G24570	AT4G24570.1	Encodes one of the mitochondrial dicarboxyla
AT4G10500	AT4G10500.1	2-oxoglutarate (2OG) and Fe(II)-dependent o
AT4G12480	AT4G12480.1	Encodes a putative lipid transfer protein, vern
AT1G64360	AT1G64360.1	SAQR is a clade specific protein present in si
AT5G61600	AT5G61600.1	encodes a member of the ERF (ethylene resp
AT2G40610	AT2G40610.1	member of Alpha-Expansin Gene Family. Nar
AT1G19250	AT1G19250.1	FMO1 is required for full expression of TIR-NI
AT2G19190	AT2G19190.1	Encodes a receptor-like protein kinase that is
AT1G56600	AT1G56600.1	GolS2 is a galactinol synthase that catalyzes
AT1G09350	AT1G09350.1	Predicted to encode a galactinol synthase
AT1G61800	AT1G61800.1	glucose6-Phosphate/phosphate transporter 2
AT1G69930	AT1G69930.1	Encodes glutathione transferase belonging to
AT2G29460	AT2G29460.1	Encodes glutathione transferase belonging to
AT3G47420	AT3G47420.1	Encodes a Pi starvation-responsive protein A
AT2G18050	AT2G18050.1	encodes a structurally divergent linker histone
AT1G51800	AT1G51800.1	The gene encodes a putative member of the
AT1G13340	AT1G13340.1	Regulator of Vps4 activity in the MVB pathwa
AT1G19180	AT1G19180.1	JAZ1 is a nuclear-localized protein involved in
AT1G73260	AT1G73260.1	Encodes a trypsin inhibitor involved in modul
AT1G72520	AT1G72520.1	PLAT/LH2 domain-containing lipoxygenase fa
AT5G52310	AT5G52310.1	cold regulated gene, the 5' region of cor78 ha
AT3G50060	AT3G50060.1	Encodes a member of the R2R3 transcription
AT2G39030	AT2G39030.1	Encodes a protein that acts as an ornithine N
AT5G39610	AT5G39610.1	Encodes a NAC-domain transcription factor. I
AT1G52890	AT1G52890.1	encodes a NAC transcription factor whose ex
AT3G04070	AT3G04070.1	NAC domain containing protein 47;(source:Ar

AT1G69490	AT1G69490.1	Encodes a member of the NAC transcription factor family.
AT1G65690	AT1G65690.1	Encodes NHL6 (NDR1/HIN1-like 6). Plays an osmotin-like protein protein_coding OSMO
AT4G11650	AT4G11650.1	Encodes a protein with sequence similarity to
AT5G05340	AT5G05340.1	Mutations in pad3 are defective in biosynthesis
AT3G26830	AT3G26830.1	Phytosulfokine 3 precursor, coding for a unique
AT3G49780	AT3G49780.1	Encodes a PINOID (PID)-binding protein containing
AT5G54490	AT5G54490.1	Encodes PNP-A (Plant Natriuretic Peptide A).
AT2G18660	AT2G18660.1	Encodes a zinc finger protein involved in high
AT5G59820	AT5G59820.1	Related to Cys2/His2-type zinc-finger proteins
AT1G27730	AT1G27730.1	Encodes SAR Deficient 1 (SARD1), a key regulator
AT1G73805	AT1G73805.1	Senescence-associated gene that is strongly
AT4G35770	AT4G35770.1	senescence-associated gene SAG13 encoding
AT2G29350	AT2G29350.1	Encodes a tyrosine aminotransferase that is related
AT2G24850	AT2G24850.1	VACUOLELESS GAMETOPHYTES (VLG) as
AT2G17740	AT2G17740.1	Pathogen-induced transcription factor. Binds to
AT4G31800	AT4G31800.1	member of WRKY Transcription Factor; Group
AT5G22570	AT5G22570.1	Pathogen-induced transcription factor. Binds to
AT1G80840	AT1G80840.1	WRKY75 is one of several transcription factors
AT5G13080	AT5G13080.1	member of WRKY Transcription Factor; Group
AT5G46350	AT5G46350.1	Encodes a protein whose sequence is similar
AT2G35980	AT2G35980.1	

Experiments

Name	Source[†]	Accession
X17days.leaf2	NCBI GEO	GSE5630
X17days.leaf4	NCBI GEO	GSE5630
ABA_1h	NASCArrays	NASCARRAYS-176
ABA_3h	NASCArrays	NASCARRAYS-176
ABA_Exp2	NCBI GEO	GSE7112
AgNO3	NASCArrays	NASCARRAYS-188
Avr_4h	NCBI GEO	GSE5685
Avr_8h	NCBI GEO	GSE5685
B.cinerea_18h	ArrayExpress	E-GEOD-5684
B.cinerea_48h	ArrayExpress	E-GEOD-5684
B.graminis	ArrayExpress	E-GEOD-12856
BTH_Exp1	IMDS	BTH time course
BTH_Exp2	IMDS	BTH experiment 2
BTH_npr1_Exp2	IMDS	BTH npr1 Exp2
coi1	NCBI GEO	GSE65740
Col.senescence	NCBI GEO	GSE5727
Cold.shoot.12h	NCBI GEO	GSE5621
Cold.shoot.1h	NCBI GEO	GSE5621
Cold.shoot.24h	NCBI GEO	GSE5621
Cold.shoot.3h	NCBI GEO	GSE5621
Cold.shoot.6h	NCBI GEO	GSE5621
ctr1	ArrayExpress	E-MEXP-174
Drought.shoot.12h	NCBI GEO	GSE5624

Drought.shoot.1h	NCBI GEO	GSE5624
Drought.shoot.30min	NCBI GEO	GSE5624
Drought.shoot.3h	NCBI GEO	GSE5624
Drought.shoot.6h	NCBI GEO	GSE5624
E.cichoracearum	NCBI GEO	GDS417
ein2	NCBI GEO	GSE5727
ET.3hr	NASCArrays	NASCARRAYS-32
Flg22_1h	NCBI GEO	GSE5615
Flg22_4h	NCBI GEO	GSE5615
GST_4h	NCBI GEO	GSE5615
H2O2	NCBI GEO	GSE5530
HrpZ_1h	NCBI GEO	GSE5615
HrpZ_4h	NCBI GEO	GSE5615
jin1.9.MeJA	ArrayExpress	E-MEXP-883
LPS_4h	NCBI GEO	GSE5615
MeJA	ArrayExpress	E-ATMX-13
MeJA_2h	ArrayExpress	E-ATMX-13
MeJA_30min	ArrayExpress	E-ATMX-13
MeJA_6h	ArrayExpress	E-ATMX-13
NahG	NCBI GEO	GSE5727
Norflurazon	NCBI GEO	GSE12887
NPP1_1h	NCBI GEO	GSE5615
NPP1_4h	NCBI GEO	GSE5615
OPDA	NCBI GEO	GSE10732
Osmotic.shoot.12h	NCBI GEO	GSE5622
Osmotic.shoot.1h	NCBI GEO	GSE5622
Osmotic.shoot.24h	NCBI GEO	GSE5622
Osmotic.shoot.3h	NCBI GEO	GSE5622
Osmotic.shoot.6h	NCBI GEO	GSE5622
Ozone	NCBI GEO	GSE5722
PQ_12h	NASCArrays	NASCARRAYS-143
PQ_24h	NASCArrays	NASCARRAYS-143
PQ_Exp2	ArrayExpress	E-ATMX-28
root_hypoxia_pCO2	NCBI GEO	GSE14578
root_hypoxia_pSCR	NCBI GEO	GSE14578
root_hypoxia_pSHR	NCBI GEO	GSE14578
root_hypoxia_pSUC2	NCBI GEO	GSE14578
root_hypoxia_pWOL	NCBI GEO	GSE14578
roottip_hypoxia_p35S	NCBI GEO	GSE14578
Rotenone_3h	ArrayExpress	E-MEXP-1797
Salt.root.12h	NCBI GEO	GSE5623
Salt.root.1h	NCBI GEO	GSE5623
Salt.root.24h	NCBI GEO	GSE5623
Salt.root.3h	NCBI GEO	GSE5623
Salt.root.6h	NCBI GEO	GSE5623
Salt.shoot.12h	NCBI GEO	GSE5623
Salt.shoot.24h	NCBI GEO	GSE5623

Salt.shoot.3h	NCBI GEO	GSE5623
Salt.shoot.6h	NCBI GEO	GSE5623
shoot_hypoxia_p35S	NCBI GEO	GSE14578
shoot_hypoxia_pRBCS	NCBI GEO	GSE14578
shoot_hypoxia_total	NCBI GEO	GSE14578
Syringolin_12h	ArrayExpress	E-MEXP-739
Vir_16h	NCBI GEO	GSE5685
Vir_24h	NCBI GEO	GSE5685
Vir_8h	NCBI GEO	GSE5685
ZAT12	NASCArrays	NASCARRAYS-353
high_light_OE63	NCBI GEO	GSE46107
high_light_W40	NCBI GEO	GSE46107
HL_OE40	NCBI GEO	GSE46107
HL_OE63	NCBI GEO	GSE46107
HL_W40	NCBI GEO	GSE46107
sen.JA_SA_ET.Co	NCBI GEO	GSE5727
sen.JA_SA_ET.Ei	NCBI GEO	GSE5727
sen.JA_SA_ET.NG	NCBI GEO	GSE5727
MLO_5	NCBI GEO	GSE17875
MLO_6	NCBI GEO	GSE17875
MLO_7	NCBI GEO	GSE17875
X1uM_IAA_for_1h	NCBI GEO	GDS743
X1uM_IAA_for_3h	NCBI GEO	GDS743
Brassinolid_1uM_1h	NCBI GEO	GDS743
Brassinolid_1uM_30min	NCBI GEO	GDS743
Brassinolid_1uM_3h	NCBI GEO	GDS743
rcd1_vs_Col_control_0h	ArrayExpress	E-MTAB-662
rcd1_vs_Col_control_1h	ArrayExpress	E-MTAB-662
rcd1_vs_Col_control_2h	ArrayExpress	E-MTAB-662
rcd1_vs_Col_control_4h	ArrayExpress	E-MTAB-662
rcd1_vs_Col_control_8h	ArrayExpress	E-MTAB-662
rcd1_vs_Col_control_24h	ArrayExpress	E-MTAB-662

/bulk/index.jsp) and represent TAIR10 Gene Annotation Data.

on Omnibus (NCBI GEO); The EMBL European Bioinformatics Institute (Array Express); The Harvard Medical School Integrated Microarray Database System (IMDS).

Primary Gene Symbol All Gene Symbols

protein_coding
6);(source:Araport11) protein_coding
ted) protein. Belongs to the plant thionin (PR-13) family with the following members: storage 2S albumin superfamily protein;(source:Araport11) protein_coding
n_coding
g
source:Araport11) protein_coding
protein_coding
rotein;(source:Araport11) protein_coding
source:Araport11) protein_coding
protein_coding
source:Araport11) protein_coding
ce:Araport11) protein_coding
ort11) protein_coding
B;(source:Araport11) protein_coding
protein_coding
ort11) protein_coding
ort11) protein_coding
osphatidyltransferase;(source:Araport11) protein_coding
d storage 2S albumin superfamily protein;(source:Araport11) protein_coding
that interacts with the 30C02 cyst nematode effector. May play a role in host defense;(source:Araport11) protein_coding
in_coding
ort11) protein_coding
|
aport11) protein_coding
ein;(source:Araport11) protein_coding
ling
ort11) protein_coding
:Araport11) protein_coding (ATBBE7) (ATBBE7)
:Araport11) protein_coding (ATBBE8) (ATBBE8)
rotein involved in plant immunity. Mutants show an increase in root length under P
ransfer proteins. Contains a PRR domain that appears to be required for localization
rotein;(source:Araport11) protein_coding (PDH2) (PDH2)
protein_coding (SBT3.3) (SBT3.3); (ATSBT3.3)
p III protein_coding (WRKY53) (ATWRKY53); (WRKY53)
g "CHITINASE, PUTATIVE" (CHI) "CHITINASE, PUTATIVE" (CHI)
ctivity; involved in ABA catabolism. Mutant analyses show that disruption in the g

ane-1-carboxylate (ACC) synthase (S-adenosyl-L-methionine methylthioadenosase (PGL) thought to be involved in the oxidative pentose-phosphate pathway (C protein_coding ALTERNATIVE OXIDASE 1D (AOX1D) ALTERNATIVE OXIDA nce gene avrRpt2 and RPS2 after infection with Pseudomonas syringae pv macleod/jasmonic acid mediated signalling pathway during systemic acquired resistance TCF, it promotes lignin biosynthesis in response to cold stress. The mRNA is c ls to BON1 in yeast two hybrid analyses. Its ability to bind to phospholipids is en component of the TAC1-mediated telomerase activation pathway. Acts redundan ein_coding CALCIUM EXCHANGER 7 (CAX7) (CCX1);CALCIUM EXCHAN n_coding CALMODULIN LIKE 37 (CML37) CALMODULIN LIKE 37 (CML37); n_coding CALMODULIN-LIKE 38 (CML38) n_coding CALMODULIN-LIKE 41 (CML41) CALMODULIN-LIKE 41 (CML41)) (also named as ATL31), a RING type ubiquitin ligase that functions in the Carb source:Araport11) protein_coding CASP-LIKE PROTEIN 1D1 (CASPL1D1) CR4-associated factor 1: AT3G44260 (CAF1a), AT5G22250 (CAF1b). Has mRN. homolog (CWLP) protein_coding CELL WALL-PLASMA MEMBRANE LINKER PRC ope of chloroplasts. Provide freezing tolerance. Expression is induced by short-t ted to the chloroplast. Cor15am protects stromal proteins from aggregation unde e involved in amino acid metabolism, providing the plant with cysteine and the ge kinase. protein_coding CYSTEINE-RICH RLK (RECEPTOR-LIKE PROTEIN K g CYTOCHROME P450, FAMILY 71, SUBFAMILY A, POLYPEPTIDE 13 (CYP7 ate carriers (DIC): DIC1 (AT2G22500), DIC2 (AT4G24570), DIC3 (AT5G09470). xygenase superfamily protein;(source:Araport11) protein_coding DMR6-LIKE alization-responsive and cold-induced. It is involved in priming the SAR and ISR ngle copy in Arabidopsis.It's expression is increased during light induced oxidativ onse factor) subfamily B-3 of ERF/AP2 transcription factor family. The protein co ming convention from the Expansin Working Group (Kende et al, 2004. Plant Mo B-LRR-conditioned resistance to avirulent pathogens and for basal resistance to involved in early defense signaling. Expression of this gene is strongly induced c the formation of galactinol from UDP-galactose and myo-inositol. GolS2 transcrip protein_coding GALACTINOL SYNTHASE 3 (GolS3) GALACTINOL SYNTHA . Expression is upregulated in the shoot of cax1/cax3 mutant. The mRNA is cell- , the tau class of GSTs. Naming convention according to Wagner et al. (2002). , the tau class of GSTs. Naming convention according to Wagner et al. (2002). tPS3. A member of the phosphate starvation-induced glycerol-3-phosphate perm whose gene expression is induced by dehydration and ABA. The mRNA is cell- LRR-RLK protein family. Expressin and mutant analysis revealed that it contribu y protein;(source:Araport11) protein_coding IST1-LIKE 6 (ISTL6) IST1-LI jasmonate signaling. JAZ1 transcript levels rise in response to a jasmonate stir ating programmed cell death in plant-pathogen interactions. protein_coding amily protein;(source:Araport11) protein_coding LIPOXYGENASE 4 (LOX4) is cis-acting regulatory elements that can impart cold-regulated gene expression factor gene family. Expressed in response to potassium deprivation and auxin. I -delta-acetyltransferase, leading to the formation of N-delta-actetylnithine. This Positively regulates aging-induced cell death and senescence in leaves. This ge pression is induced by drought, high salt, and abscisic acid. This gene binds to E araport11) protein_coding NAC DOMAIN CONTAINING PROTEIN 47 (NAC047

[illegible]

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5623>
<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5623>
<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14578>
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<https://www.ebi.ac.uk/arrayexpress/experiments/E-MEXP-739/>
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<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5685>
<http://www.webcitation.org/getfile?fileid=c2cde11f2c71cfb474c58964754c6aecc>
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<https://www.ncbi.nlm.nih.gov/sites/GDSbrowser?acc=GDS743>
<https://www.ncbi.nlm.nih.gov/sites/GDSbrowser?acc=GDS743>
<https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-662/>
<https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-662/>
<https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-662/>
<https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-662/>
<https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-662/>

press);

ers: At1g66100, At5g36910, At1g72260, At2g15010, At1g12663, At1g12660. prote

ense. protein_coding

√O stress and reduction in root length under H₂O₂ stress conditions. Mutants show in
tion to the chloroplast. protein_coding (AZI3) (AZI3)

ene results in more drought tolerance whereas overexpression results in increased tra

ine-lyase, EC 4.4.1.14) gene family The mRNA is cell-to-cell mobile. protein_coding
PPP). protein_coding 6-PHOSPHOGLUCONOLACTONASE 5 (PGL5) 6-PHOSPHOGLUCONOLACTONASE 1D (AOX1D)

ulicola strain ES4326 carrying avrRpt2 protein_coding AVRRPT2-INDUCED GENE 1
e based on expression analyses. protein_coding BASIC CHITINASE (HCHIB) (CHITINASE)
ell-to-cell mobile. protein_coding BLUE-COPPER-BINDING PROTEIN (BCB) BLU
anced by calcium ions. Involved in maintaining cell homeostasis. protein_coding BO
tly with BT3 and BT1 during female gametophyte development and with BT3 during m
GER 7 (CAX7)
(ATCML37);CALMODULIN LIKE 39 (CML39)

on/Nitrogen response for growth phase transition in Arabidopsis seedlings. The mRNA
CASP-LIKE PROTEIN 1D1 (CASPL1D1)

A deadenylation activity. Also plays a role in plant defense responses. protein_coding
TEIN (CWLP) CELL WALL-PLASMA MEMBRANE LINKER PROTEIN (CWLP)
erm cold-treatment, water deprivation, and abscisic acid treatment. protein_coding
er various stress conditions. Constitutive expression increases freezing tolerance in pr
eneration of precursors of ethylene biosynthesis. mRNA levels are elevated in respon
INASE) 7 (CRK7) CYSTEINE-RICH RLK (RECEPTOR-LIKE PROTEIN KINASE) 1
1A13) CYTOCHROME P450, FAMILY 71, SUBFAMILY A, POLYPEPTIDE 13 (CY
The mRNA is cell-to-cell mobile. protein_coding DICARBOXYLATE CARRIER 2 (DLC2)
OXYGENASE 1 (DLO1) DMR6-LIKE OXYGENASE 1 (DLO1)

responses, specifically in propagating the cell-to-cell mobile signal. protein_coding E
re stress ,drought stress and also during senescence. Promoter contains two AGL15 b
contains one AP2 domain. There are 18 members in this subfamily including ATERF-1,
l Bio). Involved in the formation of nematode-induced syncytia in roots of Arabidopsis
invasive virulent pathogens. Functions in an EDS1-regulated but SA-independent me
during leaf senescence. It is a target of the transcription factor AtWRKY6. protein_c
ot levels rise in response to methyl viologen, an oxidative damage-inducing agent. Pla
SE 3 (GoIS3);GALACTINOL SYNTHASE 3 (AtGoIS3)

to-cell mobile. protein_coding GLUCOSE-6-PHOSPHATE/PHOSPHATE TRANSFERASE
protein_coding GLUTATHIONE S-TRANSFERASE TAU 11 (GSTU11) GLUTATHIONE S-TRANSFERASE
protein_coding GLUTATHIONE S-TRANSFERASE TAU 4 (GSTU4) GLUTATHIONE S-TRANSFERASE
ase gene family: AT3G47420(G3Pp1), AT4G25220(G3Pp2), AT1G30560(G3Pp3), AT1G30560(G3Pp3),
to-cell mobile. protein_coding HISTONE H1-3 (HIS1-3) HISTONE H1-3 (HIS1-3)
tes to the interaction between Arabidopsis and Hyaloperonospora arabidopsidis. and
KE 6 (ISTL6)

ulus. JAZ1 can interact with the COI1 F-box subunit of an SCF E3 ubiquitin ligase in :
KUNITZ TRYPSIN INHIBITOR 1 (KTI1) KUNITZ TRYPSIN INHIBITOR 1 (KTI1);A
ARABIDOPSIS THALIANA LIPOXYGENASE 4 (ATLOX4);LIPOXYGENASE 4 (LOX4)
The mRNA is cell-to-cell mobile. protein_coding LOW-TEMPERATURE-INDUCED 7
nvolved in lateral root development. Interacts with ARF7 and regulates the expression
; compound is likely used in plant defense and levels of it are increased in Arabidopsis
ne is upregulated in response to salt stress in wildtype as well as NTHK1 transgenic li
RD1 promoter in vitro. protein_coding NAC DOMAIN CONTAINING PROTEIN 19
7) NAC DOMAIN CONTAINING PROTEIN 47 (anac047);SPEEDY HYPONASTIC

d PI. Its expression is associated with leaf senescence. The mRNA is cell-to-cell mobile particularly during seed germination and early seedling development. protein_coding

ow abnormal development of xylem fibers and reduced levels of lignin biosynthetic enzyme that catalyzes the conversion of dihydrocamalexin to camalexin. The mRNA (moter), develop normal cotyledons and hypocotyls but their growth, in particular that of the presence of calcium. mRNA expression is up-regulated by auxin. Not a phosphatase; their biological role has remained elusive. PNP-A contains a signal peptide domain increases the expression level of 9 cold-responsive genes and represses the expression of 8. Salt tolerance produced by ZAT10 appeared to be partially dependent on ENA1/10. protein_coding SAR DEFICIENT 1 (SARD1) SAR DEFICIENT 1 (SARD1) level of mRNA stability at different times of day. mRNAs are targets of the mRNA DEGRADATION-ASSOCIATED GENE 13 (SAG13) SENESCENCE-ASSOCIATED GENE 13 (SAG13) TRANSFERASE 3 (TAT3) TYROSINE AMINOTRANSFERASE 3 (TAT3);TYROSIN is essential for both female and male gametophyte development. protein_coding WRKY10 and WRKY60. Constitutive expression of WRKY18 enhanced resistance to P. syringae. ARABIDOPSIS THALIANA WRKY DNA-BINDING PROTEIN 38 (ATWRKY38);WRKY DNA-BINDING PROTEIN 10 and WRKY60. Coexpression with WRKY18 or WRKY60 made plants more susceptible to P. syringae during Pi starvation. RNAi mediated suppression of WRKY75 made the plants more resistant to P. syringae. ARABIDOPSIS THALIANA WRKY DNA-BINDING PROTEIN 8 (WRKY8);ARABIDOPSIS THALIANA WRKY DNA-BINDING PROTEIN 8 (WRKY8) disease resistance gene (NDR1). Expression of this gene is induced by cucumber mosaic virus.

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in_coding

creases in defense responses to pathogens including hypersensitive lesions, increased

transpiration rate and reduced drought tolerance. Gene involved in postgermination growth

ng 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE 6 (ACS6) (P)
PHOGLUCONOLACTONASE 5 (PGL5)

(AIG1) AVRRPT2-INDUCED GENE 1 (AIG1)
II-B);BASIC CHITINASE (ATHCHIB);PATHOGENESIS-RELATED 3 (PR3);PATHOGEN
UE-COPPER-BINDING PROTEIN (ATBCB);BLUE-COPPER-BINDING PROTEIN (BCB)
N ASSOCIATION PROTEIN 1 (BAP1) BON ASSOCIATION PROTEIN 1 (BAP1)
ale gametophyte development. BT2 also mediates multiple responses to nutrients, stres

is cell-to-cell mobile. protein_coding CARBON/NITROGEN INSENSITIVE 1 (CNI1)

oding CCR4- ASSOCIATED FACTOR 1A (CAF1a) CCR4- ASSOCIATED FACTOR 1

COLD REGULATED 314 INNER MEMBRANE 1 (COR413IM1) COLD REGULATED 3
otoplasts in vitro and chloroplasts in vivo. NMR and x-ray diffraction studies suggest tha
se to wounding. protein_coding CORONATINE INDUCED 1 (CORI3) JASMONIC
7 (CRK7)
P71A13)
C2) DICARBOXYLATE CARRIER 2 (DIC2)

EARLY ARABIDOPSIS ALUMINUM INDUCED 1 (EARLI1) EARLY ARABIDOPSIS ALU
inding sites. protein_coding ENESCENCE-ASSOCIATED AND QQS-RELATED (S
ATERF-2, AND ATERF-5. protein_coding ETHYLENE RESPONSE FACTOR 104 (E
thaliana. protein_coding EXPANSIN A8 (EXPA8) (EXP8); (ATEXP8);EXPANSIN A
chanism that promotes resistance and cell death at pathogen infection sites. protein_
oding FLG22-INDUCED RECEPTOR-LIKE KINASE 1 (FRK1) SE- NESCENCE-INDL
nts over-expressing GolS2 have increased tolerance to salt, chilling, and high-light stres

LOCATOR 2 (GPT2) GLUCOSE-6-PHOSPHATE/PHOSPHATE TRANSLOCATOR 2
(THIONE S-TRANSFERASE TAU 11 (GSTU11);GLUTATHIONE S-TRANSFERASE TA
NE S-TRANSFERASE TAU 4 (ATGSTU4);GLUTATHIONE S-TRANSFERASE TAU 4 (C
AT4G17550(G3Pp4) and AT2G13100(G3Pp5). Its expression is responsive to phosphat
)

The mRNA is cell-to-cell mobile. protein_coding IMPAIRED OOMYCETE SUSCEPTIB

a yeast-two-hybrid assay only in the presence of jasmonate-isoleucine (JA-ILE) or coron
RABIDOPSIS THALIANA KUNITZ TRYPSIN INHIBITOR 1 (ATKT11)

4)

'8 (LTI78) COLD REGULATED 78 (COR78);LOW-TEMPERATURE-INDUCED 78 (L
i of some auxin responsive genes. protein_coding MYB DOMAIN PROTEIN 77 (MYB77
; plants in response to MeJA and ABA. The mRNA is cell-to-cell mobile. protein_coding
nes although in the latter case the induction was drastically reduced. It was also upregul
(NAC019) (ANAC19);NAC DOMAIN CONTAINING PROTEIN 19 (NAC019);NAC D
GROWTH (SHYG);SPEEDY HYPONASTIC GROWTH (SHG);NAC DOMAIN CONTAIN

ile. protein_coding NAC-LIKE, ACTIVATED BY AP3/PI (NAP) ARABIDOPSIS NAC I
NDR1/HIN1-LIKE 6 (NHL6) NDR1/HIN1-LIKE 6 (NHL6)

xymes. protein_coding PEROXIDASE 52 (PRX52) PEROXIDASE 52 (PRX52)
is cell-to-cell mobile. protein_coding PHYTOALEXIN DEFICIENT 3 (PAD3) (CYP71
of their roots, was faster than that of wildtype. protein_coding PHYTOSULFOKINE 4 P
ylation target of PID, likely acts upstream of PID to regulate the activity of this protein in
and is secreted into the extracellular space. Co-expression analyses using microarray c
a level of 15 cold-responsive genes, including CBF genes. Also, lines overexpressing this
PMR2, a P-type ATPase required for Li⁺ and Na⁺ efflux in yeast. The protein is localized

adation pathway mediated by the downstream (DST) instability determinant. protein_c

E AMINOTRANSFERASE (TAT)

VACUOLELESS GAMETOPHYTES (VLG) VACUOLELESS GAMETOPHYTES (VLG)
igae, but its coexpression with WRKY40 or WRKY60 made plants more susceptible to b
NDING PROTEIN 38 (WRKY38)

ible to both *P. syringae* and *B. cinerea*. WRKY18, WRKY40, and WRKY60 have partially
susceptible to Pi stress as indicated by the higher accumulation of anthocyanin during
3 PROTEIN 8 (ATWRKY8)

virus, spermine and during senescence. The gene product is localized to the chloroplast

resistance and induction of SAR genes. protein_coding (ATHMAD1) (ATHMAD1

1. Plant P450 CYP707A3, ABA 8'-hydroxylase, binds enantioselectively (+)-ABA but not

(ATACS6);1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE 6 (AC

ESIS-RELATED 3 (PR-3); (B-CHI);BASIC CHITINASE (HCHIB)
;SENESCENCE ASSOCIATED GENE 14 (SAG14);BLUE COPPER BINDING PROTEIN

ses, and hormones. protein_coding BTB AND TAZ DOMAIN PROTEIN 2 (bt2) BTB

CARBON/NITROGEN INSENSITIVE 1 (CNI1);ARABIDOPSIS TOXICOS EN LEVADUR

IA (CAF1a);CCR4- ASSOCIATED FACTOR 1A (AtCAF1a)

14 INNER MEMBRANE 1 (COR413IM1);COLD REGULATED 414 THYLAKOID MEMBRANE
t COR15a alters the intrinsic curvature of the inner membrane of chloroplast envelope. I
; ACID RESPONSIVE 2 (JR2);CORONATINE INDUCED 1 (CORI3)

MINUM INDUCED 1 (EARLI1); (pEARLI 1)
;AQR) SENESCENCE-ASSOCIATED AND QQS-RELATED (SAQR)
(ERF104) ETHYLENE RESPONSE FACTOR 104 (ERF104)
8 (EXPA8); (ATHEXP ALPHA 1.11);EXPANSIN A8 (ATEXPA8)
coding FLAVIN-DEPENDENT MONOOXYGENASE 1 (FMO1) FLAVIN-DEPENDENT MONOOXYGENASE
CED RECEPTOR-LIKE KINASE (SIRK);FLG22-INDUCED RECEPTOR-LIKE KINASE
s. protein_coding GALACTINOL SYNTHASE 2 (GolS2) GALACTINOL SYNTHASE 2 (

(GPT2);ARABIDOPSIS GLUCOSE-6-PHOSPHATE/PHOSPHATE TRANSLOCATOR 2
U 11 (ATGSTU11)
;STU4);GLUTATHIONE S-TRANSFERASE 22 (GST22)
e (Pi) and not phosphite (Phi) in roots and shoots. protein_coding GLYCEROL-3-PH

ILITY 1 (IOS1) IMPAIRED OOMYCETE SUSCEPTIBILITY 1 (IOS1)

atine. Application of jasmonate methyl ester to Arabidopsis roots reduces the levels of a

TI78); (LTI140);RESPONSIVE TO DESICCATION 29A (RD29A)
) MYB DOMAIN PROTEIN 77 (MYB77)
J N-ACETYLTRANSFERASE ACTIVITY 1 (NATA1) N-ACETYLTRANSFERASE ACTIVATED BY ABA, ACC AND NAA TREATMENT, ALTHOUGH IN THE LATTER TWO CASES, THE INDUCTION OCCURS IN THE NUCLEUS
OMAIN CONTAINING PROTEIN 19 (ANAC019)
LING PROTEIN 47 (NAC047)

DOMAIN CONTAINING PROTEIN 29 (ANAC029);NAC-LIKE, ACTIVATED BY AP3/PI (I

B15);PHYTOALEXIN DEFICIENT 3 (PAD3)

RECURSOR (PSK4) PHYTOSULFOKINE 4 PRECURSOR (ATPSK4);PHYTOSULF
response to changes in calcium levels. protein_coding PINOID-BINDING PROTEIN
data suggest that PNP-A may function as a component of plant defence response and S
s gene exhibits a small but reproducible increase in freeze tolerance. Because of the rep
d to the nucleus, acts as a transcriptional repressor and is responsive to chitin oligomers

oding SENESCENCE 1 (SEN1) SENESCENCE 1 (SEN1);DARK INDUCIBLE 1 (DIN

oth *P. syringae* and *B. cinerea*. WRKY18, WRKY40, and WRKY60 have partially redun

/ redundant roles in response to the hemibiotrophic bacterial pathogen *Pseudomonas sy*
Pi starvation. protein_coding WRKY DNA-BINDING PROTEIN 75 (WRKY75) AR/

: The mRNA is cell-to-cell mobile. protein_coding YELLOW-LEAF-SPECIFIC GENE 9

l)

(-)-ABA, whereas the enzyme binds both enantiomers of AHI1 (a structural ABA analog)

OS6)

V (BCB)

AND TAZ DOMAIN PROTEIN 2 (bt2); (ATBT2)

3A 31 (ATL31)

3ANE 1 (COR414-TM1);COLD REGULATED 314 THYLAKOID MEMBRANE 1 (COR413
Late Embryogenesis abundant protein (LEA). Protects chloroplast membranes during fre

MONOOXYGENASE 1 (FMO1)

1 (FRK1)

[AtGoIS2);GALACTINOL SYNTHASE 2 (GoIS2)

! (ATGPT2)

OSPHATE PERMEASE 1 (G3Pp1) PHOSPHATE STARVATION-INDUCED GENE 3 (A'

JAZ1:GUS fusion protein, presumably by stimulating ubiquitin-proteasome-mediated d

'ITY 1 (NATA1)

ccurred relatively late when compared with NaCl or ABA treatments. Note: this protein (A

VAP);NAC-LIKE, ACTIVATED BY AP3/PI (ATNAP)

OKINE 4 PRECURSOR (PSK4); (ATPSK3 (FORMER SYMBOL))

1 (PBP1) PINOID-BINDING PROTEIN 1 (PBP1)

AR in particular, and could be classified as a newly identified PR protein. It is stress resp
ression of the CBF genes by the overexpression of this gene, the authors speculate tha
. Also involved in response to photooxidative stress. protein_coding SALT TOLERANCE

1);ARABIDOPSIS THALIANA SENESCENCE 1 (ATSEN1);SENESCENCE ASSOCIATE

dant roles in response to the hemibiotrophic bacterial pathogen *Pseudomonas syringae*

/ringae and the necrotrophic fungal pathogen *Botrytis cinerea*, with WRKY18 playing a n
ABIDOPSIS THALIANA WRKY DNA-BINDING PROTEIN 75 (ATWRKY75);WRKY DNA-

) (YLS9) YELLOW-LEAF-SPECIFIC GENE 9 (YLS9);ARABIDOPSIS NDR1/HIN1-LIK

Je used as ABA 8'-hydroxylase competitive inhibitor). protein_coding "CYTOCHROME

3-TM1)

ezing. protein_coding COLD-REGULATED 15A (COR15A) COLD-REGULATED 15A

TPS3);PHOSPHATE STARVATION-INDUCED GENE 3 (PS3);GLYCEROL-3-PHOSPH.

egradation. The Jas domain appears to be important for JAZ1-COI1 interactions in the p

tNAC6) on occasion has also been referred to as AtNAC2, not to be confused with the A

responsive and can enhance its own expression. protein_coding PLANT NATRIURETIC
It is thought that this gene may be involved in negative regulatory circuit of the CBF pathway. The mRNA
is ZINC FINGER (STZ) (ZAT10);SALT TOLERANCE ZINC FINGER (STZ)

SEN1 GENE 1 (SEN1)

and the necrotrophic fungal pathogen *Botrytis cinerea*, with WRKY18 playing a more im

portant role than the other two. The mRNA is cell-to-cell mobile. protein_coding
WRKY-BINDING PROTEIN 75 (WRKY75)

ATNHL10 (ATNHL10);NDR1/HIN1-LIKE (NHL10)

AtCOR15A (COR15A); (ATCOR15A); (COR15)

GLYCEROL-3-PHOSPHATE PERMEASE 1 (AtG3Pp1); GLYCEROL-3-PHOSPHATE PERMEASE 1 (G3Pp1)

presence of coronatine. Two positive residues (R205 and R206) in the Jas domain show

AtNAC2 found at locus AT3G15510. protein_coding NAC DOMAIN CONTAINING PRC

PEPTIDE A (PNP-A) EXPANSIN-LIKE B3 PRECURSOR (EXLB3);PLANT NATRIUR
A is cell-to-cell mobile. protein_coding RESPONSIVE TO HIGH LIGHT 41 (RHL41)

portant role than the other two. The mRNA is cell-to-cell mobile. protein_coding WRKY
WRKY DNA-BINDING PROTEIN 40 (WRKY40) (ATWRKY40);WRKY DNA-BINDING

1 to be important for coronatine -dependent COI1 binding are not required for binding AtI

ROTEIN 6 (NAC6) NAC DOMAIN CONTAINING PROTEIN 2 (ATNAC2);ORESARA 1 (OF

ETIC PEPTIDE A (PNP-A); (AtPNP-A)
(ZAT12); (ATZAT12);RESPONSIVE TO HIGH LIGHT 41 (RHL41)

7 DNA-BINDING PROTEIN 18 (WRKY18) ARABIDOPSIS THALIANA WRKY DNA-BIN
3 PROTEIN 40 (WRKY40)

MYC2. The mRNA is cell-to-cell mobile. protein_coding JASMONATE-ZIM-DOMAIN

RE1);NAC DOMAIN CONTAINING PROTEIN 6 (NAC6);NAC DOMAIN CONTAINING P

WDING PROTEIN 18 (ATWRKY18);WRKY DNA-BINDING PROTEIN 18 (WRKY18)

J PROTEIN 1 (JAZ1) JASMONATE-ZIM-DOMAIN PROTEIN 1 (JAZ1); (ATJAZ1); (TIF

ROTEIN 6 (ATNAC6);NAC DOMAIN CONTAINING PROTEIN 2 (NAC2);ARABIDOPSI

Y10A)

S NAC DOMAIN CONTAINING PROTEIN 92 (ANAC092)