The *Arabidopsis thaliana* LysM-containing Receptor-Like Kinase 2 is required for elicitor-induced resistance to pathogens

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Funding information
DFG, ERA-CAPS, Grant/Award Number: SIPIS; MIUR, ERA-CAPS; PRIN 2017, Grant/Award Number: 2017ZBBYNC; Progetti di Ricerca 2015, Grant/Award Number: C26A15CFKE; Progetti di Ricerca 2019, Grant/Award Number: RM11916B6F156C03

Abstract
In *Arabidopsis thaliana*, perception of chitin from fungal cell walls is mediated by three LysM-containing Receptor-Like Kinases (LYKs): CERK1, which is absolutely required for chitin perception, and LYK4 and LYK5, which act redundantly. The role in plant innate immunity of a fourth LYK protein, LYK2, is currently not known. Here we show that CERK1, LYK2 and LYK5 are dispensable for basal susceptibility to *B. cinerea* but are necessary for chitin-induced resistance to this pathogen. LYK2 is dispensable for chitin perception and early signalling events, though it contributes to callose deposition induced by this elicitor. Notably, LYK2 is also necessary for enhanced resistance to *B. cinerea* and *Pseudomonas syringae* induced by flagellin and for elicitor-induced priming of defence gene expression during fungal infection. Consistently, overexpression of LYK2 enhances resistance to *B. cinerea* and *P. syringae* and results in increased expression of defence-related genes during fungal infection. LYK2 appears to be required to establish a primed state in plants exposed to biotic elicitors, ensuring a robust resistance to subsequent pathogen infections.

KEYWORDS
*Botrytis cinerea*, chitin, plant innate immunity, priming

1 | INTRODUCTION

The first line of defence that plants use to fend off pathogen attacks relies on pattern recognition receptors (PRRs) on the cell surface that recognize danger signals, called elicitors, that include microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs) and damage-associated molecular patterns (DAMPs) (Boller & Felix, 2009; Gust, Pruitt, & Nürnberger, 2017). MAMPs are molecules common to all strains of a given taxonomic group of pathogens, such as flagellin, a structural protein of bacterial flagella, and chitin, the major component of fungal cell walls consisting of a polymer of N-acetylglucosamine (GlcNAc) (Boller & Felix, 2009). DAMPs are host-derived molecules produced during infection (De Lorenzo, Ferrari, Cervone, & Okun, 2018), and include, among others, pectin-derived oligogalacturonides (OGs) (Ferrari et al., 2013), Arabidopsis elicitor peptides (AtPeps) (Bartels et al., 2013) and extracellular ATP (Tanaka, Choi, Cao, & Stacey, 2014). PRR activation triggers, within minutes, very rapid downstream responses, including a transient influx of calcium ions, activation of calcium-dependent protein kinases, production of reactive oxygen species (ROS), and phosphorylation of mitogen-activated protein kinases (MAPKs) (Boudsocq et al., 2010; Kadota et al., 2014; Li et al., 2014; Nuhe, Peck, Hirt, & Boller, 2000).
These early signalling events are followed by a more delayed induction of responses, including the expression of defence-related genes, the biosynthesis of antimicrobial compounds and the deposition of callose in the cell wall, resulting in a so-called pattern-triggered immunity (PTI) effective against a broad range of pathogens (Boller & Felix, 2009; Macho & Zipfel, 2014; Tang, Wang, & Zhou, 2017).

Well-characterized plant PRRs are FLAGELLIN SENSITIVE 2 (FLS2) and EF-Tu RECEPTOR (EFR), that recognize flg22, the elicitor-active epitope of flagellin, and elf18, the epitope of bacterial Elongation Factor-Thermo-unstable (EF-Tu), respectively (Chinchilla, Bauer, Regenass, Boller, & Felix, 2006; Zipfel et al., 2006). Both FLS2 and EFR are transmembrane receptor-like kinases (RLKs) with leucine-rich repeat (LRR) motifs in their extracellular domain. After ligand perception, FLS2 and EFR interact with another LRR-RLK, BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), which induces downstream responses (Chinchilla et al., 2007). Another important class of PRRs are RLKs containing three Lysin motifs (LysMs) in their ectodomain (LysM-RLKs, or LYKs) (Buendia, Girardin, Wang, Cottret, & Lefebvre, 2018). GPI-anchored LysM-containing receptor-like proteins (LYPs) lacking an intracellular kinase domain are also found in plants (Arrighi et al., 2006). Most LYKs and LYPs that have been studied perceive structurally related GlicNAc-containing molecules and are involved in plant-microbe interactions (Buendia et al., 2018). The first identified LYKs were the Nod Factor Receptor 1 (LjnFR1) and 5 (LjnFR5) of Lotus japonicus (Limpens et al., 2003; Radutoiu et al., 2007), and MtLYK3 and MtLYK4 of Medicago truncatula (Limpens et al., 2003), that act as receptors of Nod factors, lipochitooligosaccharides (LCOs) produced by nitrogen-fixing rhizobia. While some LYKs, like LjnFR1 and MtLYK3, have a canonical RD kinase and show in vitro autophosphorylation, others carry an aberrant kinase domain lacking some conserved features and do not exhibit either auto-phosphorylation or trans-phosphorylation activities in vitro, indicating that they require one or more co-receptors to exert their function (Arrighi et al., 2006; Klaus-Heisen et al., 2011; Madsen et al., 2011).

Following the identification of LYKs involved in LCO perception, OsCEBiP, a LYP lacking an intracellular kinase domain, was found to be the rice receptor for chitin (Kaku et al., 2006). OsCEBiP acts in cooperation with the LYK co-receptor OsCERK1, that is essential for chitin signalling in rice (Shimizu et al., 2010). The Arabidopsis genome contains five predicted LYKs: AtCERK1/LysM-RLK1 (henceforth, CERK1) and AtLYK2 to AtLYK5 (henceforth, LYK2 to LYK5), of which only CERK1 and LYK3 are predicted to possess a functional kinase domain (Tanaka, Nguyen, Liang, Cao, & Stacey, 2012). Recognition of chito-oligosaccharides (COs) in Arabidopsis is mediated by CERK1, which is absolutely required for chitin perception (Miya et al., 2007; Wan et al., 2008), and by LYK4 and LYK5 (Cao et al., 2014). Direct binding of COs was demonstrated for CERK1 (Iizasa, Mitsutomi, & Nagano, 2010) and LYK5 (Cao, Liang, et al., 2014). LYK5 shows a higher affinity to COS than CERK1 (Cao, Liang, et al., 2014) and, after chitin recognition, interacts with CERK1, which homodimerizes (Liu et al., 2012) and autophosphorylates in a LYK5-dependent manner (Cao, Liang, et al., 2014). Kinase activity is necessary for CERK1 chitin-dependent phosphorylation and downstream signalling (Petutschnig, Jones, Serazetdinova, Lipka, & Lipka, 2010). Subsequent to autophosphorylation, CERK1 activates PBL27, a receptor-like cytosolic kinase that triggers downstream responses (Shinya et al., 2014; Yamada et al., 2016), whereas LYK5 is directed to endocytosis, supposedly to ensure proper receptor turnover (Erwig et al., 2017). LYK4 has a redundant role with LYK5, since lyk4 and lyk5 single mutants are still partially responsive to chitin, whereas a double mutant is entirely insensitive to this MAMP (Choi et al., 2014).

Chitin perception is thought to be necessary during fungal infection for proper activation of defences that restrict pathogen invasion, as suggested by genetic evidence. For instance, mutants with defects in CERK1, LYK4 or LYK5 show increased susceptibility to the fungal pathogen Alternaria brassicicola (Cao, Tanaka, Nguyen, & Stacey, 2014; Miya et al., 2007; Wan et al., 2008; Wan et al., 2012), and lack of CERK1 also enhances susceptibility to Glovymonics cichoracearum (Wan et al., 2008), Plectosphaera cucumerina (Mélida et al., 2018) and Fusarium oxysporum f. sp. cubense (Huaping, Xiaohui, Lunying, & Unsheng, 2016). In cotton, chitin induces the dimerization and phosphorylation of GhLYK5 and GhLYK1/GhCERK1, contributing to defence against V. dahlia and F. oxysporum f. sp. vasinfectum (Gu, Zavaliw, & Dong, 2017; Wang et al., 2020). Beside their importance in chitin signalling, LYK proteins appear to have additional roles in plant immunity. CERK1, together with two LYPs, LYM1 and LYM3, is required for bacterial peptidoglycan (PGN) perception and basal resistance to Pseudomonas syringae pv. tomato strain DC3000 (Pst DC3000) in Arabidopsis (Willmann et al., 2011). Moreover, CERK1 is involved in the perception of laminarinhexaose and β-D-cellobiosyl-(1,3)-β-D-glucose, two mixed-link glucans with elicitor activity (Mélida et al., 2018; Rebaque et al., 2021). The role of other LYK proteins is more elusive. In Arabidopsis, LYK3 acts as a negative regulator of basal immunity and a positive regulator of responses to the phytohormone abscisic acid (Paparella, Savatin, Marti, De Lorenzo, & Ferrari, 2014), whereas the function of LYK2 remains to be determined.

Most studies on the role and mode of action of PRRs in plant immunity have focused on responses occurring within minutes or hours upon elicitation (MAPK activation; oxidative burst; early gene expression). However, the establishment of a lasting status of enhanced resistance in response to treatments with MAMPs or DAMPs likely depends on long-term responses that are at least partially dependent on the accumulation of phytohormones, such as salicylic acid (SA), ethylene and jasmonates (Berens, Berry, Mine, Argueso, & Tsuda, 2017; Broekgaarden, Caarls, Vos, Pieterse, & Van Wees, 2015; De Vleesschauwer, Xu, & Höfte, 2014; Westernack, 2007). For instance, resistance against the necrotrophic fungus Botrytis cinerea induced in Arabidopsis by pre-treatments with flg22 or OGs requires the biosynthesis of camalexin (Ferrari et al., 2007) and an intact ethylene signalling pathway (Gravino, Savatin, Macone, & De Lorenzo, 2015).

Inducible defences are costly, and their improper activation might reduce plant fitness (Huo, Yao, Montgomery, & He, 2014). Plants have therefore evolved the ability to acquire a pre-conditioned state...
of defence after specific stimulation by microbial infections or environmental stresses. Exposure to MAMPs or DAMPs might increase the activation of defence responses upon subsequent perception of the same or a different elicitor, ensuring a robust resistance with a low fitness cost. However, the relative contribution of the perception and signalling mediated by specific PRRs in elicitor-induced resistance is not well understood. In this work, we have investigated the role of LYK proteins in Arabidopsis basal and elicitor-induced resistance to pathogens. Our results indicate that basal resistance to B. cinerea does not require an intact chitin perception system, but resistance to this pathogen induced by exogenous chitin is impaired by mutations in CERK1, LYK2 and LYK5. Notably, LYK2 is required for enhanced resistance to fungal infection observed after pre-treatments with different elicitors and contributes to basal and induced resistance to Pst DC3000. Our results suggest that LYK2 is largely dispensable for chitin perception and early signalling and has a more general role in the regulation of elicitor-induced priming of defence responses during pathogen infection.

2 | MATERIALS AND METHODS

2.1 | Plant material and growth conditions

Arabidopsis thaliana (L.) Heynh plants used in this work were all in the Columbia-0 (Col-0) background. Seeds of cerk1-2 (Miya et al., 2007) were a kind gift of Dr. Naoto Shibuya (Meiji University, Japan); lyk5-2 (Salk_131911C) seeds were kindly provided by Dr Elena Petutschng (Georg-August-University of Göttingen, Germany). The lyk2-1 (Salk_152226) and lyk2-2 (Salk_012441) lines were obtained from the Nottingham Arabidopsis Stock Centre (NASC) and brought to homozygosity before further characterization. For seedlings assays, Arabidopsis seeds were sterilized in 1.6% sodium hypochlorite and 0.01% sodium dodecyl sulphate (SDS) and stored at 4°C in the dark for 2 days. Then, seeds were evenly distributed on 12-well-plates containing 0.5x Murashige-Skoog (MS) basal salts, 0.5% sucrose, pH 5.6, and transferred to a growth chamber with 12 hr day/12 hr night cycle at 22°C with 50% relative humidity. For leaf assays, seeds were distributed in plates containing 0.5x MS basal salts, 0.5% sucrose, 0.7% plant agar, pH 5.6. After sowing, plates were stored at 4°C in the dark for 2 days, then transferred to a growth chamber with 12 hr day/12 hr night cycle at 22°C. Ten-day-old seedlings were transferred to soil and grown in the growth chamber under 12 hr day/12 hr night cycle at 22°C with 50% relative humidity. For co-immunoprecipitation (Co-IP) assays Nicotiana benthamiana plants were grown at 25°C, 16 hr light, 8 hr dark, 75% relative humidity, for 4–5 weeks. For bimolecular fluorescence complementation (BiFC), Agrobacterium tumefaciens (strain GV3101)-mediated transient expression was conducted on Nicotiana tabacum plants cv Petit Havana SR1 grown at 25°C, 16 hr day/8 hr night cycle, 75% relative humidity, for 4–5 weeks.

2.2 | Mutant genotyping

Genomic DNA was extracted from rosette leaves using the Edwards protocol (Edwards, Johnston, & Thompson, 1991), using the following extraction buffer: 200 mM Tris–HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS. DNA was subjected to PCR using the following primers: CCACATATTCCGAAGACAAAGC, LP12-15; GTT CTGCTTTGTAGTTGCC, RP12-15; GCTTGAATTGCACCTTTGTC, LP12-25; AAGTGTGGTGCTCTCACAGG, RP12-25; TGTTCCACGTAGTG GGCATCG, LBa1 (see Figure S1a). PCR products were resolved on 1.5% agarose gel and stained with SYBR™ Safe DNA Gel Stain (Invitrogen).

2.3 | Elicitors

OGs (DP 10–15) were obtained as previously described (Pontiggia et al., 2015). Colloidal chitin was obtained by thoroughly grinding shrimp shell chitin (Sigma Aldrich) with a pestle in a tube containing sterile milliQ water. Flg22 (QRLSTGSRINSAKDDAAGLQIA) was synthesized by EZBiolab (Carmel, IN, USA).

2.4 | Pathogen growth and infection

Botrytis cinerea (Ferrari, Plotnikova, De Lorenzo, & Ausubel, 2003) was grown for 10–15 days at 22°C on MEP medium [malt-agar 2% (w/v), peptone 1% (w/v) and micro-agar 1.5% (w/v)] until sporulation. Before plant inoculation, spores were suspended at a final concentration of 5 × 10^5 conidiospores ml^-1 in 24 g l^-1 potato dextrose broth (Difco, Detroit, USA) and incubated for 2–3 hr at room temperature (RT). Four-week-old Arabidopsis plants were inoculated placing 5 μl drops of the spore suspension on each side of the middle vein of fully expanded rosette leaves. Plants were covered with a clear plastic dome to ensure high humidity and incubated at 22°C with a 12 hr photoperiod. Lesion areas were determined 48 hr postinfection by measuring water-soaked lesions, using ImageJ software (https://imagej.nih.gov/ij/). For elicitor-induced protection, plants were sprayed with water, 200 μg ml^-1 OGs, 1 μM Flg22 or 100 μg ml^-1 colloidal chitin 24 hr before inoculation, as previously described (Ferrari et al., 2007).

Pst DC3000 was inoculated in LB liquid medium containing 25 μg ml^-1 rifampicin and grown under agitation (200 rpm) for 8–12 hr at 28°C, until OD600 = 0.6–1.0. Fully expanded rosette leaves of four-week-old plants were syringe-infiltrated with a suspension of bacteria [1 × 10^7 colony-forming units (cfu) ml^-1] as previously described (Katagiri, Thilmony, & He, 2002). Leaf discs were collected right after inoculation, 2 and 3 days after infiltration, and ground in water to collect bacteria. For each sample, a 1:10 dilution series was plated on solid LB medium containing rifampicin, and colonies were counted after incubation at 28°C for approximately 3 days.
2.5 | Determination of MAPK phosphorylation

Seedlings (about 100 mg) were homogenized in 100 μl extraction buffer [50 mM Tris–HCl pH 7.5, 200 mM NaCl, 1 mM EDTA, 10 mM NaF, 2 mM sodium orthovanadate, 1 mM sodium molybdate, 10% (v/v) glycerol, 0.1% (v/v) Tween-20, 1 mM 1,4-dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, phosphatase inhibitor cocktail P9599 (Sigma, MO)]. Total protein extracts were quantified with Bradford assay (Bio-Rad). Equal amounts of proteins were separated on 8% polyacrylamide (30% acrylamide/Bis solution, 29:1, Bio-Rad) SDS gel. Proteins were transferred to a nitrocellulose membrane using TransBlot Turbo (Bio-Rad). The filter was stained for 10 min with Ponceau-S Red (Sigma Aldrich) to assess equal loading and then blocked with 5% (w/v) bovine serum albumin (BSA, Sigma Aldrich) in Tris-Buffered Saline containing 0.1% (v/v) Tween-20 (TBS-T; Bio-Rad) for 2 hr at RT. Membranes were then incubated overnight in TBS-T containing 0.5% (w/v) BSA and primary antibodies against phospho-p44/p42 (1:2500) (Cell Signalling Technologies) or MPK6 (1:10000) (Sigma-Aldrich). Membranes were then incubated with horseradish peroxidase-conjugated anti-rabbit antibody (GE-Healthcare) diluted at 1:6000 (w/v) BSA. Signal detection was performed using Clarity™ Western ECL substrate detection kit (Bio-Rad) and a ChemiDoc MP imaging system (Bio-Rad). As controls, primary antibodies against actin (Sigma) were used.

2.6 | Oxidative burst assays

Hydrogen peroxide production was measured by a luminol-based assay as previously described (Galletti, Ferrari, & De Lorenzo, 2011). Leaf discs (0.2 cm²) from four-week-old plants were washed for 2 hr with water and incubated overnight in a 96-well plate (one disc per well). Water was then replaced with a solution of luminol (Sigma-Aldrich; 30 mg ml⁻¹) and horseradish peroxidase (Sigma-Aldrich; 20 mg ml⁻¹) containing 100 nM flg22. For chitin elicitation (100 μg ml⁻¹), discs were vacuum infiltrated with the chitin solution for 2 min before the addition of the luminol/peroxidase solution. Plates were analysed for 40 min using a GloMax 96 microplate luminometer (Promega) and a signal integration time of 1 s. Luminescence was expressed in relative light units (RLUs).

2.7 | Callose deposition assays

Rosette leaves of four-week-old plants were syringe-infiltrated with chitin (100 μg ml⁻¹), flg22 (100 nM) or water as control. After 24 hr, 10 leaves from at least four independent plants for each treatment were cleared and dehydrated with 100% (v/v) boiling ethanol. Leaves were fixed in an acetic acid: ethanol (1:3) solution for 2 hr, sequentially incubated for 15 min in 75% (v/v) ethanol, 15 min in 50% (v/v) ethanol, 15 min in 150 mM phosphate buffer pH 8.0, and then stained in 150 mM phosphate buffer pH 8.0, containing 0.01% (w/v) aniline blue for 16 hr at 4°C. After staining, leaves were mounted in 50% (v/v) glycerol and examined by a UV epifluorescence microscope (Nikon, Eclipse E200) equipped with a cooled charge-coupled device camera (DS-FI1C). Images were acquired with the Nis Elements AR software (Nikon). Fluorescence intensity in each image was calculated using ImageJ (https://imagej.nih.gov/ij/).

2.8 | Gene expression analysis

Total RNA was extracted using RNA isolation NucleoZol (Macherey-Nagel) according to the manufacturer’s instructions and treated with Turbo-DNase I (Ambion). cDNA was synthesized with ImProm-II™ Reverse Transcription System (Promega). qRT-PCR was performed with a CFX96 Real-Time PCR System (BioRad) using SYBR Green Real-Time PCR Master Mix (Promega) as recommended by the manufacturer. The amplification protocol consisted of 30 s of initial denaturation at 95°C, followed by 45 cycles of 95°C for 15 s, 58°C for 15 s and 72°C for 15 s. Melting curves were recorded to verify single product amplification. For each experiment, dilution series of pooled cDNA samples were run under the same conditions to calculate primer efficiencies. Gene expression levels were normalized to UBIQUITIN 5 (UBQ5, At4G05320). Three technical replicates were performed for each sample, and data analysis was done, with minor modifications, as previously described (Redwan et al., 2016) using LinRegPCR software (Ruijter et al., 2013). Sequences for all primers used for quantitative PCR and identifiers of the corresponding genes are listed in Table S1. Analysis of the expression of LYK genes from publicly available microarray data was performed using Genevestigator (Hruz et al., 2008).

2.9 | Generation of constructs and transgenic plants

The Red Fluorescent Protein (RFP) coding sequence was amplified by PCR from the pSAT6-mRFP-N1 plasmid (Invitrogen) with a high-fidelity DNA polymerase (Roche), using the following primers: ATCGAGAGCTCTTAAATCGGCTACGACGTCGACGGTACCG (RFP-FW) and ATCGAGAGCTCTTAGCAATGTAGGACCA (LYK2gFw) and GATGAGTTTAGGGCCATGAGGCA (LYK2gRev). The PCR product was cloned into the pGEM T-Easy Vector (Promega) and used to transform E. coli DH10B cells by electroporation. The obtained plasmid (pBl-RFP) was purified and used for the generation of the LYK2-RFP construct. The full-length LYK2 coding sequence was amplified by PCR with a high-fidelity DNA polymerase (Roche) from Col-0 genomic DNA, using the following primers: CATCAGAGATCGCGGCTGTACGACGTCGACGGTACCG (RFP-FW) and ATCGAGAGCTCTTGATGAGGCA (LYK2gRev). The PCR product was cloned into the pGEM T-Easy vector (Invitrogen) and used to transform E. coli One Shot® Omni-MAX™ 2 T1R (Invitrogen) cells by electroporation. The obtained plasmid (pGEM-LYK2) was purified and digested with KnpI and Smal and...
the insert was cloned in frame upstream the RFP coding sequence in the pBI-RFP plasmid previously digested with the same enzymes and dephosphorylated. To generate constructs for the overexpression of the untagged version of LYK2, the insert of pGEM-LYK2 was ligated with pBII121, previously digested with Knpl and Smal. The obtained plasmids (pBII121-LYK2-RFP and pBII121-LYK2) were introduced into E. coli One Shot® OmniMAX™ 2 T1R (Invitrogen) and transformed bacteria were selected on LB agar medium containing 50 μg ml⁻¹ kanamycin. To generate LYK2-GFP, the full-length coding sequence of LYK2 was cloned into the pDONR-Zeo plasmid by BP cloning using a Gateway-based system (Invitrogen). The obtained plasmid was then used for LR cloning with the destination plasmid pGWBS5 (Nakagawa et al., 2007). For generation of 35S:CERK1-GFP, 35S:CERK1-myc and 35S:LYK5-myc constructs for CoIP experiments, the full-length coding sequences of LYK5 and CERK1 were cloned into the pDONR201 plasmid (Invitrogen) using the following primers: for LYK5, ggggacaagtttgtacaaaaaagcaggcttcATGGCTGCGTGTACACTCCAGGGTGTTCCAAGAGAGCCGGAA and ggggaccacttttgcatactggaACAGGTTGAAGCAGGGTGTTCCAAGAGAGCCGGAA CGAAGA; for CERK1, ggggacaagtttgtacaaaaaagcaggcttcaCTAGTACACTCCAGGGTGTTCCAAGAGAGCCGGAA CGAAGA.

To investigate the role of LYK2 in Arabidopsis immunity, two homozygous insertional lines were obtained: lyk2-1 (SALK_152226), carrying a predicted T-DNA insertion in the second exon, corresponding to the extracellular domain of LYK2, and lyk2-2 (SALK_012441), with a predicted insertion at the very beginning of the first exon (Figure S1a, b). Untreated seedlings of both mutants showed significantly decreased levels of LYK2 transcripts, compared to the wild type (Figure S1c). In addition, we generated two independent homozygous lines (3SS:LKY2 line 1.1 and 5.15) overexpressing LYK2 (Figure S1d).

2.11 | Co-immunoprecipitation assays

A. tumefaciens was grown overnight in LB medium containing the appropriate antibiotics, collected by centrifugation, and then suspended in 10 mM MgCl₂ containing 100 mM acetosyringone. After incubation at RT for at least 2 hr, the cultures were diluted to an OD₆₀₀ = 0.5. Leaves of four-week-old N. benthamiana plants were agroinfiltrated using a needleless syringe and plants were returned to the greenhouse for 72 hr. Samples from agroinfiltrated leaves were lysed in a buffer containing 50 mM Tris (PH 7.6), 150 mM NaCl, 0.5% Triton X-100 and protease inhibitor cocktail P9599 (Sigma, MO). Extracts were centrifuged at 14,000 g for 15 min at 4°C. Anti-Myc or anti-GFP traps (Chromotek) were used for co-immunoprecipitation experiments according to the manufacturer’s instructions. Immunoblot analysis was performed as previously described (Willmann et al., 2011), using anti-myc or anti-GFP antibodies (Sigma-Aldrich) at a dilution of 1:3000.

3 | RESULTS

3.1 | LYK2 is required for resistance to Botrytis cinerea induced by different elicitors

To investigate the role of LYK2 in Arabidopsis immunity, two homozygous inserional lines were obtained: lyk2-1 (SALK_152226), carrying a predicted T-DNA insertion in the second exon, corresponding to the extracellular domain of LYK2, and lyk2-2 (SALK_012441), with a predicted insertion at the very beginning of the first exon (Figure S1a, b). Untreated seedlings of both mutants showed significantly decreased levels of LYK2 transcripts, compared to the wild type (Figure S1c). In addition, we generated two independent homozygous lines (3SS:LKY2 line 1.1 and 5.15) overexpressing LYK2 (Figure S1d).
Both lines accumulated high levels of LYK2 transcripts in the absence of any treatment (Figure S1d).

Available microarray data indicate that basal LYK2 transcript levels are quite low in seedlings and leaves of WT plants, compared to CERK1, LYK3 and LYK5, are similar to those of LYK4 (Figure S2a). We examined the expression of LYK2 in WT and mutant seedlings treated with different elicitors. Compared to water-treated seedlings, LYK2 transcripts in the wild type increased two- to four-fold after 1 hr of treatment with OGs, flg22 or chitin (Figure S2b). In contrast, expression of LYK2 in both lyk2 mutants did not significantly differ after elicitation (Figure 2b). These results indicate that lyk2-1 and lyk2-2 have impaired basal and elicitor-triggered expression of LYK2. Basal expression of CERK1 and LYK5 was comparable in WT and lyk2 seedlings; transcripts for both genes slightly increased to a similar extent after 1 h of elicitation with flg22 or chitin, though expression levels were quite variable (Figure S2c,d). Notably, compared to the WT, lyk2 mutants accumulated greater transcript levels of both CERK1 and LYK5 after 3 h of treatment with chitin, but not with flg22 (Figure S2c,d), suggesting that reduced expression of LYK2 might trigger, in response to this MAMP, a compensatory response resulting in the enhanced expression of other LYK genes.

To investigate the role of Arabidopsis LYKs in basal and chitin-induced resistance to fungal infection, we evaluated the severity of symptoms caused by B. cinerea in lyk2-1 and lyk2-2, as well as in cerk1-2 and lyk5-2, which carry loss-of-function mutations in CERK1 and LYK5, respectively, and are impaired in chitin perception (Cao, Liang, et al., 2014; Miya et al., 2007; Wan et al., 2008). Adult rosettes were sprayed with water or chitin and, after 24 hr, fully expanded leaves were inoculated with a B. cinerea spore suspension. Disease symptoms were evaluated 48 hr postinfection (hpi). None of the mutants showed increased susceptibility to B. cinerea after the water pre-treatment (Figure 1a and Figure S3a,b). Pre-treatments with chitin led to significantly smaller lesions in the wild type, but not in any of the mutants (Figure 1a and Figure S3a), indicating that induction of Botrytis resistance by chitin requires not only CERK1 and LYK5, but also LYK2. To test if these LYKs are also required for resistance induced by other MAMPs and DAMPs, WT and mutant plants were pre-treated with flg22 or OGs, that can induce resistance against B. cinerea in Arabidopsis (Ferrari et al., 2007). Both elicitors significantly increased resistance in WT, cerk1-2 and lyk5-2 plants, but not in lyk2-1 and lyk2-2 (Figure 1b and Figure S3b). Taken together, these results suggest that CERK1, LYK2 and LYK5 are all required for chitin-induced resistance, but only LYK2 is also necessary for resistance induced by the non-chitin elicitors flg22 and OGs.

To test if elevated LYK2 expression levels have an impact on resistance to B. cinerea, transgenic 35S:LYK2 line 1.1 and 5.15 adult plants were inoculated with the fungus. Both lines displayed significantly reduced lesions after B. cinerea infection (Figure 1c), indicating that high levels of expression of LYK2 increase resistance to this pathogen.

3.2 | LYK2 is dispensable for early chitin perception and signalling but is required for chitin-induced callose deposition

It was previously reported that lyk2 mutants do not show defects in chitin-induced production of ROS (Cao, Liang, et al., 2014) and
expression of WRKY53 and MPK3 (Wan et al., 2008). It is however possible that LYK2 regulates specific subsets of responses to this MAMP. After 5 and 10 min of treatment with two different doses of chitin (10 and 25 μg ml⁻¹), phosphorylation of MPK3, MPK4 and MPK6, one of the earliest responses to this elicitor (Pitzschke & Hirt, 2009; Ramonell et al., 2005), was similar in WT, lyk2-1 and lyk2-2 seedlings (Figure 2a-b). ROS production in response to chitin and flg22 was comparable in WT, lyk2-1 and lyk2-2 seedlings (Figure 2c-f). Expression of two elicitor-responsive marker genes, FLG22-INDUCED RECEPTOR-LIKE KINASE 1 (FRK1) and PHYTOALEXIN DEFICIENT 3 (PAD3) (Asai et al., 2002; Ferrari et al., 2007; Zhou, Tootle, & Glazebrook, 1999) in response to chitin was also unaffected in lyk2-1 and lyk2-2 seedlings, compared to the wild type (Figure 2g-h). We also analysed if a later response to chitin, namely callose deposition, requires LYK2. Adult rosette leaves were infiltrated with water, chitin (100 μg ml⁻¹) or flg22 (100 nM) and the presence of callose was revealed by aniline blue staining. Leaves of both lyk2 mutants, in comparison to WT plants, showed a significant reduction of the intensity and number of callose deposits in response to chitin infiltration, but not in response to flg22.

**FIGURE 2** Early chitin-induced responses are unaffected in lyk2 mutants. (a–b) 10-day-old WT, lyk2-1 and lyk2-2 seedlings were treated for 5 (a) and 10 min (b) with water (W) or chitin at the concentration of 10 μg ml⁻¹ (C10) or 25 μg ml⁻¹ (C25). Phosphorylated MPK3, MPK4, MPK6 and MPK11 were detected by immunoblot using an anti-P44/P42 antibody. Total MPK3 and MPK6 were detected by immunoblot using an anti-MPK3 and anti-MPK6 antibodies. Arrows indicate the molecular weight (in kDa) of the marker bands. Equal loading was evaluated by Ponceau-S Red staining and using an anti-Actin antibody. (c–f) Leaf discs of four-week-old WT, lyk2-1 and lyk2-2 plants were treated for the indicated times with 100 μg ml⁻¹ chitin (c,d) or 100 nM flg22 (e,f). H₂O₂ production was measured with a luminol-based assay and expressed in relative light units (RLU s⁻¹). Data points represent the average of at least 12 discs ± SE. Bars in (d) and (f) represent the average of total H₂O₂ production ± SE. Differences between total RLUs in WT and lyk2-1 or lyk2-2 were not significant (ns), according to Student’s t-test (p > .05). (g, h) FRK1 (g) and PAD3 (h) expression in WT and lyk2 seedlings treated with water or chitin (5 and 25 μg ml⁻¹) for 1 (g) and 3 h (h) was analysed by qRT-PCR using UBQ5 as control. Data are means ± SE (n = 3 biological replicates). Asterisks indicate statistically significant differences according to Student’s t-test (***, p < .001) These experiments were repeated three times with similar results [Colour figure can be viewed at wileyonlinelibrary.com]
Chitin-induced callose deposition is reduced in lyk2 mutants. (a,b) Rosette leaves of four-week-old plants of WT or lyk2-1 and lyk2-2 lines were infiltrated with water, chitin (100 µg ml⁻¹) or flg22 (100 nM) and stained with aniline blue 24 hr after infiltration. (a) Representative images for each treatment. Scale bars = 100 nm. (b) Callose deposits were quantified as fluorescence intensity per unit of the infiltrated leaf surface. Values represent means ± SE of six different leaf samples from at least five independent plants (four microscopic fields of 0.1 mm² for each leaf). Asterisks indicate statistically significant differences between mutant lines and WT according to Student’s t-test (***, p < .001). This experiment was repeated twice with similar results [Colour figure can be viewed at wileyonlinelibrary.com]

(Figure 3a,b). These data suggest that LYK2 does not contribute to the initial perception of chitin and has a minor, if any, role in early signalling events that lead to MAPK activation, oxidative burst, and early gene expression, but is necessary for full activation of callose deposition, a late response to this elicitor. Moreover, lyk2 mutants are normally responsive to flg22, though LYK2 is required for flg22-induced resistance to fungal infection.

3.3 | LYK2 is required for enhanced defence responses to chitin or fungal infection in plants pre-treated with elicitors and contributes to resistance to Pst DC3000

Basal expression of LYK2 is very low but increases in seedlings treated with different MAMPs and DAMPs (Figure S2b). To evaluate the impact of elicitor treatments on the expression of LYK genes during subsequent pathogen infection, adult Arabidopsis rosette leaves were sprayed with water, OGs or flg22 and, after 24 hr, inoculated with B. cinerea. In control-treated plants, B. cinerea infection caused a significant increase of transcripts of CERK1 at 8 hpi and of LYK2 and LYK5 at 24 hpi (Figure S4a–c). Notably, plants pre-treated with elicitors showed increased expression of these three genes even before fungal inoculation (Figure S4a–c). Moreover, B. cinerea-induced up-regulation of all three genes was faster and more robust in plants pre-treated with flg22 or OGs (Figure S4a–c). These results suggest that induction of LYK2 expression after elicitation might help plants respond more efficiently to chitin. To investigate this hypothesis, WT and lyk2 seedlings were pre-treated with water or flg22 and, after 24 h, elicited with chitin for 10 or 20 min. The pre-treatment with flg22 did not per se result in phosphorylation of MPK3, MPK4/MPK11 and MPK6 (Figure 4a,b and Figure S5). Chitin-induced MAPK phosphorylation in water-pre-treated seedlings was comparable in WT and lyk2-2 seedlings at both 10 and 20 min after elicitation (Figure 4a,b) and was slightly reduced in lyk2-1 after 20 min of elicitation (Figure 4b and Figure S5). After flg22 pre-treatment, chitin-induced phosphorylation at 10 min was comparable in all genotypes (Figure 4a and Figure S5a) but was strongly reduced at 20 min in lyk2-1 and lyk2-2 seedlings, compared to the wild type (Figure 4b and Figure S5b). As expected, chitin failed to induce any detectable activation of MAPKs in cerk1-2, even after pre-treatment with flg22 (-Figure S5a,b). These results confirm that LYK2 is dispensable for initial MAPK phosphorylation triggered by chitin treatment but suggest that, in plants previously exposed to an elicitor, MAPKs are dephosphorylated more rapidly if LYK2 is not functional.

Pre-treatments of WT plants with flg22 significantly enhanced chitin-induced expression of FRK1 and PAD3 (Figure 5); this enhanced expression was significantly impaired in lyk2-1 and lyk2-2 plants (Figure 5). In the absence of pre-treatments with flg22, lyk5-2 and cerk1-2 did not show induction of PAD3 and FRK1 in response to chitin, as expected; however, expression of both marker genes was induced by chitin in flg22-pre-treated lyk5-2 seedlings, though to a lesser extent than in the wild type, whereas cerk1-2 was almost completely unresponsive to chitin, even after previous exposure to flg22 (Figure 5). Taken together, the results described above confirm that CERK1 and LYK5, but not LYK2, are required for basal responsiveness to chitin in plants and that CERK1 is absolutely required for chitin responsiveness, and suggest that Arabidopsis, upon flg22 elicitation, acquires the ability to partially respond to chitin also in the absence of a functional LYK5. Moreover, these data indicate that LYK2 is necessary to enhance responses downstream chitin perception in plants pre-treated with flg22.

To further investigate the role of LYK2 in elicitor-induced resistance, the expression of two defence-related genes, PAD3 and PR-1,
FIGURE 5  Priming of chitin-induced gene expression in lyk mutants. 10-day-old WT, lyk2-1, lyk2-2, lyk5-2 and cerk1-2 seedlings were pre-treated with water or 10 nM flg22 for 24 h and subsequently treated with water or chitin (25 μg ml⁻¹) for 1 h. FRK1 and PAD3 expression was measured by qRT-PCR and normalized using UBQ5. (a) Bars represent mean expression ± SE (n = 3 biological replicates), asterisks indicate statistically significant differences between WT and mutants, according to Student’s t-test (*, p < .05; **, p < .01; ***, p < .001). (b) Expression of FRK1 and PAD3, relative to WT seedlings pre-treated with water and then treated with water, of the same samples as in (a). Bars represent mean fold-change ± SE (n = 3 biological replicates). Different letters indicate statistically significant differences, according to one-way ANOVA followed by Tukey’s HSD test (p < .01). This experiment was repeated three times with similar results.
was evaluated in WT and mutant plants treated with water or flg22 and subsequently inoculated with B. cinerea. Camalexin production, catalysed by the cytochrome P450 CYP71B15 encoded by PAD3 (Schuhegger et al., 2006), is necessary for elicitor-induced resistance to B. cinerea (Ferrari et al., 2007) and is primed by elicitor treatments (Gravino et al., 2015; Savatin et al., 2015). PR-1 is a well-known marker for SA-dependent responses (Delaney et al., 1994; Gaffney et al., 1993), which are also important for basal resistance to B. cinerea (Ferrari et al., 2003) and for long-lasting systemic acquired resistance to several pathogens (Cao, Liang, et al., 2014; Delaney et al., 1994; Gaffney et al., 1993). After 24 hr of infection, in water-pre-treated plants, a higher expression of PAD3 in lyk2 mutants, and of PR-1 in lyk2-1 and cerk1-2 mutants was observed, compared to the wild type, (Figure 6). In the wild type pre-treated with flg22 or OGs, expression of PAD3 and PR-1 was induced by the fungus to a greater extent than in control plants, indicating a priming effect of the elicitors (Figure 6 and Table S2), with a stronger effect observed for flg22 compared to OGs. The priming effect of the elicitors on fungal-induced PAD3 expression was also observed in cerk1-2 and lyk5 plants, though transcript levels in these mutants reached lower levels than in the WT (Figure 6 and Table S2). In infected lyk2 mutants, transcript levels of PAD3 after elicitor pre-treatments were even lower than in water-pre-treated plants, possibly because of the already high levels in the control, and were however comparable to those observed in lyk5 and cerk1-2 (Figure 6 and Table S2). Elicitor-primed expression of PR-1 during B. cinerea infection was observed in all tested genotypes but was strongly reduced in lyk2-1 and lyk2-2 plants, whereas it was comparable to the WT in lyk5 and cerk1-2 (Figure 6 and Table S2). These data indicate that LYK2, CERK1 and LYK5 are all required for full priming of PAD3 expression upon infection after elicitor pre-treatments, which is likely dependent on an increased ability of the plant to respond more efficiently to chitin, whereas priming of PR-1 expression is dependent on LYK2 but largely independent of chitin perception.

We subsequently tested if elevated LYK2 expression levels affect responses to chitin treatment or pathogen infection. Expression of PAD3 in response to low doses of chitin was only moderately enhanced in transgenic seedlings overexpressing LYK2 (Figure 7a). Consistently, MAPK activation in response to chitin was comparable in WT and 35S:LYK2 plants (Figure 7b). Notably, expression of both PAD3 and PR-1 during B. cinerea infection was significantly increased in both water- and elicitor-pre-treated 3S:LYK2 plants (Figure 7c-d). Moreover, PR-1 expression was enhanced in transgenic plants even before fungal inoculation, regardless of whether they were pre-treated with water or elicitors (Figure 7d). These data suggest that LYK2 overexpression does not affect chitin perception or early signalling, but it can increase basal (in the case of PR-1) and fungal-induced (both PR-1 and PAD3) defence gene expression.

Since lack of CERK1 or LYK5 increases susceptibility to Pst DC3000 (Cao, Liang, et al., 2014; Gimenez-Ibanez, Ntoukakis, & Rathjen, 2009), we tested whether altered levels of LYK2 also affect...
Overexpression of means (± SE, n = 35S:LYK2 line 1.1 and line 15.5 seedlings were treated with water or infection. (a) B. cinerea determined by qRT-PCR. (b) Four-week-old WT and 35S:LYK2 Line 1.1 plants were sprayed with water, 200 μg ml⁻¹ OG or 1 μM Flg22 and inoculated after 24 hr with a B. cinerea spore suspension. PAD3 (c) and PR1 (d) expression, at the indicated times, was measured by qRT-PCR and normalized using UBQ5. Data are means (± SE, n = 3 biological replicates). Asterisks indicate statistically significant differences between WT and 35S:LYK2 line 1.1 plants, according to Student’s t-test (*, p < .05; **, p < .01; ***, p < .001). The results are representative of three (a, c, and d) or two (b) independent experiments.

3.4 | LYK2 localizes in the plasma membrane and constitutively interacts with LYK5

We next investigated the subcellular localization of LYK2 and its possible interaction with other LYKs. To this aim, we generated constructs for the expression of red fluorescent protein (RFP)- and green fluorescent protein (GFP)-tagged versions of the protein (35S:LYK2-RFP and 35S:LYK2-GFP constructs, respectively). Stable overexpression of LYK2-RFP in lyk2-1 plants resulted in high basal resistance to B. cinerea (Figure 5a,b), as also observed in WT plants overexpressing the untagged LYK2 (Figure 1c), indicating that the tagged protein is functional. Confocal laser scanning microscopy (CLSM) of cotyledon epidermal cells of transgenic seedlings revealed a strong fluorescent signal at the plasma membrane (PM), as confirmed by plasmolysis experiments (Figure 7a). A similar pattern of localization could be observed in plants overexpressing LYK2-GFP (Figure 5b), supporting the conclusion that LYK2 is localized in the PM. In addition, a diffuse fluorescence could occasionally be detected in the cytosol of the transgenic plants (Figure 5a,b), which might be possibly ascribed to either leakage of the protein or cleavage of the fluorescent tag.

Since LYK5 and CERK1 reside in the PM and physically interact upon chitin elicitation to induce an immune response (Cao, Liang, et al., 2014), we hypothesized that LYK2 might associate with one or both proteins. For this purpose, co-immunoprecipitation (Co-IP) assays were performed in N. benthamiana leaves transiently co-expressing LYK2-GFP and a myc-tagged version of either LYK5 or CERK1 (LYK5-myc and CERK1-myc, respectively). Beside the expected 110 kDa band corresponding to the full-length protein, a 1 OG or 1 b band of about 70 kDa, reactive to the anti-GFP antibody, was detectable in the cytosol of the transgenic plants (Figure 5a,b), which might be possibly ascribed to either leakage of the protein or cleavage of the fluorescent tag.

To corroborate the hypothesis that LYK2 interacts with LYK5, in vivo bimolecular fluorescence complementation (BiFC) experiments...
were conducted in *N. tabacum* leaves transiently co-expressing LYK2 and LYK5 tagged with either the N-terminal or the C-terminal half of the yellow fluorescent protein (NYFP and CYFP, respectively). As shown in Figure 9c, co-transformation with 35S:LYK2-NYFP and 35S:LYK5-CYFP resulted in a YFP fluorescence signal, likely at the PM. As a positive control, homodimerization of LYK5 was confirmed by BiFC (Figure 9c). In contrast, no YFP fluorescence signal could be detected when LYK2-NYFP and LYK2-CYFP were co-expressed (Figure 9c), suggesting that LYK2 does not homodimerize under these conditions and that the signal observed when LYK2-NFP and LYK5-CFP were co-expressed was not due to non-specific YFP reconstitution.

**DISCUSSION**

In this work, we have investigated the role of LYK proteins and of chitin perception in Arabidopsis basal and elicitor-induced resistance to pathogens. Genetic evidence indicates that the contribution of chitin perception to resistance to fungi varies with different pathogens; for instance, *cerk1* mutants show increased susceptibility to *A. brassicicola*.
and G. cichoracearum, but not to Colletotrichum higginsianum (Miya et al., 2007; Wan et al., 2008). Here we have shown that mutants for CERK1 or LYK5 do not display enhanced susceptibility to B. cinerea. This is not unexpected, since this pathogen can release other MAMPs (Poinssot et al., 2003; Yang, Yu, Liang, Anderson, & Cao, 2018; Zhang, Qiu, Zeng, Guo, & Yang, 2015) and DAMPs (An et al., 2005; Voxeur et al., 2019), whose recognition is probably sufficient to confer a WT-like degree of resistance also in the absence of chitin recognition. On the other hand, activation of a strong immune response occurs when plants are exposed to purified chitin, effectively protecting against subsequent Botrytis infections (Aziz et al., 2006). We found that, in Arabidopsis, this protection requires an intact chitin perception system, since it is abolished in cerk1-2 and lyk5-2 mutants. Unexpectedly, also lyk2 mutants were impaired in chitin-induced resistance, prompting us to hypothesize that LYK2 might play a role in chitin perception and/or signalling, despite previous work failed to reveal a defect in chitin responses in mutants for this gene (Cao, Liang, et al., 2014; Wan et al., 2012). Indeed, we found that chitin-induced MAPK phosphorylation, ROS production and early marker gene expression are largely unaffected in lyk2 plants, whereas callose deposition, detected 24 hr after chitin treatment, is reduced in the mutants. These results suggest that LYK2 is not important for chitin perception and early signalling under basal conditions, though it might contribute to ensure proper induction of long-term defence responses triggered by this MAMP.

The minor role of LYK2 in early chitin responses might be due to its low basal expression levels, since lyk2 mutations impair only responses to this MAMP, like callose deposition and enhanced resistance to fungal infection, that are observed several hours after chitin treatment, when LYK2 transcripts have significantly increased above basal levels. In addition, lack of LYK2 results in greater transcript levels of CERK1 and LYK5 at 3 hr after chitin treatment, suggesting the existence of some compensatory mechanism that might partially mask a possible role of this gene in chitin signalling. We therefore hypothesized that increased LYK2 expression after elicitation might increase the ability of the plant to respond to chitin, either exogenously provided or released during fungal infection. This hypothesis was initially corroborated by the observation that gene expression in response to chitin elicitation after flg22 pre-treatment is enhanced only in WT plants, but not in plants lacking LYK2. Since the extracellular domain of LYK2 cannot be pulled down by chitin beads when overexpressed in protoplasts, in contrast to that of CERK1, LYK4 and LYK5 (Cao, Liang, et al., 2014), it is unlikely that LYK2 might directly bind chitin. Moreover, overexpression of LYK2 did not per se increase MAPK activation and only slightly increased gene expression induced by chitin, suggesting that this protein likely modulates responses downstream of, or independently of the early signalling events triggered by the initial activation of the chitin perception complex. On the other hand, CoIP and BiFC experiments suggest that LYK2 might physically interact with LYK5, hinting to a possible function of LYK2 in modulating the activity of the chitin perception complex. Interestingly, LYK4 also constitutively heterodimerizes with LYK5, and chitin treatments induce the formation of a tripartite complex comprising CERK1, LYK5 and LYK4 (Xue, Li, Xie, & Staehelin, 2019). LYK2, like LYK4, might act as a scaffold protein for LYK5, contributing, in primed plants, to increase the extent or duration of some responses downstream of the activation of the chitin perception complex. This hypothesis is suggested by the more rapid dephosphorylation of MAPKs in lyk2 mutants pre-treated with fig22. However, since lyk2 mutants, but not lyk5-2 or cerk1-2, are not protected against B. cinerea after pre-treatments with OG or flg22 and do not show priming of PR-1 expression during fungal infection, LYK2 appears to mediate elicitor-induced resistance also independently of chitin perception.

LYK2-mediated induction of resistance against fungal infection seems to involve the regulation of multiple defence responses. We have previously observed that exogenous OGs or flg22 induce a transient increase of PAD3 transcript levels, that return to basal levels within 12 hr of treatment (Denoux et al., 2008; Ferrari et al., 2007). However, camalexin accumulates to higher levels during B. cinerea infection when plants are pre-treated with elicitors (Gravino et al., 2015), suggesting that a previous elicitation primes plants to produce this phytoalexin more rapidly upon pathogen attack. Indeed, we observed elicitor-mediated priming of PAD3 expression in response to fungal infection, which requires an intact chitin perception complex, beside LYK2, since it is also reduced in mutants lacking LYK5 or CERK1. Therefore, in plants pre-treated with MAMPs or DAMPs and subsequently attacked by a fungus, enhanced activation of responses triggered by chitin (or other elicitors) released during infection might increase PAD3 expression and camalexin accumulation, enhancing resistance. We have previously observed that the Arabidopsis bak1-5 mutant, which is strongly impaired in flg22-induced responses (Roux et al., 2011; Schwessinger et al., 2011), is also compromised in both basal and flg22-induced resistance to B. cinerea (Gravino et al., 2017). Notably, BAK1 is required for responses to different MAMPs, including flg22, PGN and lipopolysaccharides, but not to chitin (Shan et al., 2008), confirming that chitin perception per se is not necessary for basal resistance to the fungus. It was subsequently reported that co-inoculation of B. cinerea with flg22 results in increased resistance to the fungus, and that this resistance depends on the BAK1-mediated phosphorylation of the juxtamembrane domain of CERK1, which in turn increases sensitivity to chitin (Gong et al., 2019). Flg22-induced priming of PAD3 expression requires both LYK2 and an intact chitin perception system, comprising CERK1 and LYK5. The lack of evidence for a role of LYK2 in direct chitin perception, and the observation that LYK2, but not CERK1 or LYK5, is required for enhanced resistance to B. cinerea and for priming of PR-1 expression when plants are pre-treated with elicitors 24 hr before inoculation indicate that LYK2 regulates, upon elicitation, a long-lasting ability of the plant to prime some defence responses, beside PAD3 expression, independently of chitin perception. Interestingly, mutations in LYK2 reduce basal resistance to Pst DC3000, similar to what observed for cerk1-2, but, in contrast to the latter, also strongly impair flg22-induced resistance against this pathogen. This is in agreement with the hypothesis that LYK2 might positively regulate long-
term defence responses downstream of recognition of different elicitors, though it cannot be ruled out that it might directly participate to PGN perception/signalling, as previously demonstrated for CERK1 and OsCERK1 (Ao et al., 2014; Buist, Steen, Kok, & Kuipers, 2008; Gust, Willmann, Desaki, Grabherr, & Nurnberger, 2012; Willmann et al., 2011). The PM-associated Ca2+ binding protein PCaP1 was recently shown to mediate OG- and fig22-induced resistance to B. cinerea and to be required for elicitor-triggered priming of defence response during infection (Giovannoni et al., 2021). Further investigation is however required to determine whether LYK2 and PCaP1 act in the same pathway.

Modulation of responses triggered by MAMPs and DAMPs is crucial to ensure proper protection against invading pathogens without excessive cost in terms of growth. In this context, LYK2 appears to be a key regulator of priming of defence responses, ensuring that plants display enhanced resistance after pre-exposure to an elicitor. Previous work suggests that LYK3, another Arabidopsis LYK, exerts an opposite role, negatively regulating defence responses, as lack of a functional protein causes constitutive expression of defence responses, including PAD3 expression, and increased resistance to B. cinerea (Paparella et al., 2014). The negative role of LYK3 in immunity might be mediated by the phytohormone abscisic acid (ABA), as this protein is required for both ABA-mediated repression of elicitor responses and for some physiological responses to this hormone (Paparella et al., 2014). Interestingly, loss of LYK3 also results in increased sensitivity to salt stress, suggesting that suppression of defence responses might contribute to properly counteract abiotic stresses (Paparella et al., 2014). Increasing evidence indeed suggests that CERK1 might regulate responses to abiotic stresses, as cerk1 mutants also show increased sensitivity to salt stress (Espinoza, Liang, & Stacey, 2017), and overexpression of a fungal chitinase causes increased tolerance to salt in a CERK1-dependent manner (Brotman et al., 2012). These observations lead to speculate that LYK proteins might have a more general function in balancing responses against different stresses beyond their role in MAMP perception. The possible function of LYK2 and other LYKs in modulating responses to different stresses therefore deserves future investigation.

The ability to mount stronger defence responses after a previous exposure to a MAMP or DAMP might avoid overactivation of plant immunity, not only preventing growth-defence trade-offs that might be triggered by the numerous microorganisms present in the environment (Yu, Pieterse, Bakker, & Berendsen, 2019), but possibly favouring the interaction with beneficial microorganisms. Increasing evidence indicates that LYKs play multiple functions in plant immunity and symbiosis. For instance, OsCERK1 is required for mycorrhizal colonization (Miyata et al., 2014; Zhang et al., 2015), and its homologs in L. japonicus (LjLYS6) and M. truncatula (Mlyk9) also play a dual role in immunity and symbiosis (Bozoki et al., 2017; Gibelin-Viala et al., 2019). Notably, rhizobial LCOs inhibit fig22 responses in Arabidopsis in a LYK3-dependent manner (Liang et al., 2013), and M. truncatula mutants for MtLYK9 are less colonized by the arbuscular mycorrhiza Rhizobagus irregularis but are more susceptible to the oomycete Aphanomyces euteiches (Gibelin-Viala et al., 2019). Future work will help determine if LYK2 is involved in the fine-tuning of plant responses to different microorganisms.

In conclusion, our results indicate that LYK2 has a limited role in chitin perception, but it is necessary to ensure a robust and durable resistance to pathogens after elicitor pre-treatments, priming activation of defence responses downstream of and/or independently of chitin perception. Future studies will help elucidate the molecular mechanism of action of LYK2, providing novel clues about how plants modulate immunity. This knowledge might be important not only to improve crop resistance to microbial diseases, but also to increase our understanding of the complexity of the interactions between plants and microbes in the environment.

ACKNOWLEDGMENTS
We thank Naoto Shibuya (Meiji University, Japan) for providing cerk1-2 seeds and Heini M. Grabherr (University of Tuebingen) for assistance with CERK1-GFP and LYK5-GFP cloning. We are grateful to Volker Lipka and Elena Petutschg (University of Goettingen, Germany) for providing lyk5-2 seeds and for critical reading of the manuscript. This work was supported by the Italian Ministry of University and Research (MIUR, ERA-CAPS 2014 second call grant “SIPI: Decoding Ligand-receptor Specificities of LysM Proteins in Plant Immunity and Symbiosis” awarded to S.F. and PRIN 2017 grant 2017ZBBYNC awarded to G.D.L.), by Sapienza Università di Roma (Ricerche Universitarie 2015 grant n. C26A15CFKE and Progetti di Ricerca 2019 grant n. RM11916B6F156C03 awarded to S.F.) and by the German Research Foundation (DFG, ERA-CAPS program, SIPI grant, awarded to T.N.). Open access funding provided by Universita degli Studi di Roma La Sapienza within the CRUI-CARE Agreement.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION
Moira Giovannoni, Damiano Lironi, Chiara Paparella, Lucia Marti, Valeria Vecchi, Andrea A. Gust and Simone Ferrari designed and performed experiments and analysed data. Moira Giovannoni, Damiano Lironi and Simone Ferrari wrote the paper. Thorsten Nürnberger, Valeria Vecchi, Andrea A. Gust and Giulia De Lorenzo contributed to revise the final version of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT
All data generated or analysed during this study are included in this published article and its supplementary information files.

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REFERENCES

An, H. J., Lurie, S., Greve, L. C., Rosenquist, D., Kimiz, C., Labavitj, J. M., & Lebrilla, C. B. (2005). Determination of pathogen-related enzyme action by mass spectrometry analysis of pectin breakdown products of plant cell walls. Analytical Biochemistry, 338(1), 71–82. https://doi.org/10.1016/j.ab.2004.11.004

Ao, Y., Li, Z., Feng, D., Xiong, F., Liu, J., Li, J. F., ... Wang, H. B. (2014). OsCERK1 and OsRLCK176 play important roles in peptidoglycan and chitin signaling in rice innate immunity. The Plant Journal, 80(6), 1072–1084. https://doi.org/10.1111/tjp.12710

Arrighi, J., Barre, A., Ben Amor, B., Boursault, A., Soriano, L., Mirabelli, R., ... Gough, C. (2006). The Medicago truncatula lym [corrected] motif-receptor-like kinase gene family includes NFP and new nodule-expressed genes. Plant Physiology, 142(1), 265–279. https://doi.org/10.1104/pp.106.084657

Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W. L., Gomez-Gomez, L., ... Sheen, J. (2002). MAP kinase signaling cascade in Arabidopsis innate immunity. Nature, 415(6875), 977–983.

Aziz, A., Trotel-Aziz, P., Dhuicco, L., Jeandet, P., Couderchet, M., & Vernet, G. (2006). Chitosan oligomers and copper sulphate induce grapevine defense reactions and resistance to grey mold and downy mildew. Phytopathology, 96(11), 1188–1194. https://doi.org/10.1094/pyth-96-1184

Bartels, S., Lori, M., Mbengue, M., van Verk, M., Klauser, D., Hander, T., ... Boller, T. (2013). The family of peas and their precursors in Arabidopsis: Differential expression and localization but similar induction of pattern-triggered immune responses. Journal of Experimental Botany, 64(7), 5309–5321.

Berens, M. L., Berry, H. M., Mine, A., Argueso, C. T., & Tsuda, K. (2017). An, H. J., Lurie, S., Greve, L. C., Rosenquist, D., Kirmiz, C., ... Bozsoki, Z., Cheng, J., Feng, F., Gysel, K., Vinther, M., Andersen, K., ... Brizard, S., Gaffney, T., Friedrich, L., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., ... Ryals, J. (1994). A central role of salicylic acid in plant disease resistance. Science, 266, 1247–1250.

Denoux, C., Galletti, R., Mammarella, N., Gopalan, S., Werck, D., De Lorenzo, G., ... Dewdney, J. (2008). Activation of defense responses by pathogens and flagellin22 elicitors in Arabidopsis seedlings. Molecular Plant, 1(3), 425–445. https://doi.org/10.1038/mp.2008.35

Dewdney, J. (2007). Resistance to Botrytis cinerea induced in Arabidopsis by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires PHYTOALEXIN DEFICIENT3. Plant Physiology, 144(1), 367–379. https://doi.org/10.1104/pp.107.095596

Ferrari, S., Plotnikova, J. M., De Lorenzo, G., & Ausubel, F. M. (2003). Arabidopsis local resistance to Botrytis cinerea involves salicylic acid and camalexin and requires EDS4 and PAD2, but not SID2, EDS5 or PAD4. The Plant Journal, 35(2), 193–205. https://doi.org/10.1046/j.1365-313x.2003.01794.x

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., ... Ryals, J. (1993). Requirement of salicylic acid for the induction of systemic acquired resistance. Science, 261, 754–756.
Iizasa, E., Mitsutomi, M., & Nagano, Y. (2010). Direct binding of a plant Hruz, T., Laule, O., Szabo, G., Wessendorp, F., Bleuler, S., Oertle, L., Gust, A. A., Pruitt, R., & Nürnberger, T. (2017). Sensing danger: Key to activating plant immunity. Plant, Cell & Environment, 44, 3078–3093. https://doi.org/10.1111/pce.11418

Gong, B. Q., Guo, J., Zhang, N., Yao, X., Wang, H. B., & Li, J. F. (2019). Cross-microbial protection via priming a conserved immune co-receptor through Juxtmembrane phosphorylation in plants. Cell Host and Microbe, (6), 810-822.e187. https://doi.org/10.1016/j.chom.2019.10.010, 26, 810–822.e7

Gravino, M., Locci, F., Tundo, S., Cervone, F., Savatin, D. V., & De Lorenzo, G. (2017). Immune responses induced by oligoglacturonides are differentially affected by AvrPto and loss of BAK1/BKK1 and PEPR1/PEPR2. Molecular Plant Pathology, 18(4), 582–595. https://doi.org/10.1111/mpp.12419

Gravino, M., Savatin, D. V., Macone, A., & De Lorenzo, G. (2015). Ethylene production in Botrytis cinerea- and oligoglacturonide-induced immunity requires calcium-dependent protein kinases. The Plant Journal, 84(6), 1073–1086. https://doi.org/10.1111/tjp.13057

Gu, Y., Zavaleev, R., & Dong, X. (2017). Membrane trafficking in plant immunity. Molecular Plant, 10(8), 1026–1034. https://doi.org/10.1101/84017.2017.07.001

Gust, A. A., Pruitt, R., & Nürnberger, T. (2017). Sensing danger: Key to activating plant immunity. Trends in Plant Science, 22(9), 779–791. https://doi.org/10.1016/j.tplants.2017.07.005

Gust, A. A., Willmann, R., Desaki, Y., Grabherr, H. M., & Numbere, T. (2012). Plant LysM proteins: Modules mediating symbiosis and immunity. Trends in Plant Science, 17(8), 495–502. https://doi.org/10.1016/j.tplants.2012.04.003

Hruz, T., Laule, O., Szabo, G., Wessendorp, F., Bleuler, S., Oertle, L., Widmayer P., Gruissem W., Zimmermann, P. (2008). Genevestigator v3: A reference expression database for the meta-analysis of transcriptional advances in Bioinformatics, 2008, 420747. https://doi.org/10.1155/2008/420747, 5

Huaping, H., Xiaohui, J., Lunying, W., & Unsheng, H. (2016). Chitin elicitor receptor kinase 1 (CERK1) is required for the non-host defense response of Arabidopsis to fusarium oxysporum f. Sp. cubense. European Journal of Plant Pathology, 147(3), 571–578. https://doi.org/10.1007/s10658-016-1026-3

Huo, B., Yao, J., Montgomery, B. L., & He, S. Y. (2014). Growth-defense tradeoffs in plants: A balancing act to optimize fitness. Molecular Plant, 7(8), 1267–1287. https://doi.org/10.1093/mp/ssu049

Iizasa, E., Mitsutomi, M., & Nagano, Y. (2010). Direct binding of a plant LysM receptor-like kinase, LysM RLK1/CERK1, to chitin in vitro. The Journal of Biological Chemistry, 285(5), 2996–3004. https://doi.org/10.1074/jbc.M109.072540

Kadota, Y., Sklenar, J., Derbyshire, P., Stransfeld, L., Asai, S., Ntoukakis, V., Zipfel, C. (2014). Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. Molecular Cell, 54(1), 43–55. https://doi.org/10.1016/j.molcel.2014.02.021

Kaku, H., Nishizawa, Y., Ishii-Minami, N., Akimoto-Tomiyama, C., Dohmae, N., Takio, K., & Shibuya, N. (2006). Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proceedings of the National Academy of Sciences of the United States of America, 103(29), 11086–11091.

Katagiri, F., Thilmony, R., & He, S. Y. (2002). The Arabidopsis thaliana - pseudomonas syringae interaction. The Arabidopsis Book (Vol. 2002. 1st edn., pp. 1–35). Rockville, MD: American Society of Plant Biologists.

Klaus-Heisen, D., Nurissso, A., Piastrzewskia-Bogiel, A., Mbengeu, M., Camut, S., Timmers, T., … Cullmore, J. V. (2011). Structure-function similarities between a plant receptor-like kinase and the human interleukin-1 receptor-associated kinase-4. The Journal of Biological Chemistry, 286(13), 11202–11210. https://doi.org/10.1074/jbc.M110.186171

Li, L., Li, M., Yu, L., Zhou, Z., Liang, X., Liu, Z., … Zhou, J. M. (2014). The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RBOHd to control plant immunity. Cell Host and Microbe, 15(3), 329–338.

Liang, Y., Cao, Y., Tanaka, K., Thibivilliers, S., Wan, J., Choi, J., … Stacey, G. (2013). Nonlegumes respond to rhizobial nod factors by suppressing the innate immune response. Science, 341(6152), 1384–1387.

Limpens, E., Franken, C., Smit, P., Willemsen, J., Bisseling, T., & Geurts, R. (2003). LysM domain receptor kinases regulating rhizobial nod factor-induced infection. Science, 302(5654), 630–633.

Liu, T., Liu, Z., Song, C., Hu, Y., Han, Z., She, J., … Chai, J. (2012). Chitin-induced dimerization activates a plant immune receptor. Science, 336(6085), 1160–1164.

Macho, A. P., & Zipfel, C. (2014). Plant PRRs and the activation of innate immune signaling. Molecular Cell, 54(2), 262–273. https://doi.org/10.1016/j.molcel.2014.03.028

Madsen, E., Antolin-Llovera, M., Grossmann, C., Ye, J., Vlesweg, S., Broghammer, A., … Paniske, M. (2011). Autophosphorylation is essential for the in vivo function of the Lotus japonicus nod factor receptor 1 and receptor-mediated signalling in cooperation with nod factor receptor 5. The Plant Journal: For Cell and Molecular Biology, 65(3), 404–417. https://doi.org/10.1111/j.1365-313X.2010.04431.x

Mérida, H., Sopena-Torres, B., Bacete, L., Garrido-Andalia, M., Jorda, L., Lopez, G., … Molina, A. (2018). Non-branched beta-1,3-glucan oligosaccharides trigger immune responses in Arabidopsis. The Plant Journal, 93(1), 34–49. https://doi.org/10.1111/tjp.13755

Miyazawa, K., Igarashi, K., Ichimura, K., Shirasu, K., … Shibuya, N. (2007). CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proceedings of the National Academy of Sciences. United States of America, 104(49), 19613–19618.

Miyata, K., Kozuki, T., Kousai, Y., Otsuka, K., Ishii, K., Amaszu, E., … Nakagawa, T. (2014). The bifunctional plant receptor, OsCERK1, regulates both chitin-triggered immunity and arbuscular mycorrhizal symbiosis in rice. Plant & Cell Physiology, 55(11), 1864–1872. https://doi.org/10.1093/pcp/pct129

Nakagawa, T., Kurose, T., Hino, T., Tanaka, K., Kawamura, M., Niwa, Y., …Kimura, T. (2007). Development of series of gateway binary vectors, pGWBS, for realizing efficient construction of fusion genes for plant transformation. Journal of Bioscience and Bioengineering, 104(1), 34–41. https://doi.org/10.1263/jbbe.104.34

Nakagawa, Y., Katagiri, T., Shinozaki, K., Qi, Z., Tatsumi, H., Furuichi, T., … Iida, H. (2007). Arabidopsis plasma membrane protein crucial for Ca(2+) influx and touch sensing in roots. Proceedings of the National Academy of Sciences. United States of America, 104(9), 3639–3644.

Nuhse, T. S., Peck, S. C., Hirt, H., & Boller, T. (2000). Microbial elicitors induce activation and dual phosphorylation of the Arabidopsis thaliana MAPK 15. The Journal of Biological Chemistry, 275(11), 7521–7526.

Paparella, C., Savatin, D. V., Marti, L., De Lorenzo, G., & Ferrari, S. (2014). The Arabidopsis LYSIN MOTIF-CONTAINING RECEPTOR-LIKE KINASE3 regulates the cross talk between immunity and abscisic acid responses. Plant Physiology, 165(1), 262–276.
Petutschnig, E., Jones, A., Serazetdinova, L., Lipka, U., & Lipka, V. (2010). The lysin motif receptor-like kinase (LysM-RLK) CERK1 is a major chitin-binding protein in Arabidopsis thaliana and subject to chitin-induced phosphorylation. The Journal of Biological Chemistry, 285(37), 28902–28911. https://doi.org/10.1074/jbc.M110.116657

Pitzschke, A., & Hirt, H. (2009). Disentangling the complexity of mitogen-activated protein kinases and reactive oxygen species signaling. Plant Physiology, 149(2), 606–615.

Poinso, B., Vandelle, E., Beneteau, M., Adrian, M., Levis, C., Brygo, Y., ... Poinssot, B., Vandelle, E., Bentejac, M., Adrian, M., Levis, C., Brygoo, Y., ... Shimizu, T., Nakano, T., Takamizawa, D., Desaki, Y., Ishii-Minami, N., Nishizawa, Y., ... Shibuya, N. (2010). Two LysM receptor molecules, CEBIP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. The Plant Journal, 64(2), 204–214.

Shinya, T., Yamaguchi, K., Desaki, Y., Yamada, K., Narisawa, T., Kobayashi, Y., ... Shibuya, N. (2014). Selective regulation of the chitin-induced defense response by the Arabidopsis receptor-like cytoplasmic kinase PBL27. The Plant Journal, 79(1), 56–66. https://doi.org/10.1111/tjp.12535

Tanaka, K., Choi, J., Cao, Y., & Stacey, G. (2014). Extracellular ATP acts as a damage-associated molecular pattern (DAMP) signal in plants. Frontiers in Plant Science, 5, 446.

Tanaka, K., Nguyen, C.T., Liang, Y., Cao, Y., & Stacey, G. (2013). Role of LysM receptors in chitin-triggered plant innate immunity. Plant Signaling & Behavior, 8(1), e22598. https://doi.org/10.4161/psb.22598

Tang, D., Wang, G., & Zhou, J. M. (2017). Receptor kinases in plant-pathogen interactions: More than pattern recognition. Plant Cell, 29(4), 618–637.

Voipue, A., Habrylo, O., Guenin, S., Mlart, F., Soulie, M. C., Riouyou, C., ... Vernhettes, S. (2019). Oligogalacturonide production upon Arabidopsis thaliana-Botrytis cinerea cinerea interaction. Proceedings of the National Academy of Sciences of the United States of America, 116(39), 19743–19752. https://doi.org/10.1073/pnas.1900317116

Walter, M., Chaban, C., Schutze, K., Batistic, O., Weckermann, K., Nake, C., ... Kudla, J. (2004). Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. The Plant Journal, 40(3), 428–439.

Wan, J., Tanaka, K., Zhang, X. C., Son, G. H., Brechenmacher, L., Nguyen, T. H., & Stacey, G. (2012). LYK4, a lysin motif receptor-like kinase, is important for chitin signaling and plant innate immunity in Arabidopsis. Plant Physiology, 160(1), 396–406.

Wan, J., Zhang, X. C., Neece, D., Ramonell, K. M., Clough, S., Kim, S. Y., ... Stacey, G. (2008). A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis. Plant Cell, 20(2), 471–481. https://doi.org/10.100107.105.056754

Wang, P., Zhou, L., Jamieson, P., Zhang, L., Zhao, Z., Babilonia, K., ... Shan, L. (2020). The cotton all-associated kinase GHWAK7A mediates responses to fungal wilt pathogens by complexing with the chitin sensing receptors. The Plant Cell, 32(12), 3978–4001. https://doi.org/10.1105/tpc.19.00950

Wasternack, C. (2007). Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. Annals of Botany, 100(4), 681–697.

Willmann, R., Lajunen, H. M., Erbs, G., Newman, M. A., Kolb, D., Tsuda, K., ... Stacey, G. (2012). LYK4, a LysM receptor-like kinase, is important for chitin signaling and plant innate immunity in Arabidopsis. Plant Physiology, 160(1), 396–406.

Xue, D., Li, C., Xie, Z., & Staehelin, C. (2019). LYK4 is a component of a tripartite chitin receptor complex in Arabidopsis thaliana. Journal of Experimental Botany, 70(19), 5507–5516. https://doi.org/10.1093/jxb/erz213

Yamada, K., Yamaguchi, K., Shirakawa, T., Nakagami, H., Mine, A., Ishikawa, K., ... Kawasaki, T. (2016). The Arabidopsis CERK1-associated kinase PBL27 connects chitin perception to MAPK activation. The EMBO Journal, 35(22), 2468–2483. https://doi.org/10.15252/embj.201694248

Yang, Y., Yu, Y., Liang, Y., Anderson, C. T., & Cao, J. (2018). A profusion of molecular scissors for pectins: Classification, expression, and functions of plant polygalacturonases. Frontiers in Plant Science, 9, 1208. https://doi.org/10.3389/fpls.2018.01208

Yu, K., Pieterse, C., Bakker, P., & Berendse, R. (2019). Beneficial microbes going underground of root immunity. Plant, Cell & Environment, 42(10), 2860–2870. https://doi.org/10.1111/pce.13632

Zhang, X., Dong, W., Sun, J., Feng, F., Deng, Y., He, Z., ... Wang, E. (2015). The receptor kinase CERK1 has dual functions in symbiosis and...
immunity signalling. The Plant Journal: For Cell and Molecular Biology, 81(2), 258–267. https://doi.org/10.1111/tpj.12723
Zhang, Y., Qiu, D., Zeng, H., Guo, L., & Yang, X. (2015). BcGs1, a glycoprotein from Botrytis cinerea, elicits defence response and improves disease resistance in host plants. Biochemical and Biophysical Research Communications, 457(4), 627–634. https://doi.org/10.1016/j.bbrc.2015.01.038
Zhou, N., Tootle, T. L., & Glazebrook, J. (1999). Arabidopsis PAD3, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. Plant Cell, 11(12), 2419–2428.
Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D. G., Boller, T., & Felix, G. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell, 125(4), 749–760. https://doi.org/10.1016/j.cell.2006.03.037

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How to cite this article: Giovannoni, M., Lironi, D., Marti, L., Paparella, C., Vecchi, V., Gust, A. A., De Lorenzo, G., Nürnberg, T., & Ferrari, S. (2021). The Arabidopsis thaliana LysM-containing Receptor-Like Kinase 2 is required for elicitor-induced resistance to pathogens. Plant, Cell & Environment, 44, 3775–3792. https://doi.org/10.1111/pce.14192