Short title: Characterisation of a novel CRK10 mutant

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Title: A point mutation in the kinase domain of CRK10 leads to xylem vessel collapse and activates defence responses

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One sentence summary: A mutated receptor-like kinase affects the xylem vasculature of roots and hypocotyls and induces constitutive defence responses to pathogens.

S. K. and M.C.M planned and designed the project. M.P. and M.C.M. performed most of the experiments. R.B. performed the kinase domain modelling. M.J.D. performed the protein mass spectrometry and analysis. E.C. provided the hormone data. A.K.M.W. assisted with the pathogen assays. D.P.S performed the RNA-Seq analysis. M.P, S.K., J.A.N. and M.C.M. wrote the manuscript.

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ABSTRACT

Cysteine-rich receptor-like kinases (CRKs) are a large family of plasma membrane-bound receptors ubiquitous in higher plants. They are transcriptionally regulated by a wide variety of environmental cues and stresses, however their precise biological roles remain largely unknown. Here we report a novel mutant isolated for the CYSTEINE-RICH RECEPTOR-LIKE KINASE 10 (CRK10) of *Arabidopsis thaliana* which harbours the substitution of alanine 397 by a threonine in the α C-helix of its kinase domain and which we registered as *crk10-A397T* in the community database. *In situ* phosphorylation assays with the His-tagged wild type (WT) and *crk10-A397T* versions of the CRK10 kinase domain revealed that both alleles are active kinases capable of auto-phosphorylation with the newly introduced threonine acting as an additional phosphorylation site in *crk10-A397T*. Phenotypically the mutant is a dwarf and the analysis of thin cross sections with light and transmission electron microscopy revealed that collapsed xylem vessels in roots and hypocotyls are very likely the cause for this reduction in stature. Transcriptomic analysis of WT and mutant hypocotyls revealed that predominantly biotic and abiotic stress-responsive genes are constitutively up-regulated in the mutant. Root-infection assays with the vascular

pathogen *Fusarium oxysporum* demonstrated that the *crk10-A397T* mutant has enhanced resistance to this pathogen compared to WT plants. Taken together our results suggest that *crk10-A397T* is a gain-of-function allele of *CRK10* and open up new avenues for the investigation of this elusive receptor-like kinase family.

INTRODUCTION

Plant growth and development is modulated by intrinsic growth regulators as well as by beneficial and adverse environmental cues. Factors regulating development and environmental and pathogenic signals are mostly recognised by receptor-like kinases (RLKs), membrane-localized receptors which perceive and transduce these signals inside plant cells to activate programmes directing appropriate responses. Similar to animal receptor tyrosine kinases (RTKs), these receptors consist of an extracellular domain which perceives specific ligands, a single pass transmembrane domain, and a cytoplasmic kinase domain which transduces the signal via phosphorylation of downstream target proteins in the cytoplasm in order to tailor a cellular response (Shiu & Bleecker, 2001; De Smet et al., 2009). Their highly variable extracellular domains are used to classify RLKs into subgroups, the largest of which (~200 genes in *Arabidopsis thaliana*) being characterised by leucine-rich repeats (LRR-RLKs) (Diévert & Clark, 2004). The well-studied brassinosteroid receptor BRASSINOSTEROID INSENSITIVE 1 (BRI1; AT4G39400) and the microbial pattern recognition receptors (PRR) FLAGELLIN SENSING 2 (FLS2; AT5G46330) and EF-TU RECEPTOR (EFR; AT5G20480) belong to this subgroup (Friedrichsen et al., 2000; Chinchilla et al., 2006; Zipfel et al., 2006).

Among the several subfamilies of RLKs found in plant genomes, the cysteine-rich receptor-like kinases (CRKs) is one of the largest with over 40 members in *Arabidopsis*. The signature motif for CRKs is the presence of, in most cases, two repeats of the DOMAIN OF UNKNOWN FUNCTION 26 (DUF26) in their extracellular domain, which contains three cysteine residues in the conserved configuration C-X8-C-X2-C (Chen, 2001). The functional significance of the DUF26 domain remains to be elucidated but was originally suggested to participate in ROS or redox sensing (Wrzaczek et al., 2010; Bourdais et al., 2015). However, more recent data obtained from the crystallographic analysis of the DUF26-containing ectodomain of the plasmodesmata localised proteins (PLDPs) PLDP5 and PLDP8 point

towards the involvement of the cysteine residues in forming disulfide bonds for the structural stabilisation of the protein, rather than redox sensing (Vaattovaara et al., 2019). The same study also revealed that the DUF26 domain shows strong structural similarity to fungal carbohydrate-binding lectins, which suggests that DUF26-containing proteins might constitute a group of carbohydrate-binding proteins in plants (Vaattovaara et al., 2019). That DUF26-containing proteins do interact with carbohydrates has been shown for the secreted antifungal protein from *Ginkgo biloba*, Ginkbilobin2 (Gnk2), which contains a single DUF26 domain and acts as a mannose binding lectin (Miyakawa et al., 2009; Miyakawa et al., 2014), and for the secreted antifungal proteins AFP1 and AFP2 from maize, which contain tandem DUF26 domains also binding mannose (Ma et al., 2018). Ligands for the CRKs, however, still remain to be discovered.

Despite the prominence of the CRKs among the RLKs, very little is known about their specific functions and the regulation of downstream signalling events. Efforts to assign functions to CRKs involved a comprehensive analysis of a collection of T-DNA knockout lines for 41 CRKs of *Arabidopsis*, which suggested a role for several members in the fine-tuning of stress adaptation and plant development. Most knockout lines, however, did not display obvious phenotypes, as is expected for a large gene family due to redundancy amongst its members (Bourdais et al., 2015). Studies in *Arabidopsis* also revealed that several CRKs are transcriptionally regulated by a wide variety of biotic and abiotic factors such as ozone, ultraviolet light (UV), reactive oxygen species (ROS), the hormone salicylic acid (SA) and elicitation with pathogen-derived molecules (Czernic et al., 1999; Du & Chen, 2000; Ohtake et al., 2000; Wrzaczek et al., 2010). Functionally, CRKs belong to the RD subclass of Ser/Thr kinases (Vaattovaara et al., 2019), which typically carry a conserved arginine (R) immediately preceding the invariant aspartate (D) in subdomain VI required for catalytic activity and are in most cases activated through auto-phosphorylation of the activation loop (Nolen et al., 2004). Although the ability to auto-phosphorylate as well as to phosphorylate substrates *in situ* has been demonstrated for CRK2 and CRK7 (Idänheimo et al., 2014; Kimura et al., 2020), for example, detailed studies on the *in vivo* and *in vitro* role of phosphorylation sites are still outstanding for CRKs.

Gain-of-function mutations of a kinase are a valuable tool to study specific effects on cells and organisms, as in these instances the signalling cascade is initiated spontaneously without requiring the presence of an extracellular ligand. Although much is known about the

structure–function properties of eukaryotic kinases, predicting which point mutations will lead to a spontaneous activation of the signalling cascade is not straightforward and mutations are generally fortuitously isolated from mutagenic screens. In this study we describe the characterisation of such a gain-of-function mutant, *crk10-A397T*, obtained for the cysteine-rich receptor-like kinase CRK10 (AT4G23180) of *Arabidopsis*. This mutant contains a single amino acid substitution from alanine to threonine in the α C-helix of the kinase domain of the protein, with the newly introduced Thr-397 acting as an additional *in situ* phosphorylation site. Defense responses are constitutively activated in the *crk10-A397T* mutant, as detected by transcriptome profiling, and resistance to a soil-borne vascular pathogen is significantly enhanced. In addition, we could link the dwarf phenotype of the mutant to the severe collapse of the xylem vessel elements which we showed to occur in the root and hypocotyl but, surprisingly, not in the shoot.

RESULTS

crk10-A397T is a semi-dominant mutant allele of CRK10

The *Arabidopsis thaliana* mutant characterised in this report was isolated in a forward genetic ethyl methanesulfonate (EMS) screen performed for an unrelated study. In brief, six rounds of backcrosses to the wild-type (WT) Col-0 parent were performed in order to clean the genetic background of the mutant before an in-depth characterisation. The homozygous mutant has a strong dwarf phenotype and observation of the segregating F2 progeny of the sixth backcross revealed the semi-dominant nature of the mutation, as WT, intermediate and dwarf phenotypes segregated according to a 1:2:1 ratio with heterozygous plants being clearly discernible (Supplemental Figure S1A; $\chi^2_2 = 2.36$, $p = 0.308$). In order to determine the underlying mutation responsible for the dwarf phenotype, whole genome sequencing was performed on bulk segregants derived from the sixth backcross. This returned a list of 15 candidate genes containing point mutations in coding regions. We noticed that a substitution of G>A in the 4th exon of the *CYSTEINE-RICH RECEPTOR-LIKE KINASE 10* (*CRK10*; AT4G23180) lead to the substitution of alanine 397 by a threonine in the kinase domain of CRK10 (Supplemental Figure S1B). As we considered this receptor-like kinase to be the most likely candidate among the 15 identified genes, we tested whether the dwarf phenotype could be rescued by constitutive expression of the WT cDNA sequence of *CRK10* under the

control of the 35S promoter. All T1 transformants showed a WT phenotype (Supplemental Figure S1C), suggesting that we had identified the correct gene. To further confirm that the mutation in *CRK10* causes the dwarf morphology, we recreated the G>A substitution by *in vitro* mutagenesis in the cDNA sequence of *CRK10*, and introduced this ORF into a *crk10* KO background (*crk10-2*, SAIL_427_E09, characterisation of KO lines to follow) under the control of the 1 kb genomic region containing the putative native promoter of *CRK10*. A total of 25% of the recovered transformants were dwarfs, establishing a direct link between the dwarf phenotype and the mutant allele (Supplemental Figure S1D). Subsequently, we will refer to this mutant as *crk10-A397T*.

The A397T substitution is localised in the α C-helix of the kinase domain of CRK10

According to the subdivision of eukaryotic kinase domains into 12 conserved subdomains (Hanks & Hunter, 1995), the A397T substitution is localised in subdomain III of the kinase domain of CRK10 (Figure 1A), which corresponds to the α C-Helix motif in the three-dimensional structure of the protein. Homology modelling to the active kinase domain of the Arabidopsis BRASSINOSTEROID INSENSITIVE 1 (BRI1) positions Thr-397 at the C-terminal end of the helix, with its side chain likely to be exposed on the surface of the protein (Figure 1B).

The cytoplasmic kinase domains of CRK10 and CRK10-A397T are active kinases

With CRKs being classified as Ser/Thr kinases, the substitution of Ala-397 by a threonine (A397T) in the CRK10 kinase domain could have introduced a potential additional phosphorylation site. We therefore wanted to determine whether WT and mutant CRK10 are enzymatically active kinases and if differences in their auto-phosphorylation pattern can be detected. We addressed this question by investigating the auto-phosphorylation activity of the cytoplasmic kinase domain of CRK10 *in situ* when expressed in *Escherichia coli* cells (Taylor et al., 2013). We purified the CRK10 WT cytoplasmic kinase domain as an N-terminal 6x His-tag fusion protein (His-CRK10kd^{WT}) from *E. coli* cells, as well as its “dead” kinase counterpart which harboured the substitution of the essential aspartic acid 473 with an asparagine residue (His-CRK10kd^{WT-D473N}). Following separation of the recombinant proteins

by SDS-PAGE and detection by anti-His immunoblotting, the dead kinase version His-CRK10kd^{WT-D473N} migrated at the predicted molecular weight (M_r) of 40 kDa, while the WT kinase version His-CRK10kd^{WT} showed an electrophoretic mobility shift to larger M_r , known to occur for phosphorylated proteins (Wegener & Jones, 1984) (Figure 1C). In order to determine whether the A397T substitution in the CRK10 kinase domain affects its auto-phosphorylation activity, we generated constructs in which Ala-397 was replaced by threonine through *in vitro* mutagenesis (His-CRK10kd^{A397T} and His-CRK10kd^{A397T-D473N}). Although the dead kinase version His-CRK10kd^{A397T-D473N} migrated at the same M_r as His-CRK10kd^{WT-D473N} on SDS-PAGE gels, the mobility shift of the His-CRK10kd^{A397T} was increased when compared to the one observed for His-CRK10kd^{WT} (Figure 1C), suggesting additional sites were phosphorylated in His-CRK10kd^{A397T}. In order to confirm that the electrophoretic mobility shift was due to the presence of phosphorylated residues, the purified recombinant proteins were treated with λ -phosphatase prior to separation by SDS-PAGE. Irrespective of the treatments, the dead kinase versions CRK10kd^{WT-D473N} and His-CRK10kd^{A397T-D473N} migrated at the predicted M_r as confirmed by SDS-PAGE and anti-His immunoblotting (Figure 1D). However, λ -phosphatase treatment of His-CRK10kd^{WT} and His-CRK10kd^{A397T} resulted in a clearly detectable shift to a lower M_r , consistent with the auto-phosphorylation of recombinant His-CRK10kd^{WT} and His-CRK10kd^{A397T} as being responsible for their electrophoretic mobility shift. Taken together, these results confirm that both His-CRK10kd^{WT} and His-CRK10kd^{A397T} are active kinases capable of auto-phosphorylation. Furthermore, the increased mobility shift of His-CRK10kd^{A397T} compared to His-CRK10kd^{WT} suggested the presence of additional phosphorylation sites in His-CRK10kd^{A397T}.

The kinase domain of CRK10 auto-phosphorylates highly conserved residues in the activation loop and Thr-397 is an additional phosphorylation site in His-CRK10kd^{A397T}

We next proceeded to identify which sites in the kinase domain of CRK10 are being phosphorylated by subjecting tryptic peptides of His-CRK10kd^{WT} and His-CRK10kd^{A397T} to analysis by liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS). The Mascot probability-based algorithm was used to confirm the peptides match to the CRK10 kinase domain sequence. Individual MS/MS spectra were inspected for confirmation of

phosphorylation sites, which led to the unambiguous identification of Thr-340, Tyr-363, Thr-507, Ser-508, Tyr-514, Thr-625, Ser-662 and Thr-664 as phosphosites in both His-CRK10kd^{WT} and His-CRK10kd^{A397T} proteins (Figure 1E). Interestingly, Thr-507, Ser-508, and Tyr-514 align to conserved phosphorylation sites in the activation loop of several RLKs, known to be essential for the activation of RD kinases (Supplemental Figure S2). Phosphorylated residues were also detected in the juxta-membrane region (Thr-340) as well as in the C-terminal tail of CRK10 (Thr-625, Ser-662, and Thr-664), which are predicted to act as regulatory sites for interaction with binding partners. In addition, the identification of two phosphorylated tyrosine residues (Tyr-363 and Tyr-514) classifies CRK10 as dual specificity kinase and constitutes the first instance in which such activity has been reported for a CRK. Interestingly, Thr-397 itself was identified as a phosphorylation site in the His-CRK10kd^{A397T} kinase domain *in situ* (Figure 1F), however, whether Thr-397 also acts as a phosphorylation site *in vivo* remains to be determined.

The *crk10-A397T* mutant is a dwarf

WT Col-0 and *crk10-A397T* plants were phenotypically characterised for the duration of one life cycle. Although germination rate and establishment of seedlings is accelerated in the mutant (Supplemental Figure S3), one week after sowing these differences are no longer apparent. No other obvious differences in growth were observed between WT and *crk10-A397T* seedlings until between weeks two and three, when leaf expansion becomes restricted in the *crk10-A397T* mutant as small, dark green leaves are formed, and a reduction of more than 70% in rosette size is observed at four weeks after sowing compared to the WT (Figure 2A-G; Supplemental Figure S4A). Despite the dwarf phenotype displayed during vegetative growth, the onset of flowering occurs simultaneously in *crk10-A397T* mutant and WT plants, although the shoot apical meristem is frequently aborted in the mutant (Supplemental Figure S4B), and the main inflorescence remains stunted (Figure 2H). At later stages, mutant plants develop numerous lateral inflorescences with smaller, stunted siliques filled with viable seeds which are in general larger than those of WT plants (Figure 2-IK).

The *crk10-A397T* mutant has collapsed xylem vessels in roots and hypocotyls

Dwarfism in plants is often caused by defects in the vascular system. To investigate whether the vasculature of the *crk10-A397T* mutant develops normally, we prepared

transverse cross sections of resin-embedded hypocotyl, root and stem samples of 5-week-old plants. The sections were stained with potassium permanganate, a lignin-specific dye which allows the observation of lignified xylem vessels and fibres. Imaging of the cross sections revealed that xylem vessels in the root and hypocotyl of the mutant plants are severely collapsed, whereas the vasculature in the stem is not morphologically altered (Figure 3A-F). Interestingly, the collapsed xylem vessels displayed darker brown staining in response to the dye compared to their WT counterparts, which suggests that their secondary cell walls are more heavily lignified (Figure 3G-H). This hypothesis was reinforced by the visualization of auto-fluorescence of lignin of these cells using confocal microscopy, as the xylem vessels in the mutant hypocotyl consistently exhibited a more intense auto-fluorescence signal compared to the WT (Figure 3I-J). Transmission electron microscopy (TEM) was used to investigate potential defects in the ultrastructure of the secondary cell wall of the collapsed xylem vessels in 3-week-old hypocotyls, but no differences were observed in electron density and general appearance of the cell walls compared to that of intact vessels in the WT (Supplemental Figure S5).

To understand the progression of the phenotype, a developmental time series of hypocotyl cross sections spanning weeks one to five after sowing was analysed. Cross sections of one- and two-week-old hypocotyls revealed the disorganization of xylem vessels in *crk10-A397T* plants at early developmental stages, as they do not proceed to form the typical radial patterning observed in the hypocotyl vasculature of WT plants (Supplemental Figure S6A-D). At three weeks of age, the first deformed and collapsed xylem vessels become apparent in the mutant hypocotyls, a phenotype which is even more severe in 4-week-old plants (Supplemental Figure S6E-H). At 5 weeks of age, following the onset of flowering, cross sections revealed the absence of fully differentiated xylem fibres in the hypocotyl of the *crk10-A397T* plants, in contrast to the WT (Supplemental Figure S6I-L). Differentiation of xylary fibres in *Arabidopsis* hypocotyls is tightly associated with the switch to growth phase II of xylem development, which is triggered by the transition to flowering. We conclude that this switch is delayed in the *crk10-A397T* plants, despite flowering occurring simultaneously to WT plants.

Loss of function or overexpression of *CRK10* does not cause a phenotype in *Arabidopsis*

In order to investigate whether increased levels of *CRK10* expression has observable phenotypic effects, we introduced a construct carrying the WT cDNA of *CRK10* under the control of the constitutive 35S promoter in WT *Arabidopsis* plants. Two independent homozygous lines were generated and selected for further analysis (*CRK10* OE-1 and OE-2). Compared to WT, qPCR performed on 4-week-old leaves detected a *CRK10* transcript increase of 15 and 6 times for *CRK10* OE-1 and OE-2, respectively, although growth and development were not altered (Supplemental Figure S7). In order to investigate whether the absence of the *CRK10* transcript affects the phenotype of *Arabidopsis* plants, two homozygous T-DNA knockout lines for the *CRK10* gene were isolated, *crk10-2* (SAIL_427_E09) and *crk10-4* (SALK_116653). Quantification of *CRK10* transcript levels from leaves of 4-week-old plants by qPCR confirmed that *crk10-2* and *crk10-4* are a knockout and knockdown line of *CRK10*, respectively, however growth and development of both lines were indistinguishable from WT plants (Supplemental Figure S8). Cross sections of hypocotyls of 4-week-old *crk10-2* and *CRK10* OE-1 plants were imaged to rule out the presence of collapsed xylem vessels (Supplemental Figure S9). Lines *CRK10* OE-1 and *crk10-2* were used for the pathogen assays which are described in a subsequent section.

***CRK10* is expressed in close association with vascular tissues and the protein localises to the plasma membrane**

Tissue specific expression of *CRK10* was determined by placing the reporter β -glucuronidase under the control of the 1 kb genomic sequence containing the putative promoter of *CRK10* (*CRK10*_{Pro}:GUS). *GUS* expression was detected in the vasculature of the roots, cotyledons, petioles, leaves, hypocotyls and inflorescence stem (Figure 4A-B; Supplemental Figure S10A-D). In hypocotyls of 2-week-old seedlings, expression was localised to differentiating xylem vessels and parenchyma cells surrounding the vessel elements (Figure 4B). The presence of the *CRK10* transcript in hypocotyls and inflorescence stems of 3- and 6-week-old WT and *crk10-A397T* mutant plants, respectively, was confirmed by qPCR (Supplemental Figure S10E).

Subcellular localisation of *CRK10* was determined by analysing lines expressing a construct carrying the C-terminal translational fusion of *CRK10* with the fluorescent protein mCherry under the control of the constitutive 35S promoter (*35S:CRK10-mCherry*). Both transient expression of the construct in *N. benthamiana* leaves and stable expression in

transgenic *Arabidopsis* plants indicated that the fusion protein localises to the plasma membrane (Supplemental Figure S10F; Figure 4C). The presence of Hechtian strands, characteristic of the retracting plasma membrane from the cell wall following plasmolysis (Oparka, 1994), further confirmed this subcellular localisation of the protein (Figure 4D). Therefore, we conclude *CRK10* is expressed in close association with vascular tissues of below and aboveground organs, and the protein localises to the plasma membrane of plant cells.

Collapsed xylem vessels in the root and hypocotyl are responsible for the dwarf phenotype of the *crk10-A397T* mutant

Although *CRK10* is expressed in tissues associated with vasculature in the stem, hypocotyl and roots, as demonstrated by *CRK10_{Pro}:GUS* analysis, it is intriguing that in the *crk10-A397T* plants xylem vessel collapse occurs only in roots and hypocotyls. To investigate if the dwarf phenotype is solely due to the defects of the below ground tissues, or whether it is a “whole-plant” response, we performed a micrografting experiment (Turnbull et al., 2002). *In vitro* grown 4-day-old seedlings were used to generate combinations of WT rootstocks and *crk10-A397T* scions (WT/*crk10-A397T*) and vice-versa (*crk10-A397T*/WT), as well as self-grafted plants as controls (WT/WT and *crk10-A397T/crk10-A397T*). Phenotypic assessment of successful grafts revealed that a WT scion grafted onto a *crk10-A397T* rootstock develops the characteristic dwarf phenotype of the mutant, whereas a mutant scion develops into a WT-like plant when grafted onto a WT rootstock (Figure 5). Our observations show that the root and hypocotyl system of the *crk10-A397T* plants are responsible for their dwarf phenotype, which is likely due to the presence of collapsed xylem vessels in these tissues.

The hypocotyl transcriptome of *crk10-A397T* carries the signature of a plant responding to stress

The effect of the *crk10-A397T* mutation on the transcriptome was analysed by RNA-Seq. Total RNA was isolated from 15-, 21- and 35-day-old WT and mutant hypocotyls, which corresponds to the time points used for morphological analysis. Principal component analysis (PCA) showed good clustering of replicates according to genotypes and developmental time points (Supplemental Figure S11A). Following normalization and statistical analysis of the sequencing results ($q \leq 0.05$; log₂ fold change threshold of ± 1), we

obtained 523 (15 days), 1836 (21 days) and 913 (35 days) differentially expressed genes (DEG), of which 274 were common to all time points. These DEGs were selected as the core set and taken forward for analysis (Figure 6A; Supplemental Figure S11B; Supplemental Tables S1-S4). Comparison to public datasets using the GENEVESTIGATOR® Signature tool (Hruz et al., 2008) revealed that the transcriptome signature of the *crk10-A397T* mutant was most similar to Arabidopsis plants challenged by fungal and bacterial pathogens (*Sclerotinia sclerotorum*, *Plectosphaerella cucumerina* and *Pseudomonas syringae*) and exposed to abiotic stress (treatment with fenclorim and sulfometuron methyl) (Figure 6C). Equally, Gene Ontology (GO) enrichment analysis of the up-regulated genes within the core set (246 genes) with AgriGO v.20 (Tian et al., 2017; False Discovery Rate < 0.05) revealed that terms associated with the biological functions “Defense response” (GO:0006952; p=2.30E-26), “Response to stimulus” (GO:0050896; p=1.40E-24) and “Response to stress” (GO:0006950; p=2.00E-24) are significantly overrepresented (Figure 6B; Supplemental Tables S5-S6). In accordance with the whole transcriptome data, marker genes indicative for the activation of the salicylic acid (SA)- and jasmonic acid (JA)-regulated defense pathways, such as pathogenesis-related and camalexin biosynthetic genes (Uknes et al., 1992; Thomma et al., 1998; Ahuja et al., 2012), are highly up-regulated in the *crk10-A397T* mutant (Supplemental Table S7). Transcription factors belonging to the WRKY, MYB and NAC-domain containing families are prominent among the regulatory genes induced by *crk10-A397T*, many of which have been associated with the modulation of stress responses (Supplemental Table S8). Therefore, biotic and abiotic stress-responsive pathways are constitutively activated in the *crk10-A397T* mutant, supporting our hypothesis that *crk10-A397T* is a gain-of-function allele of *CRK10*.

Furthermore, analysis of the lists of DEGs for each individual time point revealed that, specifically at 21 days, genes involved in the biosynthesis, signalling and homeostasis of the hormone abscisic acid (ABA) were also significantly up-regulated in the *crk10-A397T* mutant (Supplemental Table S9).

***crk10-A397T* mutant hypocotyls contain increased levels of the stress hormones salicylic acid (SA) and abscisic acid (ABA)**

In order to corroborate the transcriptomic data we quantified the levels of the stress hormones salicylic acid (SA), abscisic acid (ABA) and jasmonic acid (JA) from the

hypocotyls of 3-week-old WT and *crk10-A397T* plants (Supplemental Table S10). In agreement with the transcriptional induction of stress-responsive pathways, the levels of free SA and ABA were increased approximately 3 and 1.5 times, respectively, in the mutant hypocotyls. In contrast, JA levels were not significantly different to the WT.

The *crk10-A397T* mutant displays enhanced resistant to infection by the vascular pathogen *Fusarium oxysporum* f. sp. *conglutinans* 699

With defense responses constitutively up-regulated in the mutant, we next wanted to investigate whether this is reflected in an enhanced resistance to pathogens. Since *CRK10* expression is detected mainly in the vasculature, we chose the vascular pathogen *Fusarium oxysporum* f. sp. *conglutinans* 699, an isolate known to infect Arabidopsis for the assay (Masachis et al., 2016). *CRK10* OE-1 and *crk10-2* lines were also included in the experiment as overexpression and knockout of other CRKs often showed enhanced/decreased resistance phenotype to pathogens (Acharya et al., 2007, Yeh et al., 2015; Yadeta et al., 2017). The progression of the infection was observed (Figure 7A-D) and a time-mortality curve was recorded from 7 to 20 days post inoculation (Figure 7E). Our results showed that the susceptibility of WT and *crk10-2* plants to the pathogens was very similar, with both genotypes reaching over 65% of mortality at the end of the experiment. *CRK10* OE-1 and *crk10-A397T* plants exhibit a similarly low mortality rate until 10 days post inoculation, although *CRK10* OE-1 plants reach a final death toll of 47.5% in contrast to the lowest overall death count displayed by the *crk10-A397T* mutant of around 18%. Statistical analysis (Deviance test, $\chi^2_3=19.68$, $p < 0.001$) confirmed the differences in the probability of survival between genotypes, with the *crk10-A397T* mutant having the highest chance of survival of 81.25%, followed by 52.5% for the *CRK10* OE-1 plants and just over 30% for both *crk10-2* and WT (Figure 7F). Fungal burden quantification by qPCR showed increased *F. oxysporum* biomass in WT and *crk10-2* plants compared to *CRK10* OE-1 and *crk10-A397T* mutant at 7 days post inoculation, in agreement with the mortality trend results (Supplemental Figure S12). The experiment was performed twice with similar results. Therefore, our results strongly suggest that *crk10-A397T* mutant plants are more resistant to infection with *F. oxysporum*, reinforcing our hypothesis that the transcriptional responses induced by the *crk10-A397T* mutant allele are effective at reducing the spread of root-infecting vascular pathogens.

DISCUSSION

Previous efforts to assign functions to specific CRKs mainly focused on the characterisation of T-DNA and overexpression lines. However, being a large multigene family, effects of knocking out one specific member are often masked by redundancy and, in the absence of known stimulants, overexpression lines are generally phenotypically indistinguishable from WT. Here we report the characterisation of the *crk10-A397T* mutant which harbours a gain-of-function allele of CRK10, to our knowledge the first such mutant obtained for this class of receptors in *Arabidopsis*.

The *crk10_A397T* mutation lies in an important regulatory subdomain of the kinase domain of CRK10

The point mutation responsible for the conversion of CRK10 into a gain-of-function allele (alanine 397 by threonine) lies in kinase subdomain III, right at the C-terminus of the α C-helix and at the start of the short α C- β 4 loop which links the α C-helix to the β strand 4. The importance of this region for kinase regulation has been studied in numerous mammalian kinases with mutations residing in this area often leading to kinase de-regulation and disease, but equivalent studies in plant kinases are absent. The combined α C-helix and α C- β 4 loop has been shown to be a critical allosteric docking site which plays a crucial part in kinase regulation (Yeung et al., 2020). As the 3D model of the CRK10 kinase domain suggests, the substituted amino acid lies on that surface of the helix, which faces away from the active site of the kinase and which is known to provide an interface for interactions with many regulatory domains and proteins. For human CDK2, for example, it was shown that residue Lys-56, equivalent to the position of Thr-397 in *crk10-A397T*, is involved in its interaction with cyclin necessary for the stabilisation of the active form of the kinase (Jeffrey et al., 1995), whereas in the case of the Ser/Thr-protein kinase B-Raf (BRaf), where dimerisation is thought to be an important part of the activation mechanism, the α C-Helix/ α C- β 4 loop provides the interface for dimer formation (Rajakulendran et al., 2009). This region can also function as a *cis*-regulatory site as shown for the human leucine-rich repeat kinase 2 (LRRK2), where it provides a firm docking site for the C-terminal residues of the kinase which keeps it in an inactive conformation (Deniston et al., 2020, Taylor et al., 2020). As the

α C-helix/ α C- β 4 loop is a feature common to all eukaryotic kinases, it seems reasonable to speculate that the amino acid substitution in *crk10-A397T* perturbs the interaction of CRK10 with a regulatory partner. It is also noteworthy that the α C helix and α C- β 4 loop is a highly conserved region within the family of CRKs and only three residues are found to occupy the position equivalent to Ala-397 of CRK10 among all the members: alanine, threonine or serine (Supplemental Figures S13 and S14). It now remains to be seen whether other members of the CRK-family are similarly affected by an analogous mutation in their α C-helix/ α C- β 4 loops.

Threonine 397 in the kinase domain of CRK10 is an auto-phosphorylation site *in situ*

In situ phosphorylation analysis of the cytoplasmic kinase domains of WT and mutant CRK10 by LC-MS/MS determined that CRK10, being an RD kinase, shows the typical auto-phosphorylation pattern of conserved phosphorylation sites in the activation loop, on which the activity of this class of kinases is dependant (Nolen et al., 2004, Beenstock et al., 2016). Thr-507 and Ser-508 were identified as unambiguous phosphorylation sites, with Ser-508 likely to be functionally equivalent to Thr-450 of BAK1, which plays a key role in its activation by maintaining the active conformation of the activation loop (Yan et al., 2012). However, we also detected phosphorylation of tyrosine residues (Tyr-363 and Tyr-514), with Tyr-514 residing in the activation loop, which establishes CRK10 as a dual specificity kinase. Additional phosphorylated residues were detected in the juxtamembrane domain (Thr-340) and at the C-terminus (Thr-625, Ser-662 and Thr-664). Phosphorylation of residues in these non-catalytic regions have been shown to play an important role in the recognition and/or phosphorylation of downstream substrates, although these are unknown for CRKs (Wang et al., 2005; Oh et al., 2012; Zhou et al., 2020). The phosphorylation pattern of His-CRK100kd^{WT} and His-CRK10kd^{A397T} was identical, with the notable exception of the substituted Thr-397 functioning as an additional phosphorylation site in His-CRK10kd^{A397T}. Based on the experimental evidence, it is currently not possible to distinguish whether Thr-397 *per se* or whether its phosphorylated form is the underlying cause for the phenotypic effects observed in the *crk10-A397T* mutant. The significance of the identified *in situ* phosphorylation sites for CRK10 activity *in vivo* also remains to be determined.

The dwarf phenotype of the *crk10-A397T* mutant is linked to the collapse of xylem vessels in roots and hypocotyls

Phenotypically, the *crk10-A397T* mutant is a dwarf, which we showed to be the consequence of collapsed xylem elements in the roots and hypocotyl. Collapsed xylem vessels are thought to occur due to alterations in the cell wall composition, leading to cell wall weakening and a consequent inability to withstand the negative pressure generated by transpiration, as reported in the *eskimo* and *irx* mutants, for example (Turner & Somerville, 1997; Lefebvre et al., 2011). Although secondary cell wall defects are generally readily observed when analysed by TEM, we could not detect any obvious differences in the vessel cell wall ultrastructure in cross sections of 3-week-old hypocotyls of the *crk10-A397T* mutant and WT. Considering the severity of the vessel collapse in the mutant this was unexpected and future analysis of the composition of the cell wall will be necessary in order to determine the biochemical defects. Intriguingly, although *CRK10* is expressed in vascular-associated tissues in both stem and hypocotyl as shown by reporter lines and qPCR analysis, xylem elements in the stem seem to develop normally in the mutant. Why xylem elements are defective in one organ but not the other can, therefore, not simply be explained by a restricted expression pattern of *CRK10* but, considering that RLKs usually reside in large complexes at the plasma membrane, might be due to tissue-specific composition of the “receptorsomes”. However, grafting experiment strongly suggest that it is the defective vasculature in the belowground organs causing the dwarf phenotype, as WT scions grafted onto *crk10-A397T* rootstocks become similarly stunted whereas *crk10-A397T* scions grafted on WT rootstocks develop normally.

Stress responses are constitutively activated in the hypocotyl of *crk10-A397T*

Collapsed vessel elements are thought to impede proper water transport which is likely to be perceived as drought stress leading to an increase of ABA. This could explain the elevated levels of ABA which we detected in the hypocotyl of *crk10-A397T* and the up-regulation of genes involved in ABA synthesis, perception and response (Supplemental Table S9). A similar increase in ABA levels has been observed in other cell wall mutants such as *esk1* and *irx1-6* whereas *irx3*, *irx5* and *irx9* contained numerous constitutively up-regulated ABA-responsive genes (Chen et al., 2005; Hernández-Blanco et al., 2007; Lefebvre et al., 2011; Faria-Blanc et al., 2018; Xu et al., 2020). The stunting of these mutants has been

suggested to be the consequence of the response to drought signalling hormones resulting in the suppression of growth, which could also explain the dwarfism of *crk10-A397T*. It will be interesting to investigate whether dwarfing of *crk10-A397T* can be alleviated by an ABA insensitive mutant such as *abi1* (Koornneef et al., 1984).

The severe collapse of the xylem elements in the roots and hypocotyl of *crk10-A397T* is most likely due to alterations of the composition of the cell walls and it is increasingly being reported that modification of cell wall composition by genetic or chemical means leads to the constitutive activation of defense pathways and an altered resistance to pathogens. The primary cell wall mutant *ixr1/cev1*, for example, with reduced crystalline cellulose content due to the defective cellulose synthase CESA3, displays constitutive activation of jasmonic acid and ethylene signalling (Ellis et al., 2001; Ellis et al., 2002). Transcriptomic data obtained for the secondary cell wall mutants *irx1-6*, *irx5-5* and *irx9* showed that defense-related genes are constitutively expressed in these mutants (Hernández-Blanco et al., 2007; Faria-Blanc et al., 2018). In line with these reports, our data showed that the signature of the core set of DEGs of *crk10-A397T* was most similar to the transcriptome of plants responding to biotic and abiotic stress. Canonical SA-dependent marker genes (PR1, PR2 and PR5) and genes involved in the synthesis of the tryptophan-derived antimicrobial compounds (camalexin, glucosinolates) are significantly up-regulated in the mutant, as are numerous transcription factors usually associated with coordinating stress responses (Supplemental Tables S7 and S8). Concomitant with gene induction, SA levels in the *crk10-A397T* mutant hypocotyls are increased, whereas changes in JA levels were not significant. Induction of defense pathways due to cell wall impairment manifests itself frequently in alteration of disease resistance to a wide range of pathogens (Houston et al., 2016; Bacete et al., 2018). Molina et al. (2021), for example, showed that from a panel of 34 cell wall mutants affecting a wide range of different cell wall compounds, 29 had an altered, mainly enhanced, resistance response to pathogens comprising different parasitic lifestyles. In order to assess disease resistance of *crk10-A397T* we chose the root-infecting, hemi-biotrophic vascular wilt pathogen *F. oxysporum* to perform a pathogen assay, bearing in mind the vasculature associated expression of *CRK10*. In agreement with the studies linking cell wall modification to altered disease resistance, we found that the *crk10-A397T* mutant was significantly more resistant to the pathogen, part of which could be due to the fact that collapsed xylem vessels act as physical barriers slowing pathogen progression in the roots.

Conclusion

Taken together, the experimental evidence provided in this report suggests that *crk10-A397T* is a mutant which contains a gain-of function allele of the cysteine rich receptor-like kinase CRK10. The transcriptome of the mutant carries the typical signature of a plant responding to stress, although at this stage it is not possible to differentiate whether the stress response is the direct consequence of spontaneous signalling occurring because of the mutated CRK10 receptor or whether it is the response to the perception of the defective xylem vessel cell walls. The mutation lies in the α C-Helix/ α C- β 4 loop which is emerging as a key structural element in the regulation of kinase function and regulation and a hot-spot for post-translational modifications (Yeung et al., 2020). It is noteworthy that the only other semi-dominant mutant reported for a CRK in rice, *als1*, which develops spontaneous lesions on leaf blades and sheaths also localises to this loop (Du et al., 2019). Future work will now need to address the consequence of the *A397T* mutation for kinase activity. The dwarf and collapsed xylem phenotype of the *crk10-A397T* mutant could, for example, be caused by CRK10 having been converted into a constitutively active kinase and thereby reflect the function of wild type CRK10. However, another scenario which needs to be taken into consideration is that CRK10 *A397T* has assumed a novel function and interferes with an endogenous pathway distinct from the normal mode of action of wild type CRK10.

FIGURE LEGENDS

Figure 1 CRK10 and CRK10-A397 are active kinases and the A397T substitution introduces an additional auto-phosphorylation site in the kinase domain of the protein. A, Amino acid sequence of the cytoplasmic kinase domain of CRK10 used for in situ phosphorylation studies (CRK10 amino acid residue numbering shown on the left). Subdomains I-XI are indicated by roman numerals. Ala/Thr 397 site is highlighted in red, and the RD motif is underlined and highlighted in bold. The conserved glycine-rich loop, catalytic loop and activation segment motifs are also shown. B, Structure of the CRK10 kinase domain generated by homology modelling to the active kinase domain of BRI1. A molecule of an ATP analogue, coloured blue, occupies the active site. The α C-helix is coloured red, and the atoms in the side chain of the mutated threonine residue are depicted as

red spheres. C, Recombinant His-CRK10kd^{WT} and His-CRK10kd^{A397T} extracts and their respective dead kinase controls (His-CRK10kd^{WT}-D473N and His-CRK10kd^{A397T}-D473N) resolved by SDS-PAGE; detection by Western blot with HRP-conjugated anti-His antibody is shown. D, SDS-PAGE and Western blot following treatment of purified His-tagged proteins with λ -phosphatase (PPase); control: untreated protein. E, In situ auto-phosphorylation sites in His-CRK10kd^{WT} and His-CRK10kd^{A397T} identified by LC-MS/MS analysis of the recombinant protein kinase domain. Threonine 397 is highlighted in red. His: 6x His-tag; JM: juxta-membrane domain; KD: kinase domain; C-term tail: C-terminal tail. (f) MS/MS spectrum of the doubly charged (m/z 758.4) tryptic phosphopeptide NEVVLVTKLQHR in which the threonine residue is phosphorylated. Neutral losses of phosphoric acid from both the precursor ion and the C-terminal y ions are observed.

Figure 2 *crk10-A397T* mutant is a dwarf. A-F, Rosette morphology of WT (A, B, C) and *crk10-A397T* (D, E, F) plants at 2 (A, D), 3 (B, E) and 4 (C, F) weeks after sowing. Bar = 1 cm. G, Leaf series of 4-week-old WT (top) and *crk10-A397T* (bottom) plants. Bar = 1 cm. H, Main inflorescence stem of 5-week-old WT (left) and *crk10-A397T* (right) plants. Bar = 1 cm. I, 10-week-old WT (left) and *crk10-A397T* (right) plants. Bar = 2 cm. J, Siliques of WT (left) and *crk10-A397T* (right) plants. Bar = 1 cm. K, Seeds of WT (left) and *crk10-A397T* (right) plants. Bar = 500 μ m.

Figure 3 Xylem vessels collapse in the root and hypocotyl of *crk10-A397T* plants, but not in the stem. A-F, Transverse cross sections of the base of stem (A, B), hypocotyl (C, D) and roots (E, F) of 5-week-old WT (A, C, E) and *crk10-A397T* (B, D, F) plants. Stain: potassium permanganate. Bars = (A, B, C, E, F) 100 μ m; (D) 50 μ m. G-H, Detail of xylem vessels in hypocotyls of 4-week-old WT (G) and *crk10-A397T* (H) mutant plants. Stain: potassium permanganate. Insert in top left corner of image shows original micrographs. Bars = (insert G) 50 μ m; (insert H) 25 μ m. I-J, Detection of auto-fluorescence of lignin on resin-embedded cross sections of 4-week-old hypocotyls of WT (I) and *crk10-A397T* (J) plants. Bars = 10 μ m.

Figure 4 CRK10 is a plasma membrane-localised protein expressed in association with the vasculature in the hypocotyl. A-B, Histochemical staining of reporter lines

expressing the *CRK10*_{Pro}:*GUS* construct showed expression of the reporter gene in the vasculature of stem and hypocotyls, as shown by free-hand cross section of 8-week-old inflorescence stem (A) and cross section of 2-week-old hypocotyl embedded in resin (B). Bars = (A) 50 μ m; (B) 25 μ m. C, Detail of cross section in B shows presence of histochemical staining in xylem parenchyma cells (asterisks) and differentiating xylem vessels (arrows) in the hypocotyl. C-D, Hypocotyl of 4-day-old seedling of transgenic *Arabidopsis* plant expressing 35S:*CRK10-mCherry* before (C) and after (D) plasmolysis. Bars = 20 μ m.

Figure 5 The root-hypocotyl system is responsible for the dwarf phenotype of *crk10-A397T* mutant plants. Images of non-grafted plants (A), self-graft controls (B) and graft combinations (C) of WT and *crk10-A397T* mutant. Plants were imaged 3 weeks after micrografting was performed. The phenotype observed for the reciprocal grafting combinations were consistently observed in two independent repetitions of the experiment. An average number of 10 grafts per combination was recovered each time. Annotation: scion / rootstock.

Figure 6 Transcriptional reprogramming in the *crk10-A397T* mutant shows activation of defense responses to biotic and abiotic stresses. A, Venn diagram displaying the number of differentially expressed genes (DEGs) in the *crk10-A397T* hypocotyls compared to the WT at each developmental time point. B, Top 15 enriched GO terms (Biological Process) for the up-regulated core DEGs in the *crk10-A397T* mutant plotted against their respective $-\log_{10}$ FDR. C, Top 15 perturbations showing highest overall similarity to *crk10-A397T* mutant expression signature (analysis performed using the GENEVESTIGATOR® SIGNATURE tool; $\log_2$ fold change values of time point 21 days was used as input for 274 core genes).

Figure 7 *crk10-A397T* mutant plants are more resistant to infection with *Fusarium oxysporum* f. sp. *conglutinans* 699. A-D, Representative images of WT (A), *crk10-2* (B), *CRK10* OE-1 (C) and *crk10-A397T* mutant (D) plants at 11 days post inoculation with *F. oxysporum*. E, Mortality curve of WT, *crk10-A397T*, *CRK10* OE-1 and *crk10-2* plants from day 7 to 20 post inoculation with *F. oxysporum*. Mortality is shown in percentage. The experiment was repeated twice with similar results. F, Probability of survival of each

genotype following inoculation with the pathogen *F. oxysporum*. Associated 95% confidence intervals are shown. Deviance test, $\chi^2_3=19.68$, $p < 0.001$.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Arabidopsis thaliana ecotype Col-0 plants were grown in Grobanks cabinets (CLF 2006, Plant Climatics, Germany) in Levington F2 + Sand compost in long day conditions (16h/8h), 23/18 °C day/night temperature, 200 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ of light intensity. For *in vitro* experiments, surface sterilized seeds were placed on ½ MS plates (Murashige & Skoog, Duchefa Biochemie).

RNA-Seq

Total RNA was extracted from hypocotyls of *Arabidopsis* plants using the RNeasy Mini Kit (QIAGEN), and samples were subsequently treated with DNase Turbo DNA-free kit (Thermo Fisher Scientific). Each biological replicate consisted of a pool of 50-60 hypocotyls, and four biological replicates were isolated per genotype per time point. RNA quality was assessed on a 2100 Bioanalyzer (Agilent Technologies). Library preparation and paired-end sequencing, Illumina HiSeq 125 PE sequencing was performed by Exeter Sequencing Service (University of Exeter, UK). The Rothamsted instance of the Galaxy (<https://usegalaxy.org/>) bioinformatics web pipeline was used to perform quality control (MultiQC; <https://multiqc.info/>), trimming (Trimmomatic; Bolger et al., 2014) and mapping to the reference genome (HiSAT2; Kim et al., 2015). The table of counts was acquired using the featureCounts functions in the Subread (Liao et al., 2019; <https://bioconductor.org/packages/release/bioc/html/Rsubread.html>) on the R Bioconductor platform (<https://bioconductor.org/>). Genes with less than 3 samples with counts ≥ 5 were discarded. Differentially expressed genes were identified (using the default Wald test) in the R (v3.6.1) Bioconductor package DESeq2 (Love et al., 2014; <https://bioconductor.org/packages/release/bioc/html/DESeq2.html>). Gene Ontology (GO) enrichment analysis (Single Enrichment Tool, AgriGO v2.0; Tian et al., 2017) and similarity comparison with deposited micro-array datasets (Signature tool, GENEVESTIGATOR®, Hruz et al., 2008) were performed for the set of 274 core differentially expressed genes.

Quantitative PCR

RNA was isolated using TRI Reagent® (Merck) and treated with DNase I, Amplification Grade (Thermo Fisher Scientific) prior to cDNA synthesis with SuperScript™ III Reverse Transcriptase (Thermo Fisher Scientific). All qPCR reactions were performed in a LightCycler® 96 Real-Time PCR System (Roche Diagnostics) using the FastStart Essential DNA Green Master (Roche Diagnostics). *ACTIN2* (AT3G18780) and *UBC21* (AT5G25760) were used as internal controls (Supplemental Table S11), and the $2^{-\Delta\Delta C_t}$ method (Livak & Schmittgen, 2001) was used to calculate relative expression.

Quantification of plant hormones

Hypocotyl samples (75 – 100 mg fresh weight) isolated from 3-week-old plants were used for the quantification of hormones as described in Camut et al., 2019. A 2.6 µm Accucore RP-MS column (100 mm length x 2.1 mm i.d.; ThermoFisher Scientific) and a Q-Exactive mass spectrometer (Orbitrap detector; ThermoFisher Scientific) were used. The concentrations of hormones in the extracts were determined using embedded calibration curves and the Xcalibur 4.0 and TraceFinder 4.1 SP1 programs. Three biological replicates per genotype were analysed.

Cloning of genetic constructs

The list of primers used in this study can be found in Supplemental Table S11. To generate the construct for the complementation of the *crk10-A397T* mutant and overexpression of *CRK10* in the WT plants, the cDNA sequence of *CRK10* was amplified from the cDNA clone U60398 with *SalI* and *SacI* restriction sites and cloned into pJD330 downstream of the 35S promoter. The construct 35S:*CRK10*-NOS was then amplified with *AscI* restriction sites and cloned into the RS 3GSeedDSRed MCS vector. To obtain the *CRK10_{Pro}:crk10-A397T*-NOS construct, the 1 kb genomic sequence upstream of *CRK10* (*CRK10_{Pro}*) was amplified from Arabidopsis Col-0 genomic DNA with *SphI* and *SalI* restriction sites, and was cloned in pJD330 upstream of the *CRK10* cDNA sequence. The G>A mutation harboured by the *crk10-A397T* mutant was introduced by *in vitro* mutagenesis.

The *CRK10*_{Pro} sequence was amplified with *Sph*I and *Nco*I restriction sites, and the fragment was ligated upstream of the β -glucuronidase (*GUS*) gene in pJD330. The *CRK10*_{Pro}:*GUS*-NOS construct was amplified with *Asc*I restriction sites and cloned into the RS 3GSeedDSRed MCS vector. To obtain a translational fusion of CRK10 with the fluorescent protein mCherry, the stop codon of *CRK10* was removed and a *Sac*I restriction site was introduced by *in vitro* mutagenesis between *CRK10* and the NOS terminator sequence in pJD330. The coding sequence of *mCherry* was cloned with *Sac*I restriction sites on both ends and ligated in frame with *CRK10*. The construct *CRK10-mCherry*-NOS was then amplified with *Sal*II and *Not*I restriction sites and cloned into pENTR1A Dual Selection Vector (Invitrogen). Following LR reaction (Gateway) with the binary vector pB2GW7 (Invitrogen), the construct 35S:*CRK10-mCherry*-NOS was obtained. Finally, to generate the constructs for recombinant protein in *E. coli*, the coding sequence of the kinase domain of *CRK10* was amplified with *Sal*II and *Not*I restriction sites and cloned into pENTR1A Dual Selection Vector (Invitrogen) prior to recombination with pDEST17 (Invitrogen), where it was cloned in frame with an N-terminal 6x His-tag. *In vitro* mutagenesis was used to generate the gain-of-function mutation of CRK10 (A397T) as well as the dead kinase variant by mutation of the invariant aspartic acid in the active site to an asparagine (D473N). All constructs used for the generation of transgenic plants were introduced in *A. tumefaciens* cells strain GV3101 using the freeze and thaw protocol (An et al., 1988), and the floral dip method (Clough and Bent, 1998) was used to transform *Arabidopsis* plants.

Transient expression of fluorescent fusion in *N. benthamiana*

Protocol was adapted from Sparkes et al., 2006. Transient expression of the fusion protein was observed 72 hours post-infiltration.

Microscopy

For the preparation of thin sections for light microscopy, plant tissue samples were fixed in 4% paraformaldehyde / 2.5% glutaraldehyde (0.05 M phosphate buffer pH 7.2), dehydrated in graded ethanol series (10 – 100%) and infiltrated with LRWhite resin (Agar Scientific). Sections were prepared using a Reichert Ultramicrotome (section thickness: 1-2 μ m) and stained with 0.5% potassium permanganate. Thin sections were observed with a Zeiss Axiophot equipped with a Q-Imaging Retiga EXi CCD mono digital camera with RGB

filter wheel (QImaging, Canada) coupled with the Metamorph software (Molecular Devices, USA). Non-stained resin embedded thin sections were also used for the detection of the auto-fluorescence of lignin using the confocal microscope (excitation: 405 nm; emission: 451-480 nm and 560-612 nm). For the detection of mCherry fluorescence from *N. benthamiana* leaves and hypocotyls of Arabidopsis, a small piece of fresh tissue sample was mounted on a glass slide with glass cover slip and water, and the fluorescence was detected using the confocal microscope (excitation: 561 nm laser; emission: 578 - 639 nm). Plasmolysis was performed using a 0.8M mannitol solution for 40 minutes and images acquired prior to and after treatment. Fluorescent signals were detected from thin sections and whole plant samples using a Zeiss 780 LSM confocal microscope. For the preparation of samples for transmission electron microscopy (TEM), plant material was fixed by high-pressure freezing in 0.1 M sucrose using a Leica HPM100, followed by freeze substitution with 100% ethanol (Leica EM Auto Freeze substitution) and infiltration with LRWhite resin (Agar Scientific). Ultra-thin sections prepared with a Leica EM UCT Ultramicrotome (section thickness: 90 nm) were collected on pioloform/carbon-coated nickel grids (Agar Scientific) and stained with 2.5% uranyl acetate and Reynolds lead citrate (Reynolds, 1963). Ultrathin sections were imaged using a JEOL-2100Plus Transmission Electron Microscope (JEOL, Japan) equipped with a Gatan OneView IS Camera (Gatan, USA).

Micrografting of Arabidopsis seedlings

Grafting protocol was performed as previously described (Turnbull et al., 2002). Successful grafts were transferred to soil 7-10 days post-grafting.

GUS staining

Plant tissue was incubated overnight in X-Gluc (Melford) solution at 37 °C. Chlorophyll was removed with 80% ethanol prior to imaging using a Leica M205 FA Stereomicroscope (Leica Microsystems).

Recombinant protein expression, purification and analysis

BL21 AI One-Shot E. coli cells (Invitrogen) were used for the expression of recombinant 6x His-tag CRK10 kinase domain variants. Singles colonies were inoculated in liquid media supplemented with antibiotics and 0.1% glucose and grown overnight at 37 °C

shaking at 225 rpm. A 250 μ L aliquot of the overnight culture was used to inoculate 50 mL of fresh selective media, and cells were grown until $OD_{600} = 0.4-0.5$ was obtained. L-arabinose was added to the cultures to a final concentration of 0.2% to induce expression of the recombinant protein, and cultures were grown for an additional 3 hours. Cells were harvested by centrifugation (5,000 g x g for 10 minutes), resuspended in equilibration buffer (50 mM sodium phosphate pH 8.0, 0.3 M sodium chloride) and lysed by sonication. The cell lysate was centrifuged for 30 minutes at 5,000 x g and the supernatant was recovered and mixed with HIS-Select Nickel Affinity Gel (Sigma Aldrich) for protein purification (manufacturer's instructions were followed). The concentration of the purified protein extracts was measured using the Bradford method (Protein Assay Dye Reagent, Bio-Rad). Purified His-tagged proteins were resolved by SDS-PAGE (NuPAGE 4-12% Bis-Tris Protein Gels, Invitrogen) and gels were stained with Quick Coomassie Stain (Generon). Alternatively, proteins were transferred to a PVDF membrane (iBlot Transfer Stack, PVDF, Invitrogen) for Western blotting. The membrane was blocked with 5% powder milk in PBS-T, and incubated with His-probe (H-3) HRP monoclonal antibody (Santa Cruz Biotechnology) for 1 hour. The membrane was washed and incubated with Amersham ECL Western Blotting Detection Reagent (GE Healthcare) and developed in the dark. Protein extracts were also treated with Lambda Protein Phosphatase (Lambda PP, New England BioLabs) according to the manufacturer's protocol. Briefly, 5 μ g of purified protein were incubated with Lambda PP for 1h30min at 30 $^{\circ}$ C. Phosphatase-treated samples were then resolved on SDS-PAGE and detected by Western blotting.

Liquid chromatography – Mass Spectrometry/Mass Spectrometry (LC-MS/MS) and MASCOT database search

Gel bands were transferred into a 96-well PCR plate. Peptide bands were cut into 1mm² pieces, destained, reduced (DTT) and alkylated (iodoacetamide) and subjected to enzymatic digestion with sequencing grade trypsin (Promega, Madison, WI, USA) overnight at 37 $^{\circ}$ C. After digestion, the supernatant was pipetted into a sample vial and loaded onto an autosampler for automated LC-MS/MS analysis. All LC-MS/MS experiments were performed using a Dionex Ultimate 3000 RSLC nanoUPLC (Thermo Fisher Scientific Inc, Waltham, MA, USA) system and a QExactive Orbitrap mass spectrometer (Thermo Fisher Scientific Inc, Waltham, MA, USA). Separation of peptides was performed by reverse-phase

chromatography at a flow rate of 300 nL/min and a Thermo Scientific reverse-phase nano Easy-spray column (Thermo Scientific PepMap C18, 2 µm particle size, 100A pore size, 75 µm i.d. x 50cm length). Peptides were loaded onto a pre-column (Thermo Scientific PepMap 100 C18, 5 µm particle size, 100A pore size, 300 µm i.d. x 5mm length) from the Ultimate 3000 autosampler with 0.1% formic acid for 3 minutes at a flow rate of 10 µL/min. After this period, the column valve was switched to allow elution of peptides from the pre-column onto the analytical column. Solvent A was water + 0.1% formic acid and solvent B was 80% acetonitrile, 20% water + 0.1% formic acid. The linear gradient employed was 2-40% B in 30 minutes. Further wash and equilibration steps gave a total run time of 60 minutes. The LC eluant was sprayed into the mass spectrometer by means of an Easy-Spray source (Thermo Fisher Scientific Inc.). All m/z values of eluting ions were measured in an Orbitrap mass analyzer, set at a resolution of 70000 and was scanned between m/z 380-1500. Data dependent scans (Top 20) were employed to automatically isolate and generate fragment ions by higher energy collisional dissociation (HCD, NCE:25%) in the HCD collision cell and measurement of the resulting fragment ions was performed in the Orbitrap analyser, set at a resolution of 17500. Singly charged ions and ions with unassigned charge states were excluded from being selected for MS/MS and a dynamic exclusion window of 20 seconds was employed.

Protein modelling

A structural model of the kinase domain of CRK10 was generated by homology modelling using PyMOD 3.0 with default parameters (Janson et al., 2017). The kinase domain of BRI1 (PDB code 5LPV) (Bojar et al., 2014) was used as template retaining the ATP analogue (phosphoaminophosphonic acid-adenylate ester) but no other heteroatoms.

Plant infection assay

Root infection assay was modified from Masachis et al., 2016. Arabidopsis seedlings (12-days-old) grown *in vitro* were inoculated by immersing their roots for 20 minutes in a suspension of 1×10^6 microconidia ml⁻¹ of *F. oxysporum* f. sp. *conglutinans* 699. Seedlings were transferred to Levington F2 + Sand compost with vermiculite (3:1) and incubated in growth chamber under long day conditions (16/8h), 28/25 °C day/night temperature. Eighty plants per genotype were planted in a randomised blocked tray design, and mortality was

assessed daily between 7 and 20 days post-inoculation. For determination of fungal burden, three independent replications of the experiment were performed. Seedlings were inoculated as described above and collected at 2 and 7 days post-inoculation. Each repetition of the experiment included one pool of eight seedlings per genotype which were processed as one biological replicate. Total DNA extraction was performed according to Yu et al., 2019 and the relative amount of fungal DNA was quantified by qPCR using the *F. oxysporum ACTIN1* normalized to the Arabidopsis *ACTIN2* gene. Results were expressed relative to WT at 2 days post inoculation.

Statistical analysis

Statistical tests were performed using the Genstat software (Genstat for Windows 21st Edition; VSN International, Hemel Hempstead, UK). Student's t-test were used to assess statistical differences between two variants. To assess whether the pattern of segregation of the dwarf phenotype followed the expected 1:2:1 ratio, the $\chi^2 = \sum_{i=1}^r \frac{(O_i - E_i)^2}{E_i}$ chi-square statistic was used, where O_i is the observed count for group i and E_i is the expected count for group i . Under the null hypothesis of 1:2:1 segregation this test statistic should follow a chi-square distribution with 2 degrees of freedom. The probability of survival of each genotype in the bioassay with *F. oxysporum* was assessed with a generalized linear model (Bernoulli distribution; logit link function fitted to the final mortality outcome of each plant); statistical significance of the genotypic effect was tested after removing variation associated with plant position within rows of different trays and quantified through a Chi-squared statistic of the difference in deviance. Statistical significance of the differences in fungal burden between genotypes were tested by ANOVA (expression levels were log-transformed to meet the ANOVA requirements, and each individual experiment was considered as a block).

Accession numbers

Gene sequence data for this article can be found in The Arabidopsis Information Resource (TAIR) under the accession number AT4G23180 (*CRK10*). The *CRK10* cDNA clone (U60398) was obtained from the Arabidopsis Biological Resource Center (ABRC). The T-DNA lines SAIL_427_E09 and SALK_116653 were obtained from the Nottingham Arabidopsis Stock Centre (NASC; plants were genotyped according to instructions provided in <http://signal.salk.edu>). The mass spectrometry proteomics data have been deposited to the

ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2019) partner repository with the dataset identifier PXD023831.

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REFERENCES

- Acharya BR, Raina S, Maqbool SB, Jagadeeswaran G, Mosher SL, Appel HM, Schultz JC, Klessig DF, Raina R** (2007) Overexpression of *CRK13*, an Arabidopsis cysteine-rich receptor-like kinase, results in enhanced resistance to *Pseudomonas syringae*. *Plant Journal* **50**: 488-499
- Ahuja I, Kissen R, Bones AM** (2012) Phytoalexins in defense against pathogens. *Trends in Plant Sciences* **17**: 73-90
- An G, Ebert PR, Mitra A, Ha SB** (1988) Binary vectors. In: Gelvin SB, Schilperoot RA eds. *Plant Molecular Biology Manual*. Kluwer, Dordrecht, Netherlands, A3:1-19
- Bacete L, Melida H, Miedes E, Molina A** (2018) Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. *Plant Journal* **93**: 614-636

887 **Beenstock J, Mooshayef N, Engelberg D** (2016) How Do Protein Kinases Take a Selfie
888 (Autophosphorylate)? Trends in Biochemical Sciences **41**: 938-953

889 **Bojar D, Martinez J, Santiago J, Rybin V, Bayliss R, Hothorn M** (2014) Crystal
890 structures of the phosphorylated BRI1 kinase domain and implications for brassinosteroid
891 signal initiation. Plant Journal **78**: 31-43

892 **Bolger AM, Lohse M, Usadel B** (2014) Trimmomatic: a flexible trimmer for Illumina
893 sequence data. Bioinformatics **30**: 2114-2120

894 **Bourdais G et al. CRK Consortium** (2015) Large-Scale Phenomics Identifies Primary and
895 Fine-Tuning Roles for CRKs in Responses Related to Oxidative Stress. PLoS Genetics **11**:
896 e1005373

897 **Camut L, Regnault T, Sirlin-Josserand M, Sakvarelidze-Achard L, Carrera E, Zumsteg
898 J, Heintz D, Leonhardt N, Lange MJP, Lange T et al.** (2019) Root-derived GA12
899 contributes to temperature-induced shoot growth in Arabidopsis. Nature Plants **5**:1216-1221

900 **Chen Z.** (2001) A Superfamily of Proteins with Novel Cysteine-Rich Repeats. Plant
901 Physiology **126**: 473-476

902 **Chen Z, Hong X, Zhang H, Wang Y, Li X, Zhu JK, Gong Z** (2005) Disruption of the
903 cellulose synthase gene, *AtCesA8/IRX1*, enhances drought and osmotic stress tolerance in
904 Arabidopsis. Plant Journal **43**: 273-283

905 **Chinchilla D, Bauer Z, Regenass M, Boller T, Felix G** (2006) The Arabidopsis receptor
906 kinase FLS2 binds flg22 and determines the specificity of flagellin perception. Plant Cell **18**:
907 465-476

908 **Clough S, Bent A** (1998) Floral dip: A simplified method for Agrobacterium-mediated
909 transformation of Arabidopsis thaliana. Plant Journal **16**: 735-743

910 **Czernic P, Visser B, Sun W, Savouré A, Deslandes L, Marco Y, Van Montagu M,
911 Verbruggen N** (1999) Characterization of an *Arabidopsis thaliana* receptor-like protein
912 kinase gene activated by oxidative stress and pathogen attack. The Plant Journal **18**: 321-327

913 **De Smet I, Voß U, Jürgens G, Beeckman T** (2009) Receptor-like kinases shape the plant.
914 Nature Cell Biology **11**: 1166-1173

915 **Deniston CK, Salogiannis J, Mathea S, Snead DM, Lahiri I, Matyszewski M, Donosa O,
916 Watanabe R, Böhning J, Shiao AK et al.** (2020) Structure of LRRK2 in Parkinson's
917 disease and model for microtubule interaction. Nature **588**: 344–349

918 **Diévarit A, Clark SE** (2004) LRR-containing receptors regulating plant development and
919 defense. *Development* **131**: 251-261

920 **Du D, Liu M, Xing Y, Chen X, Zhang Y, Zhu M, Lu X, Zhang Q, Ling Y, Sang X, Li Y,**
921 **Zhang C, He G** (2019) Semi-dominant mutation in the cysteine-rich receptor-like kinase
922 gene, ALS1, conducts constitutive defence response in rice. *Plant Biology* **21**:25-34

923 **Du L, Chen Z** (2000) Identification of genes encoding receptor-like protein kinases as
924 possible targets of pathogen-and salicylic acid-induced WRKY DNA-binding proteins in
925 Arabidopsis. *Plant Journal* **24**: 837-847

926 **Ellis C, Turner JG** (2001) The Arabidopsis mutant *cev1* has constitutively active jasmonate
927 and ethylene signal pathways and enhanced resistance to pathogens. *Plant Cell* **13**: 1025-1033

928 **Ellis C, Karafyllidis I, Wasternack C, Turner JG** (2002) The Arabidopsis Mutant *cev1*
929 Links Cell Wall Signaling to Jasmonate and Ethylene Responses. *Plant Cell* **14**: 1557-1566

930 **Faria-Blanc N, Mortimer JC, Dupree P** (2018) A Transcriptomic Analysis of Xylan
931 Mutants Does Not Support the Existence of a Secondary Cell Wall Integrity System in
932 Arabidopsis. *Frontiers in Plant Science* **9**: article 384

933 **Friedrichsen DM, Joazeiro CAP, Li J, Hunter T, Chory J** (2000) Brassinosteroid-
934 Insensitive-1 Is a Ubiquitously Expressed Leucine-Rich Repeat Receptor Serine/Threonine
935 Kinase. *Plant Physiology* **123**: 1247-1255

936 **Hanks SK, Hunter T** (1995) The eukaryotic protein kinase superfamily: kinase (catalytic)
937 domain structure and classification. *The FASEB Journal* **9**: 576-596

938 **Hernández-Blanco C, Feng DX, Hu J, Sanchez-Vallet A, Deslandes L, Llorente F,**
939 **Berrocal-Lobo M, Keller H, Barlet X, Sanchez-Rodriguez C et al.** (2007) Impairment of
940 Cellulose Synthases Required for Arabidopsis Secondary Cell Wall Formation Enhances
941 Disease Resistance. *Plant Cell* **19**: 890–903

942 **Houston K, Tucker MR, Chowdhury J, Shirley N, Little A** (2016) The Plant Cell Wall: A
943 Complex and Dynamic Structure As Revealed by the Responses of Genes under Stress
944 Conditions. *Frontiers in Plant Science* **7**: article 984

945 **Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem**
946 **W, Zimmermann P** (2008) Genevestigator V3: A Reference Expression Database for the
947 Meta-Analysis of Transcriptomes. *Advances in Bioinformatics* **2008**: article 420747

948 **Idänheimo N, Gauthier A, Salojärvi J, Siligato R, Brosché M, Kollist H, Mähönen AP,**
949 **Kangasjärvi J, Wrzaczek M** (2014) The *Arabidopsis thaliana* cysteine-rich receptor-like

kinases CRK6 and CRK7 protect against apoplastic oxidative stress. *Biochemical and Biophysical Research Communications* **445**: 457-462

Janson G, Zhang C, Prado MG, Paiardini A (2017) PyMod 2.0: improvements in protein sequence-structure analysis and homology modeling within PyMOL. *Bioinformatics* **33**: 444-446

Jeffrey PD, Russo AA, Polyak K, Gibbs E, Hurwitz J, Massagé J, Pavletich NP (1995) Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex. *Nature* **376**: 313-320

Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. *Nature Methods* **12**: 357-360.

Kimura S, Hunter K, Vaahtera L, Tran HC, Citterico M, Vaattovaara A, Rokka A, Stolze SC, Harzen A, Meißner L et al. (2020) CRK2 and C-terminal phosphorylation of NADPH oxidase RBOHD regulate reactive oxygen species production in Arabidopsis. *Plant Cell* **32**: 1063-1080

Koornneef M, Reuling G, Karssen CM (1984) The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. *Physiologia Plantarum* **61**: 377-383

Lefebvre V, Fortabat MN, Ducamp A, North HM, Maia-Grondard A, Trouverie J, Boursiac Y, Mouille G, Durand-Tardif M (2011) *ESKIMO1* disruption in Arabidopsis alters vascular tissue and impairs water transport. *PLoS ONE* **6**: e16645

Liao Y, Smyth GK, Shi W (2019) The R package R subread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. *Nucleic Acids Research* **47**: e47

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods* **25**: 402-408

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**: 550.

Ma LS, Wang L, Trippel C, Mendoza-Mendoza A, Ullmann S, Moretti M, Carsten A, Kahnt J, Reissmann S, Zechmann B, Bange G et al. (2018) The *Ustilago maydis* repetitive effector Rsp3 blocks the antifungal activity of mannose-binding maize proteins. *Nature Communications* **9**: 1711

980 **Masachis S, Segorbe D, Turrà D, Leon-Ruiz M, Fürst U, El Ghalid M, Leonard G,**
981 **Lopez-Berges MS, Richards TA, Felix G et al. (2016)** A fungal pathogen secretes plant
982 alkalizing peptides to increase infection. *Nature Microbiology* **1**: 16043
983 **Miyakawa T, Miyazono K, Sawano Y, Hatano K, Tanokura M (2009)** Crystal structure of
984 ginkbilobin-2 with homology to the extracellular domain of plant cysteine-rich receptor-like
985 kinases. *Proteins* **77**: 247-251
986 **Miyakawa T, Hatano K, Miyauchi Y, Suwa Y, Sawano Y, Tanokura M (2014)** A
987 secreted protein with plant-specific cysteine-rich motif functions as a mannose-binding lectin
988 that exhibits antifungal activity. *Plant Physiology* **166**: 766-778
989 **Molina A, Miedes E, Bacete L, Rodríguez T, Mélida H, Denancé N, Sánchez-Vallet A,**
990 **Rivière MP, López G, Freydier A et al. (2021)** Arabidopsis cell wall composition
991 determines disease resistance specificity and fitness. *PNAS* **118**: e2010243118
992 **Nolen B, Taylor S, Gourisankar G (2004)** Regulation of Protein Kinases: Controlling
993 Activity through Activation Segment Conformation. *Molecular Cell* **15**: 661-675
994 **Oh MH, Clouse SD, Huber SC (2012)** Tyrosine Phosphorylation of the BRI1 Receptor
995 Kinase Occurs via a Post-Translational Modification and is Activated by the Juxtamembrane
996 Domain. *Frontiers in Plant Science* **3**: 175
997 **Ohtake Y, Takahashi T, Komeda Y (2000)** Salicylic Acid Induces the Expression of a
998 Number of Receptor-Like Kinase Genes in *Arabidopsis thaliana*. *Plant & Cell Physiology* **41**:
999 1038-1044
1000 **Oparka K (1994)** Plasmolysis: New Insights into an Old Process. *New Phytologist* **126**: 571-
1001 591
1002 **Perez-Riverol Y, Csordas A, Bai J, Bernal-Llinares M, Hewapathirana S, Kundu DJ,**
1003 **Inuganti A, Griss J, Mayer G, Eisenacher M et al. (2019)** The PRIDE database and related
1004 tools and resources in 2019: improving support for quantification data. *Nucleic Acids*
1005 *Research* **47**: D442-D450
1006 **Rajakulendran T, Sahmi M, Lefrançois M, Sicheri F, Therrien M (2009)** A dimerization-
1007 dependent mechanism drives RAF catalytic activation. *Nature* **461**: 542-545
1008 **Reynolds ES (1963)** The use of lead citrate at high pH as an electron-opaque stain in electron
1009 microscopy. *Journal of Cell Biology* **17**: 208-212.
1010 **Seo M, Jikumar Y, Kamiya Y (2011)** Profiling of Hormones and Related Metabolites in
1011 Seed Dormancy and Germination Studies. *Methods in Molecular Biology* **773**: 99-111.

1012 **Shiu SH, Bleecker AB** (2001) Plant Receptor-Like Kinase Gene Family: Diversity,
1013 Function, and Signaling. *Science Signaling* **2001**: RE22

1014 **Sparkes IA, Runions J, Hawes C** (2006) Rapid, transient expression of fluorescent fusion
1015 proteins in tobacco plants and generation of stably transformed plants. *Nature Protocols* **1**:
1016 2019-2025

1017 **Taylor I, Seitz K, Bennewitz S, Walker JC** (2013) A simple in vitro method to measure
1018 autophosphorylation of protein kinases. *Plant Methods* **9**: 22

1019 **Taylor SS, Kaila-Sharma P, Weng JH, Aoto P, Schmidt SH, Knapp S, Mathea S,**
1020 **Herberg FW** (2020) Kinase Domain Is a Dynamic Hub for Driving LRRK2 Allosteric.
1021 *Frontiers in Molecular Neuroscience* **13**: 538219

1022 **Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch-Mani B, Vogelsang R,**
1023 **Cammue BPA, Broekaert WF** (1998) Separate jasmonate-dependent and salicylate-
1024 dependent defense-response pathways in Arabidopsis are essential for resistance to distinct
1025 microbial pathogens. *Plant Biology* **95**: 15107-15111

1026 **Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, Xu W, Su Z** (2017) agriGO v2.0: a GO analysis
1027 toolkit for the agricultural community, 2017 update. *Nucleic Acids Research* **45**: W122-
1028 W129

1029 **Turnbull CGN, Booker JP, Leyser HM** (2002) Micrografting techniques for testing long-
1030 distance signalling in Arabidopsis. *Plant Journal* **32**: 255-262

1031 **Turner SR, Somerville CR** (1997) Collapsed Xylem Phenotype of Arabidopsis identifies
1032 Mutants Deficient in Cellulose Deposition in the Secondary Cell Wall. *The Plant Cell* **9**: 689-
1033 701

1034 **Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D,**
1035 **Slusarenko A, Ward E, Ryals J** (1992) Acquired Resistance in Arabidopsis. *The Plant Cell*
1036 **4**: 645-656

1037 **Vaattovaara A, Brandt B, Rajaraman S, Safronov O, Veidenberg A, Luklová M,**
1038 **Kangasjärvi J, Löytynoja A, Hothorn M, Salojärvi J et al.** (2019) Mechanistic insights
1039 into the evolution of DUF26-containing proteins in land plants. *Communications Biology*
1040 **2**:56

1041 **Xu D, Dhiman R, Garibay A, Mock HP, Leister D, Kleine T** (2019) Cellulose defects in
1042 the Arabidopsis secondary cell wall promote early chloroplast development. *Plant Journal*
1043 **101**: 156-170

- Wang X, Goshe MB, Soderblom EJ, Phinney BS, Kuchar JA, Li J, Asami T, Yoshida S, Huber SC, Clouse SD** (2005) Identification and functional analysis of in vivo phosphorylation sites of the Arabidopsis BRASSINOSTEROID-INSENSITIVE1 receptor kinase. *The Plant Cell* **17**: 1685-1703
- Wegener AD, Jones LR** (1984) Phosphorylation-induced mobility shift in phospholambdan in sodium dodecyl sulfate-polyacrylamide gels. Evidence for a protein structure consisting of multiple identical phosphorylatable subunits. *Journal of Biological Chemistry* **259**: 1834-1841
- Wrzaczek M, Brosché M, Salojärvi J, Kangasjärvi S, Idänheimo N, Mersmann S, Robatzek S, Karpiński S, Karpińska B, Kangasjärvi J** (2010) Transcriptional regulation of the CRK/DUF26 group of Receptor-like protein kinases by ozone and plant hormones in Arabidopsis. *BMC Plant Biology* **10**: 95
- Yadeta KA, Elmore JM, Creer AY, Feng B, Franco JY, Rufian JS, He P, Phinney B, Coaker G** (2017) A cysteine-rich protein kinase associates with a membrane immune complex and the cysteine residues are required for cell death. *Plant Physiology* **173**: 771-787
- Yan L, Ma Y, Liu D, Wei X, Sun Y, Chen X, Zhao H, Zhou J, Wang Z, Shui W et al.** (2012) Structural basis for the impact of phosphorylation on the activation of plant receptor-like kinase BAK1. *Cell Research* **22**: 1304-1308
- Yeung W, Ruan Z, Kannan N** (2020) Emerging roles of the α C- β 4 loop in protein kinase structure, function, evolution, and disease. *IUBMB Life* **72**: 1189-1202
- Yeh YH, Chang YH, Huang PY, Huang JB, Zimmerli L** (2015) Enhanced Arabidopsis pattern-triggered immunity by overexpression of cysteine-rich receptor-like kinases. *Frontiers in Plant Science* **6**: 1-12
- Zhou Q, Liu J, Wang J, Chen S, Chen L, Wang J, Wang HB, Liu B** (2020) The juxtamembrane domains of Arabidopsis CERK1, BAK1, and FLS2 play a conserved role in chitin-induced signaling. *Journal of Integrative Plant Biology* **62**: 556-562
- Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JDG, Boller T, Felix G** (2006) Perception of the Bacterial PAMP EF-Tu by the Receptor EFR Restricts Agrobacterium-Mediated Transformation. *Cell* **125**: 749-760

Figure 7 *crk10-A397T* mutant plants are more resistant to infection with *Fusarium oxysporum* f. sp. *conglutinans* 699. A-D, Representative images of WT (A), *crk10-2* (B), CRK10 OE-1 (C) and *crk10-A397T* mutant (D) plants at 11 days post inoculation with *F. oxysporum*. E, Mortality curve of WT, *crk10-A397T*, CRK10 OE-1 and *crk10-2* plants from day 7 to 20 post inoculation with *F. oxysporum*. Mortality is shown in percentage. The experiment was repeated twice with similar results. F, Probability of survival of each genotype following inoculation with the pathogen *F. oxysporum*. Associated 95% confidence intervals are shown. Deviance test, $\chi^2_3=19.68$, $p < 0.001$.

Figure 1 CRK10 and CRK10-A397 are active kinases and the A397T substitution introduces an additional auto-phosphorylation site in the kinase domain of the protein. A, Amino acid sequence of the cytoplasmic kinase domain of CRK10 used for in situ phosphorylation studies (CRK10 amino acid residue numbering shown on the left). Subdomains I-XI are indicated by roman numerals. Ala/Thr 397 site is highlighted in red, and the RD motif is underlined and highlighted in bold. The conserved glycine-rich loop, catalytic loop and activation segment motifs are also shown. B, Structure of the CRK10 kinase domain generated by homology modelling to the active kinase domain of BRI1. A molecule of an ATP analogue, coloured blue, occupies the active site. The α C-helix is coloured red, and the atoms in the side chain of the mutated threonine residue are depicted as red spheres. C, Recombinant His-CRK10kd^{WT} and His-CRK10kd^{A397T} extracts and their respective dead kinase controls (His-CRK10kd^{WT}-D473N and His-CRK10kd^{A397T}-D473N) resolved by SDS-PAGE; detection by Western blot with HRP-conjugated anti-His antibody is shown. D, SDS-PAGE and Western blot following treatment of purified His-tagged proteins with λ -phosphatase (PPase); control: untreated protein. E, In situ auto-phosphorylation sites in His-CRK10kd^{WT} and His-CRK10kd^{A397T} identified by LC-MS/MS analysis of the recombinant protein kinase domain. Threonine 397 is

highlighted in red. His: 6x His-tag; JM: juxta-membrane domain; KD: kinase domain; C-term tail: C-terminal tail. (f) MS/MS spectrum of the doubly charged (m/z 758.4) tryptic phosphopeptide NEVVLVTKLQHR in which the threonine residue is phosphorylated. Neutral losses of phosphoric acid from both the precursor ion and the C-terminal y ions are observed.

Figure 2 *crk10-A397T* mutant is a dwarf. A-F, Rosette morphology of WT (A, B, C) and *crk10-A397T* (D, E, F) plants at 2 (A, D), 3 (B, E) and 4 (C, F) weeks after sowing. Bar = 1 cm. G, Leaf series of 4-week-old WT (top) and *crk10-A397T* (bottom) plants. Bar = 1 cm. H, Main inflorescence stem of 5-week-old WT (left) and *crk10-A397T* (right) plants. Bar = 1 cm. I, 10-week-old WT (left) and *crk10-A397T* (right) plants. Bar = 2 cm. J, Siliques of WT (left) and *crk10-A397T* (right) plants. Bar = 1 cm. K, Seeds of WT (left) and *crk10-A397T* (right) plants. Bar = 500 μm.

Figure 3 Xylem vessels collapse in the root and hypocotyl of *crk10-A397T* plants, but not in the stem. A-F, Transverse cross sections of the base of stem (A, B), hypocotyl (C, D) and roots (E, F) of 5-week-old WT (A, C, E) and *crk10-A397T* (B, D, F) plants. Stain: potassium permanganate. Bars = (A, B, C, E, F) 100 μ m; (D) 50 μ m. G-H, Detail of xylem vessels in hypocotyls of 4-week-old WT (G) and *crk10-A397T* (H) mutant plants. Stain: potassium permanganate. Insert in top left corner of image shows original micrographs. Bars = (insert G) 50 μ m; (insert H) 25 μ m. I-J, Detection of auto-fluorescence of lignin on resin-embedded cross sections of 4-week-old hypocotyls of WT (I) and *crk10-A397T* (J) plants. Bars = 10 μ m.

Figure 4 CRK10 is a plasma membrane-localised protein expressed in association with the vasculature in the hypocotyl. A-B, Histochemical staining of reporter lines expressing the *CRK10*_{Pro}:*GUS* construct showed expression of the reporter gene in the vasculature of stem and hypocotyls, as shown by free-hand cross section of 8-week-old inflorescence stem (A) and cross section of 2-week-old hypocotyl embedded in resin (B). Bars = (A) 50 μm; (B) 25 μm. C, Detail of cross section in B shows presence of histochemical staining in xylem parenchyma cells (asterisks) and differentiating xylem vessels (arrows) in the hypocotyl. C-D, Hypocotyl of 4-day-old seedling of transgenic *Arabidopsis* plant expressing 35S:*CRK10-mCherry* before (C) and after (D) plasmolysis. Bars = 20 μm.

Figure 5 The root-hypocotyl system is responsible for the dwarf phenotype of *crk10-A397T* mutant plants. Images of non-grafted plants (A), self-graft controls (B) and graft combinations (C) of WT and *crk10-A397T* mutant. Plants were imaged 3 weeks after micrografting was performed. The phenotype observed for the reciprocal grafting combinations were consistently observed in two independent repetitions of the experiment. An average number of 10 grafts per combination was recovered each time. Annotation: scion / rootstock.

Figure 6 Transcriptional reprogramming in the *crk10-A397T* mutant shows activation of defense responses to biotic and abiotic stresses. A, Venn diagram displaying the number of differentially expressed genes (DEGs) in the *crk10-A397T* hypocotyls compared to the WT at each developmental time point. B, Top 15 enriched GO terms (Biological Process) for the up-regulated core DEGs in the *crk10-A397T* mutant plotted against their respective $-\log_{10}$ FDR. C, Top 15 perturbations showing highest overall similarity to *crk10-A397T* mutant expression signature (analysis performed using the GENEVESTIGATOR® SIGNATURE tool; $\log_2$ fold change values of time point 21 days was used as input for 274 core genes).

Parsed Citations

Acharya BR, Raina S, Maqbool SB, Jagadeeswaran G, Mosher SL, Appel HM, Schultz JC, Klessig DF, Raina R (2007) Overexpression of CRK13, an Arabidopsis cysteine-rich receptor-like kinase, results in enhanced resistance to Pseudomonas syringae. Plant Journal 50: 488-499

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ahuja I, Kissen R, Bones AM (2012) Phytoalexins in defense against pathogens. Trends in Plant Sciences 17: 73-90

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

An G, Ebert PR, Mitra A, Ha SB (1988) Binary vectors. In: Gelvin SB, Schilperoot RA eds. Plant Molecular Biology Manual. Kluwer, Dordrecht, Netherlands, A3:1-19

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Bacete L, Melida H, Miedes E, Molina A (2018) Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. Plant Journal 93: 614-636

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Beenstock J, Mooshayef N, Engelberg D (2016) How Do Protein Kinases Take a Selfie (Autophosphorylate)? Trends in Biochemical Sciences 41: 938-953

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Bojar D, Martinez J, Santiago J, Rybin V, Bayliss R, Hothorn M (2014) Crystal structures of the phosphorylated BRI1 kinase domain and implications for brassinosteroid signal initiation. Plant Journal 78: 31-43

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114-2120

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Bourdais G et al. CRK Consortium (2015) Large-Scale Phenomics Identifies Primary and Fine-Tuning Roles for CRKs in Responses Related to Oxidative Stress. PLoS Genetics 11: e1005373

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Camut L, Regnault T, Sirlin-Josserand M, Sakvarelidze-Achard L, Carrera E, Zumsteg J, Heintz D, Leonhardt N, Lange MJP, Lange T et al. (2019) Root-derived GA12 contributes to temperature-induced shoot growth in Arabidopsis. Nature Plants 5:1216-1221

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Chen Z (2001) A Superfamily of Proteins with Novel Cysteine-Rich Repeats. Plant Physiology 126: 473-476

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Chen Z, Hong X, Zhang H, Wang Y, Li X, Zhu JK, Gong Z (2005) Disruption of the cellulose synthase gene, AtCesA8/IRX1, enhances drought and osmotic stress tolerance in Arabidopsis. Plant Journal 43: 273-283

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Chinchilla D, Bauer Z, Regenass M, Boller T, Felix G (2006) The Arabidopsis receptor kinase FLS2 binds flg22 and determines the specificity of flagellin perception. Plant Cell 18: 465-476

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Clough S, Bent A (1998) Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant Journal 16: 735-743

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Czerniec P, Visser B, Sun W, Savouré A, Deslandes L, Marco Y, Van Montagu M, Verbruggen N (1999) Characterization of an Arabidopsis thaliana receptor-like protein kinase gene activated by oxidative stress and pathogen attack. The Plant Journal 18: 321-327

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

De Smet I, Voß U, Jürgens G, Beeckman T (2009) Receptor-like kinases shape the plant. Nature Cell Biology 11: 1166-1173

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Deniston CK, Salogiannis J, Mathea S, Snead DM, Lahiri I, Matyszewski M, Donosa O, Watanabe R, Böhning J, Shiao AK et al. (2020) Structure of LRRK2 in Parkinson's disease and model for microtubule interaction. Nature 588: 344-349

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Diévert A, Clark SE (2004) LRR-containing receptors regulating plant development and defense. Development 131: 251-261

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Du D, Liu M, Xing Y, Chen X, Zhang Y, Zhu M, Lu X, Zhang Q, Ling Y, Sang X, Li Y, Zhang C, He G (2019) Semi-dominant mutation in the cysteine-rich receptor-like kinase gene, ALS1, conducts constitutive defence response in rice. Plant Biology 21:25-34

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Du L, Chen Z (2000) Identification of genes encoding receptor-like protein kinases as possible targets of pathogen-and salicylic acid-induced WRKY DNA-binding proteins in Arabidopsis. Plant Journal 24: 837-847

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ellis C, Turner JG (2001) The Arabidopsis mutant cev1 has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. Plant Cell 13: 1025-1033

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ellis C, Karafyllidis I, Wasternack C, Turner JG (2002) The Arabidopsis Mutant cev1 Links Cell Wall Signaling to Jasmonate and Ethylene Responses. Plant Cell 14: 1557-1566

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Faria-Blanc N, Mortimer JC, Dupree P (2018) A Transcriptomic Analysis of Xylan Mutants Does Not Support the Existence of a Secondary Cell Wall Integrity System in Arabidopsis. Frontiers in Plant Science 9: article 384

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Friedrichsen DM, Joazeiro CAP, Li J, Hunter T, Chory J (2000) Brassinosteroid-Insensitive-1 Is a Ubiquitously Expressed Leucine-Rich Repeat Receptor Serine/Threonine Kinase. Plant Physiology 123: 1247-1255

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hanks SK, Hunter T (1995) The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. The FASEB Journal 9: 576-596

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hernández-Blanco C, Feng DX, Hu J, Sanchez-Vallet A, Deslandes L, Llorente F, Berrocal-Lobo M, Keller H, Barlet X, Sanchez-Rodriguez C et al. (2007) Impairment of Cellulose Synthases Required for Arabidopsis Secondary Cell Wall Formation Enhances Disease Resistance. Plant Cell 19: 890-903

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Houston K, Tucker MR, Chowdhury J, Shirley N, Little A (2016) The Plant Cell Wall: A Complex and Dynamic Structure As Revealed by the Responses of Genes under Stress Conditions. Frontiers in Plant Science 7: article 984

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

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 Transcriptomes. Advances in Bioinformatics 2008: article 420747

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Idänheimo N, Gauthier A, Salojärvi J, Siligato R, Brosché M, Kollist H, Mähönen AP, Kangasjärvi J, Wrzaczek M (2014) The Arabidopsis thaliana cysteine-rich receptor-like kinases CRK6 and CRK7 protect against apoplastic oxidative stress. Biochemical and Biophysical Research Communications 445: 457-462

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Janson G, Zhang C, Prado MG, Paiardini A (2017) PyMod 2.0: improvements in protein sequence-structure analysis and homology modeling within PyMOL. Bioinformatics 33: 444-446

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jeffrey PD, Russo AA, Polyak K, Gibbs E, Hurwitz J, Massag J, Pavletich NP (1995) Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex. Nature 376: 313-320

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nature Methods 12: 357-360.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kimura S, Hunter K, Vaahtera L, Tran HC, Citterico M, Vaattovaara A, Rokka A, Stolze SC, Harzen A, Meißner L et al. (2020) CRK2 and C-terminal phosphorylation of NADPH oxidase RBOHD regulate reactive oxygen species production in Arabidopsis. Plant Cell 32: 1063-1080

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Koornneef M, Reuling G, Karssen CM (1984) The isolation and characterization of abscisic acid-insensitive mutants of Arabidopsis thaliana. Physiologia Plantarum 61: 377-383

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lefebvre V, Fortabat MN, Ducamp A, North HM, Maia-Grondard A, Trouverie J, Boursiac Y, Mouille G, Durand-Tardif M (2011) ESKIMO1 disruption in Arabidopsis alters vascular tissue and impairs water transport. PLoS ONE 6: e16645

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Liao Y, Smyth GK, Shi W (2019) The R package R subread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. Nucleic Acids Research 47: e47

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. Methods 25: 402-408

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15: 550.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ma LS, Wang L, Trippel C, Mendoza-Mendoza A, Ullmann S, Moretti M, Carsten A, Kahnt J, Reissmann S, Zechmann B, Bange G et al. (2018) The *Ustilago maydis* repetitive effector Rsp3 blocks the antifungal activity of mannose-binding maize proteins. *Nature Communications* 9: 1711

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Masachis S, Segorbe D, Turrà D, Leon-Ruiz M, Fürst U, El Ghalid M, Leonard G, Lopez-Berges MS, Richards TA, Felix G et al. (2016) A fungal pathogen secretes plant alkalizing peptides to increase infection. *Nature Microbiology* 1: 16043

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Miyakawa T, Miyazono K, Sawano Y, Hatano K, Tanokura M (2009) Crystal structure of ginkbilobin-2 with homology to the extracellular domain of plant cysteine-rich receptor-like kinases. *Proteins* 77: 247-251

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Miyakawa T, Hatano K, Miyauchi Y, Suwa Y, Sawano Y, Tanokura M (2014) A secreted protein with plant-specific cysteine-rich motif functions as a mannose-binding lectin that exhibits antifungal activity. *Plant Physiology* 166: 766-778

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Molina A, Miedes E, Bacete L, Rodríguez T, Mérida H, Denancé N, Sánchez-Vallet A, Rivière MP, López G, Freydisier A et al. (2021) *Arabidopsis* cell wall composition determines disease resistance specificity and fitness. *PNAS* 118: e2010243118

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Nolen B, Taylor S, Gourisankar G (2004) Regulation of Protein Kinases: Controlling Activity through Activation Segment Conformation. *Molecular Cell* 15: 661-675

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Oh MH, Clouse SD, Huber SC (2012) Tyrosine Phosphorylation of the BRI1 Receptor Kinase Occurs via a Post-Translational Modification and is Activated by the Juxtamembrane Domain. *Frontiers in Plant Science* 3: 175

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ohtake Y, Takahashi T, Komeda Y (2000) Salicylic Acid Induces the Expression of a Number of Receptor-Like Kinase Genes in *Arabidopsis thaliana*. *Plant & Cell Physiology* 41: 1038-1044

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Oparka K (1994) Plasmolysis: New Insights into an Old Process. *New Phytologist* 126: 571-591

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Perez-Riverol Y, Csordas A, Bai J, Bernal-Llinares M, Hewapathirana S, Kundu DJ, Inuganti A, Griss J, Mayer G, Eisenacher M et al. (2019) The PRIDE database and related tools and resources in 2019: improving support for quantification data. *Nucleic Acids Research* 47: D442-D450

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Rajakulendran T, Sahmi M, Lefrançois M, Sicheri F, Therrien M (2009) A dimerization-dependent mechanism drives RAF catalytic activation. *Nature* 461: 542-545

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* 17: 208-212.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Seo M, Jikumaru Y, Kamiya Y (2011) Profiling of Hormones and Related Metabolites in Seed Dormancy and Germination Studies. *Methods in Molecular Biology* 773: 99-111.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Shiu SH, Bleecker AB (2001) Plant Receptor-Like Kinase Gene Family: Diversity, Function, and Signaling. *Science Signaling* 2001: RE22

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Sparkes IA, Runions J, Hawes C (2006) Rapid, transient expression of fluorescent fusion proteins in tobacco plants and generation of stably transformed plants. *Nature Protocols* 1: 2019-2025

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Taylor I, Seitz K, Bennewitz S, Walker JC (2013) A simple in vitro method to measure autophosphorylation of protein kinases. *Plant Methods* 9: 22

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Taylor SS, Kaila-Sharma P, Weng JH, Aoto P, Schmidt SH, Knapp S, Mathea S, Herberg FW (2020) Kinase Domain Is a Dynamic Hub for Driving LRRK2 Allostery. *Frontiers in Molecular Neuroscience* 13: 538219

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch-Mani B, Vogelsang R, Cammue BPA, Broekaert WF (1998) Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Plant Biology* 95: 15107-15111

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, Xu W, Su Z (2017) agriGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. Nucleic Acids Research 45: W122-W129

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Turnbull CGN, Booker JP, Leyser HM (2002) Micrografting techniques for testing long-distance signalling in Arabidopsis. Plant Journal 32: 255-262

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Turner SR, Somerville CR (1997) Collapsed Xylem Phenotype of Arabidopsis identifies Mutants Deficient in Cellulose Deposition in the Secondary Cell Wall. The Plant Cell 9: 689-701

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D, Slusarenko A, Ward E, Ryals J (1992) Acquired Resistance in Arabidopsis. The Plant Cell 4: 645-656

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Vaattovaara A, Brandt B, Rajaraman S, Safronov O, Veidenberg A, Luklová M, Kangasjärvi J, Löytynoja A, Hothorn M, Salojärvi J et al. (2019) Mechanistic insights into the evolution of DUF26-containing proteins in land plants. Communications Biology 2:56

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Xu D, Dhiman R, Garibay A, Mock HP, Leister D, Kleine T (2019) Cellulose defects in the Arabidopsis secondary cell wall promote early chloroplast development. Plant Journal 101: 156-170

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wang X, Goshe MB, Soderblom EJ, Phinney BS, Kuchar JA, Li J, Asami T, Yoshida S, Huber SC, Clouse SD (2005) Identification and functional analysis of in vivo phosphorylation sites of the Arabidopsis BRASSINOSTEROID-INSENSITIVE1 receptor kinase. The Plant Cell 17: 1685-1703

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wegener AD, Jones LR (1984) Phosphorylation-induced mobility shift in phospholamban in sodium dodecyl sulfate-polyacrylamide gels. Evidence for a protein structure consisting of multiple identical phosphorylatable subunits. Journal of Biological Chemistry 259: 1834-1841

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wrzaczek M, Brosché M, Salojärvi J, Kangasjärvi S, Idänheimo N, Mersmann S, Robatzek S, Karpiński S, Karpińska B, Kangasjärvi J (2010) Transcriptional regulation of the CRK/DUF26 group of Receptor-like protein kinases by ozone and plant hormones in Arabidopsis. BMC Plant Biology 10: 95

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yadeta KA, Elmore JM, Creer AY, Feng B, Franco JY, Rufian JS, He P, Phinney B, Coaker G (2017) A cysteine-rich protein kinase associates with a membrane immune complex and the cysteine residues are required for cell death. Plant Physiology 173: 771-787

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yan L, Ma Y, Liu D, Wei X, Sun Y, Chen X, Zhao H, Zhou J, Wang Z, Shui W et al. (2012) Structural basis for the impact of phosphorylation on the activation of plant receptor-like kinase BAK1. Cell Research 22: 1304-1308

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yeung W, Ruan Z, Kannan N (2020) Emerging roles of the α C- β 4 loop in protein kinase structure, function, evolution, and disease. IUBMB Life 72: 1189-1202

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yeh YH, Chang YH, Huang PY, Huang JB, Zimmerli L (2015) Enhanced Arabidopsis pattern-triggered immunity by overexpression of cysteine-rich receptor-like kinases. Frontiers in Plant Science 6: 1-12

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zhou Q, Liu J, Wang J, Chen S, Chen L, Wang J, Wang HB, Liu B (2020) The juxtamembrane domains of Arabidopsis CERK1, BAK1, and FLS2 play a conserved role in chitin-induced signaling. Journal of Integrative Plant Biology 62: 556-562

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JDG, Boller T, Felix G (2006) Perception of the Bacterial PAMP EF-Tu by the Receptor EFR Restricts Agrobacterium-Mediated Transformation. Cell 125: 749-760

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)